Development of Eco-friendly Technique for the Bioclarification of Highly Turbid Drinking Water In Mining Region of Goa Using Freshwater Yeast Cultures

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Under the guidance of

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September, 2022

CERTIFICATE

This is to certify that **SHEELA PAL** has satisfactorily completed the thesis entitled "**Development of Eco-friendly Technique for the Bioclarification of Highly Turbid Drinking Water In Mining Region of Goa Using Fresh Water Yeast Cultures**" submitted to Goa University for theaward of the degree of **DOCTOR OF PHILOSOPHY IN BOTANY** is a record of original and independent work carried out during the period of February 2016 – 2022 in the **DEPARTMENT OF BOTANY, SCHOOL OF BIOLOGICAL SCIENCES**

GOA UNIVERSITY under my supervision and that it has not previously formed the basis for theaward of any Degree, Diploma, Associateship or Fellowship or any other similar title to any candidate of this or any other university. I affirm that the thesis submitted by **SHEELA PAL** is completely independent research work carried by her under my supervision.

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It is hereby declared that the thesis entitled "**Development of Eco-friendly Technique for the Bioclarification of Highly Turbid Drinking Water In Mining Region of Goa Using Fresh water Yeast Cultures**" submitted to Goa University for the award of the degree of DOCTOR OF PHILOSOPHY IN BOTANY is a record of original and independent work carried out during the period of February 2016 – 2022 in the DEPARTMENT OF BOTANY, GOA UNIVERSITY under the supervision of Dr. NANDKUMAR MUKUND KAMAT, and that it has not previously formed the basis for the award of any Degree, Diploma, Associateship or Fellowship or any other similar title to any candidate of this or any other university.

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Dedicated

to Almighty Lord Shiva

My family, who have trusted on me

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ABBREVIATIONS

NTU	Nephelometric Turbidity unit
gm	Gram
L	Liter
mm	Millimeter
Tem	Temperature
rpm	Revolution per minute
Mm	Milimole
F.d.	Fractal dimension
MEA	Malt extract agar
ABR	Aclian blue retention
YMB	Yeast mediated biosedimentation
qt	Adsorption efficiency
Ce	NTU at equilibrium
Co	Initial concentration
Cf	NTU final
SMTTW	Simulated mine tailing turbid water

Thesis is organized into four chapters. Chapter I consists of brief introduction to the work presented, review of literature and objectives. Chapter II includes materials and methods involved, Chapter III consist of results obtained and final Chapter IV presents discussion with reference to important results concluding with prospects / scope for future work. At the end are placed associated sections such as summary, bibliography of literature cited, appendices regarding media, reagents, stains used, papers published / communicated in peer-reviewed journals, papers presented at national / international conferences, courses, workshops / other conference participation

CHAPTER I

Introduction

Review Of Literature

and

Objective

INTRODUCTION

The present work attempts to address the problem of pollution of drinking water resources, in the iron ore mining region of Goa.

1.1. Pollution of drinking water resources

Water on the earth is one of the important needs of living being. While 1.1 billion people live without clean drinking water, 2.6 billion people lack adequate sanitation in the world (Source: World water Council, 2022,).

In India approximately 77 million people lack access to safe water, 769 million lack adequate sanitation (Source: water.org, 2022). Around the world, the water quality in mining area is degrading in response to mining activity.

Organic solvents, petroleum products, and heavy metals from disposal sites or storage facilities can migrate into aquifers. Pesticides and fertilizers. Runoffs of mine dump can be carried into lakes and streams by rainfall runoffs or snowmelt, or can percolate into aquifers (US Environmental Protection Agency., 2021).

30% people of Goa get polluted water encouraging health problems like diarrhoea, dysentery mainly among children and the aged. The presence of excess raw iron is responsible for neurodegenerative diseases (George., 2010).

1.1.1. Open cast mining causes pollution in natural water bodies

The water sources on which we all rely are being threatened more and more by human activities like mining. Water pollution from mine wastewater dumped into the environment, seepage from tailings and waste rock impoundments, and heavy water use in ore processing are all effects of mining that have an impact on fresh water.

Open cast mines affect both surface, water body and agriculture field (Okolo., et al 2018).The environmental impact of mining operations that were conducted with little regard for the environment is becoming more widely known. We have occasionally paid a very high price for using minerals in our daily lives. By its very nature, mining uses up, diverts, and potentially dangerously pollutes water resources. For the purification of surface water there is a need to develop cost effective, easier and eco-friendly process.

Table. 1.1 Nomenclature related to ecofriendly sedimentation technique

Nomenclature	Definition	Reference
Bioclarification	Clarification is an essential step in a water or wastewater treatment process to remove suspended solids through gravity settling, providing a clarified liquid effluent. The process to remove suspended solids by biological means followed by gravity settling, is known as bioclarification.	https://www.evoqua.com/en/markets/applications/clarification/.
Biosorption	Both absorption and adsorption are described by the term "sorption,". The assimilation of a substance from one state into another is called absorption (i.e., liquids being absorbed by a solid or gases being absorbed by water). Ions and molecules physically bind to the surface of the solid material through adsorption. In this instance, the solid surface is the adsorbent and the material that has gathered at the interface is the adsorbate. In biosorption, a biological matrix is used as the sorbent.	Izabela et al., 2013
Bioadhesion	The phenomena of natural and artificial materials adhering to biological surfaces is referred to as bioadhesion.	Manuel et al., 2012
Bioflocculation	The term "bioflocculation" refers to a method where flocculants are mediated by microbes or biodegradable macromolecular flocculants produced by microorganisms.	Zayad et al., 2018
Biosedimentation	Sedimentation is the process in which particles separate from a liquid because of gravity. Biosedimentation is the process in which particles separate from a liquid mediated by biological agents followed by gravity.	Haan (2020)

1.2. Problems caused by colloidal Turbidity

Turbidity is the cloudiness or haziness of a fluid. Turbidity in water results from the presence of colloidal particles that scatter light. As a result, objects in water become indistinct. The turbidity of water measured with Turbidity meter, is calculated based on the amount of light scattered by particles in the water column.

Turbidity of water is a prevalent problem these days near industrial regions, such as mining and textile industries. Chemical and physical coagulants are now widely used to treat turbidity. Chemical procedures are unfriendly to the environment, whereas physical approaches cover a vast region. The use of microorganisms to treat turbidity hasn't been much applied. Previously, bacterial and fungal strains were utilized to remove turbidity, but they were found to be low range tolerant and produce a large amount of biomass, respectively (Keenan., 2000, Nazareth et al. 2001).

Soil colloids with size range 0.001µm to 1 µm are complex mixtures of organic and inorganic entities (Everett., 1972) and causes turbidity in solution (Sakhawoth et al., 2017).

Table.1.2 Classification of textural classes

S.N.	Textural Class	Particle size diameter (mm)
1	**Sand**	0.02 - 2.0 mm
2	**Silt**	0.002 - 0.02 mm
3	**Clay**	0.002 mm

1.2.1. Suspended particulate matter (SPM)

More the particles present in water, more will be the light scattered. Hence, turbidity and total suspended solids are related.

LITERATURE REVIEW

1.3. Goa

Goa is an important Indian state having natural magnificent beaches, a plateau, estuaries, and mangrove environment, as well as a wide range of plant and animal species. It is rich in minerals such as iron, manganese, bauxite, limestone, dolomite, refractory clays, limonite sand, steatite, silica sand, feldspar, graphite, talc, quartz, soapstone, etc. Due to the abundance of minerals, the mining sector in Goa is vital to the state's economy (Nayak., 2002).

Mining industry in Goa is mainly centered in four talukas: Bicholim, Sattari, Quepem and Sanguem, accounting for around 16 percent of the overall geographical area (AEQM.,1997). Aside from development, mining has irreversibly harmed forests, agriculture and water resources. Ore was retrieved from turbid pits below the water table in some mines using water pumped from the pits. Pumped water has the potential to b detrimental to both biotic and human life. Metals such as Fe, Mn, Cr, Pb, Ni, and Zn are adsorbed on clay and transported from mines (Nayak et al., 1995). During the rainy season, mines discharge runoff into local water bodies, causing turbidity. The Selaulim and Bicholim rivers were clogged with silt, while the Zuari and Mandovi rivers were poisoned with arsenic (levels as high as 50 micrograms/gram due to mining activity) (Nayak et al., 1995). Approximately 90% of Goa's iron and ferromanganese is carried to the Mormugao seaport via these two rivers. The Mollem National Park, the Bhagwan Mahaveer animal sanctuary, and protected regions in Bondla, Neturlim, and Cotigao wildlife sanctuaries are among the sensitive zones where mining is steadily making inroads (AEQM., 1997).

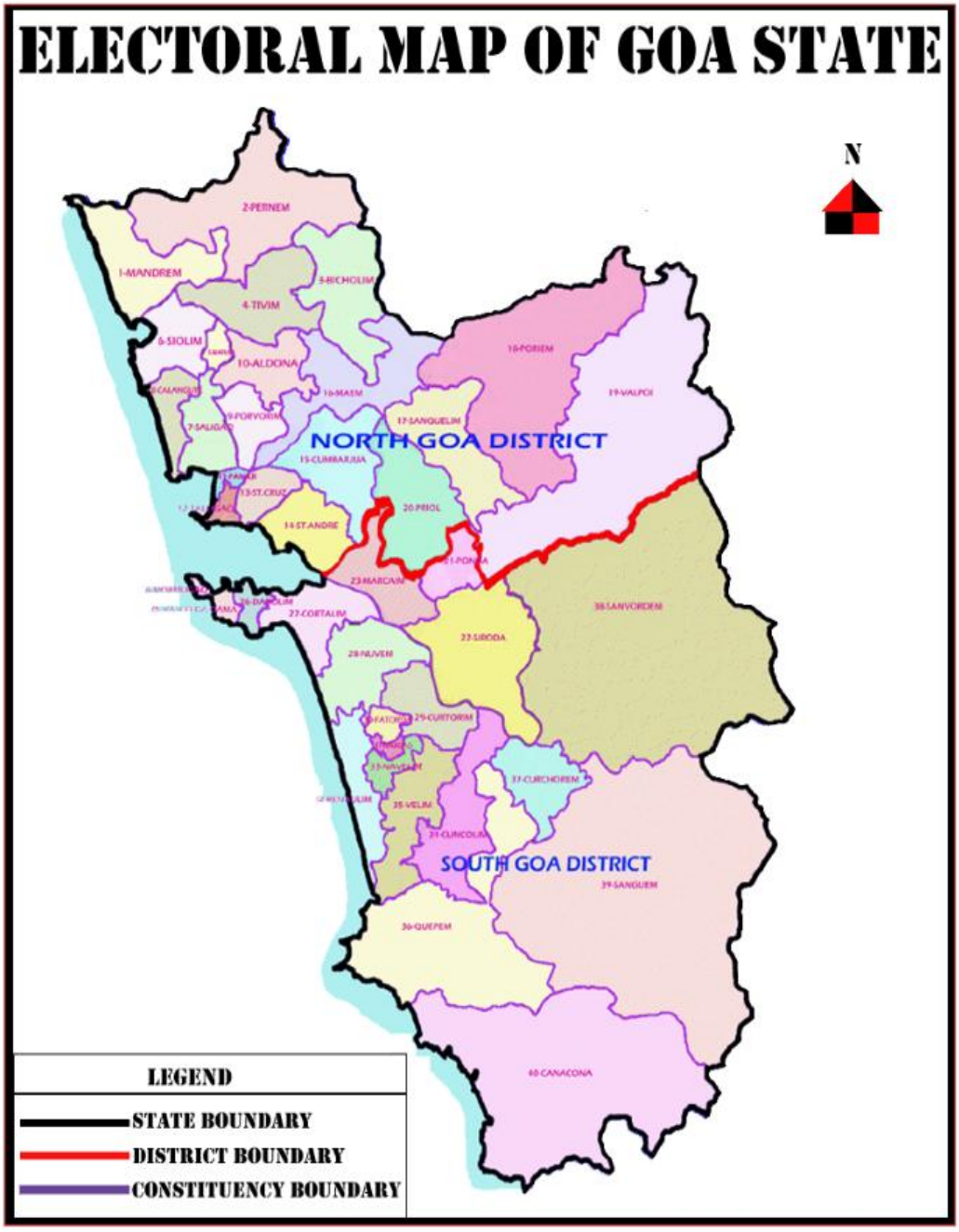


Figure.1.1: Map of Goa

(Image courtesy:https://ceogoa.nic.in/appln/uil/GoaMap.aspx)

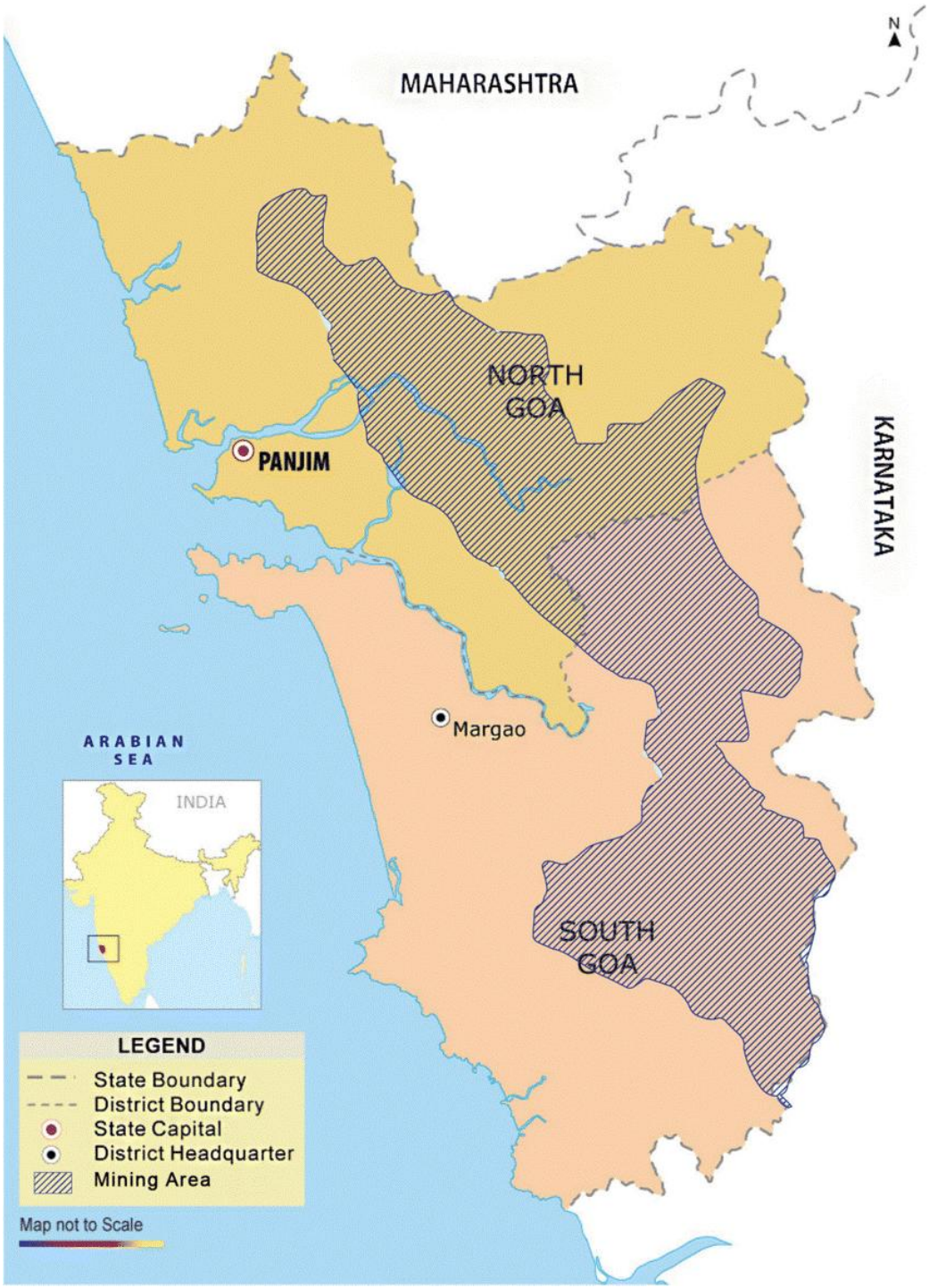


Figure.1.2: Mining area of Goa (Image courtesy: Sarupria et al., 2018)

1.3.1. Freshwater bodies in Goa

Goa is blessed with a plethora of freshwater bodies including lentic and lotic e.g. natural springs, lakes, ponds, waterfalls and streams. The water reservoirs having low salinity concentration are known as freshwater bodies.

1.3.2. Present status of Fresh water bodies in Goa

Freshwater bodies have a lot of biodiversity. It depicts seasonal changes in the environment (Udayashankara.,2015). Water quality monitoring using physiochemical indicators reflect contemporary water quality. However biotic parameters created in recent years have proven to be a great tool for predicting water quality, trophic level evolution and pollution status. Biological monitoring could be a good and affordable way to assess the effects of environmental influencers (Robert 1974). The abundance of phytoplanktons and other bacteria has an impact on trophic levels in the environment (Krishnamurthy et al., 2000). Many bacteria can be extracted from fresh water bodies for potential application in human wellbeing. Nearly all of the freshwater bodies in the area are affected by mining operations.

The Western Ghats`s Freshwater Biodiversity Assessment was conducted by the IUCN Global Species Programme's Freshwater Biodiversity Unit in collaboration with the Zoo Outreach Organization (ZOO) to review the global conservation status and distribution of 1,146 freshwater species belonging to four taxonomic groups: fishes (290 taxa), mollusks (77 taxa), odonates (171 taxa) and aquatic plants (608 taxa) in response to this need for information and raised awareness (Molur., 2011).

During summer, the temperature of the water in Goa's temple tanks was higher than usual (Pariolkar, 2014). The presence of yeasts in seasonal freshwater bodies in Goa has received little attention. The ideal time for this type of fungi to recuperate is during the monsoon season (Naik KS., 2016). There is a scarcity of information about freshwater biodiversity.

Table 1.3 Water parameters values in freshwater bodies of Goa

Water body	Parameter	Temp (°C)	pH	Alkalinity	Hardness	Ca^{+2}	Cl^{-1}	Fe^{+3}	Mg^{+2}
Vaddem lake	Vikrant et al., 2015	28.5		166.1	317.4	169.3	218.7	0.32	120.9
Mayem lake	-do-	28.6	6.56	59	34.1	20.1	30.7	0.46	13.9
Santacruz lake	-do-	28.7	6.66	95.6	103	51.5	123.3	0.40	49
Someshwa tank	-do-	25.5	7.38	168.9	123.8	67.3	65.4	0.4	52.9
Syngenta lake	Sawaiker et al., 2016	28	6.20	-	-	-	-	-	-
Khandola Pond	-do-	25	6	-	-	-	--	-	-
Lotus lake	-do-	25.5	6.3	-	-	-	-	-	-
Curtorim lake	-do-	26.2	6.72	-	-	-	-	-	-

PO_4^{-3}	0.478	0.27	0.43	0.37	0.1	0.07	0.01-0.7	0.49
SO_4^{-2}	225	84.8	103.2	170.1	-	-	-	-
TSS	-	-	-	-	-	-	-	-
TDS	-	-	-	--	600	45	616	1210
DO	-	-	-	-	8-12	9	6.60	9.19
NO_3^{-1}		-	-	-	0.20-0.7	0.4	2.5	1.43

*Temp=Temperature ,ºC All values are in mg/L except temperature, electrical conductivity,pH

1.3.3. Natural factors affecting water quality in Goa

Natural factors, affecting water quality are related to the hydrography and the topography of the region. Lakes, ponds, ditches, water reservoirs, tanks etc receive pollutants from catchment area and are less diverse in pollutants as compared to rivers, stream, creeks, brooks, etc.

Stream frequency are often subjected to a greater degree of pollution. More the amount of runoff making its way into the water, more will be the increase in pollutant diversity.

The intensity of rainfall varies in Goa from place to place. Rainfall is the main cause of aquatic pollution of water bodies as it causes runoff from sand, laterite and bauxite in mining areas.

The topography of Goa is highly undulating with vast areas of uneven terrain. Mining activity is considered as the primary cause of altering the natural topography in some mining areas of Goa. Due to resulting heavy siltation, the health of water bodies is degrading (Nayak., 2002).

Physico-chemical analysis of seven riverine water samples from Bicholim taluka carried out by Nayak (1995), show that Velguem and Harvalem rivers exhibit a marked seasonal variation in pH and TSM as they run through active mine areas. Locals in some rural areas of Goa use tank water (natural spring water) for drinking, and they suffer greatly during the wet season due to lack of clean drinking water, as it gets contaminated with run offs of silt or from mine dumps.

Water treatment is a critical issue in the mining industry because it is used in the mineral ore treatment process. Mine dump runoff pollutes water by contaminating it with heavy metals, producing leaching, erosion, and sedimentation. Clay, colloids, and minerals are commonly found in mine effluent (Ghose et al., 2000), which increase water turbidity and hence have an influence on public health.

Studies are being conducted to remove colloidal turbidity caused by mining operations. Heavy metal contamination and its effects on the environment and living creatures are being examined by research institutions in various parts of Goa. Chemical, physical, and biological approaches are commonly employed to eliminate colloidal turbidity.

Some chemical flocculants used in water purification such as polyacrylamide are non-biodegradable. Chemical flocculants also produce colours in treated water effluent and build fragile flocs that are difficult to settle (Bhatti et al., 2009).

Physical wastewater treatment technologies have been criticised for having problems such as sludge settleability, a big footprint, a long retention time and high-volume inefficiency (Odegard et al., 1990). Heavy metals are being removed from aqueous solutions using polymer-based nano composites (Guixia et al., 2018). Wang (2018) examined the research on removing heavy metals from wastewater using nanoscale zero valent iron. Metal organic framework compounds can be thought of as an alternative

method to get heavy metals out of water (Paulina et al., 2018). Yihan (2018) examined the work on heavy metal removal from wastewater utilizing novel materials such as metal organic framework.

Because of their economic viability and ecological superiority, biological techniques have got a lot of attention. Microbial species are being employed as sustainable ways in the biotreatment of very turbid water resources, utilizing the ability of diverse species of bacteria, fungi, algae, and other microorganisms to aid the flocculation process and remove turbidity. When it comes to reducing colloidal turbidity, coagulation and flocculation have an advantage. Microbial treatment plants take up less room and are environmentally favourable. Microbes work by contacting positively charged particles with negatively charged microbial cell surfaces, resulting in the formation of clumps, that settle down quickly (Hattori et al., 1970; Verspagen., 2006).

1.4. Composition of muddy water and soil in mining area and mine tailing soil

Goa is rich in natural mineral wealth. Iron ore, Bauxite, China clay and silica sand are important deposits. Iron ore and manganese ore have resulted from the residual concentration of banded manganiferrous quartzites and phyllites respectively. Mining reserves are prominent in Bicholim, Sanguem and Sattari. Important mineral formations in Goa is significantly observed in the Vageri formation, Bicholim formation, Sanvordem formation and Barcem formation (Nayak et al., 1995).

Table1.4 Types of clays and their composition in Iron ore mining (Nayak et al., 1995).

Name of clay	Colour	Percentage of Metal Elements (W/W)
Intrusive	Pale Pink with yellow spots	Fe: 18-25; Al_2o_3: 30-35; Mno: 0.02- 0.5 SiO_2: 10-15
Lateritic	Brown pink	Fe: 40- 45; Al_2o_3: 20- 25; Mno: 0.25- 0.5 Sio_2:10-20
Limonitic	Yellowish orange	Fe: 45- 56; Al_2o_3: 14- 17 Mno: 0.25- 0.5; Sio_2 : 7-9
Manganiferous	Black, yellow, brown sticky with oily appearance	Fe: 35- 43; Al_2o_3 : 5- 10 Mno: 5- 12; Si0 2: 5- 9
Phyllitic	Pink	Fe: 11- 12; Al_2o_3: 17 Mno : traces; Sio_2: 25- 30

Table 1.5 Physical Properties of natural soils dump waste and mine tailings dump (Nayak et al., 1995)

Soil Sample	Particle Size Analysis			Bulk Density g/cm³	Particle Bulk Density g/cm³	Porosity%	Water holding capacity %
	Sand%	Silt%	Clay %				
Natural Soil	51.2	24.7	20.3	0.96	1.9	49.48	44.1
Dump Waste (Sandy Loams)	56.9	21.9	18.1	1.3	2.8	53.5	40.6
Tailings (Sandy Clay Loam)	48.6	25.0	26.1	1.7	3.0	44.4	31.7

Table 1.6 Metals found in agricultural soil from mining and non mining areas (Ratha et., al 1994)

Metal found in agricultural soil affected by mining				Metal found in agricultural soil non affected by mining			
Element	Concentration	Element	Concentration	Element	Concentration	Element	Concentration
Na %	0.35	Ca %	0.208	Na %	0.275	Ca %	0.274
Mg %	0.77	Ti %	1.581	Mg %	1.035	Ti %	1.295
Al %	17.92	Mn %	0.360	Al %	17.405	Mn %	0.247
Si %	45.688	Fe %	31.924	Si %	43.00	Fe %	34.824
P %	0.147	Ni ppm	1.422	P %	0.162	Ni ppm	1.345
K %	1.045	Pb ppm	0.641	K %	1.168	Pb ppm	0.642
Cr %	0.253	Zn ppm	0.210	Cr %	0.204	Zn ppm	0.188
Co%	0.087	-	-	Co%	0.066	-	

1.5. Rainfall as a factor for transportation of mine tailing dump in water bodies

Goa received annual rainfall of 3663.9 mm in 2016, 3443.4 mm, in 2017, 2671.3 mm in 2018 (IMD, https://hydro.imd.gov.in/hydrometweb) so average annual rainfall of Goa is approximately 3500 mm (Kamal et al., 2015). During rainy season, mine dump or ore runoff enters into the freshwater bodies of these areas. The high concentration of ore tailing cause turbidity in the water bodies. According to analytical reports of mining companies and IBM Nagpur, suspended solids were in the range of 102 to 4212 mg/L in the river stream nallahs of Goa (AEQM, 1997).

1.6. Conventional and natural methods for removal of colloidal turbidity

Coagulation or flocculation, sedimentation, and sand filtration, followed by disinfection, are some of the traditional methods for removing turbidity from water (Raus et al., 2016). Chitosan and its derivatives are utilised as flocculants in a variety of applications (Yang et al., 2016). Among all chemical-based flocculants, such as ferric chloride, aluminium sulphate, copper sulphate, and ferrous sulphate; Al-based flocculants are frequently used because they can change the surface's charge property, causing colloidal particles to coagulate and form flocs (Wue et al., 2007). However, aluminum residues have been linked to Alzheimer's disease in studies (Xu et al, 2014).

Table.1.7 Turbidity removal efficiency of different natural plant coagulants

Name of coagulants from plant	Turbidity removal efficiency (%)	References
Okra mucilage	98.7%	Ani et al., 2012
Bean seed	80%	Antov et al., 2012
Moringa oleifera seeds	97%	Azni et al., 2006
Date palm rachis	95%	Mhenni., 2010
Hibiscus rosa sinensis	99%	Jinisha et al., 2017
Chestnut and acorn	90%	Skirbic et al., 2009
Cactus and hyacinth beans peels	89.03 %, 77.10 %	Girish., 2012
Plantago ovata	95.6%	Ramavandi et al., 2014
Water hyacinth (*Eichhornia crassipes*)	99.5% Cr (IV) removal)	Shaha et al., 2017 Shaha et al., 2017

Natural coagulants, based on plants researched for removing turbidity from wastewater, produce huge biomass, making it difficult to remove from drinking water. Seaweeds have been examined for their biosorption qualities due to their adsorbent properties and the fact that they are readily available in large quantities (Davis et al., 2000). Cellulosic algal cell walls contain carboxylate, amine, imidazole, phosphate, sulphydril sulphate and hydroxyl moieties (Christ et al., 1981). The decrease of NTU of colloidal turbidity by *Plantago ovate* seed extract has been researched, and displayed a substantial reduction rate of NTU after doping with NaCl (Ramesh et al., 2017). Table 1.7 contains a list of natural coagulants as reported by various studies.

1.7. Algae as bioremediation agents

Because algae undergo photosynthesis, they transform solar energy into useful biomass and absorb nutrients like nitrogen and phosphorus, creating eutrophication. Microalgae are an important part of the aquatic food chain because they produce C and N. (Lange et al., 1993). In wastewater treatment, the bio-treatment of sewage using algae is particularly appealing. The use of algae to clean wastewater has been studied for over forty years, with Oswald et al. (1957) reporting one of the first descriptions of this application. In the presence of Lemanea, Stigeoclonium and other algae, wastewater treatment is mediated by a combination of nutrient intake, higher pH and high dissolved oxygen concentration. *Micrasterias*, *Staurastrum*, *Pinnularia*, *Meridionand*, *Surirella*,

and other *Micrasterias* species have been extensively used in this area (Al-Homaidan et al., 2014).

Algae have the ability to remove large amounts of nitrogen, sulphur, and phosphorus from the environment (Chevalier et al., 2000; Mulbry et al., 2008; Su, Mennerich, & Urban, 2012; Woertz et al., 2009). The presence of *Botryococcusbraunnii sp*. was reported in wastewaters contaminated with nitrogen, phosphorous, sulphur, ammonium phosphate and nitrate (An et al.,2003; Salloum et al., 2002), as it blooms when these elements are present in high concentrations in wastewater (An et al., 2003; Salloum et al.,) *Chlorella sp*. removed phosphate from wastewater and decreased nitrates in amines (Wang et al., 2010). Walker et al. (1975). Semple et al. (1996) investigated *Prothecazopfii* for the degradation of aromatic compounds in oil and crude oil reduction. The bioremediation process of *Chlorella spp*. has been researched in both live and dried (frozen) cell stages (Wang et al., 2011).

Ribson (1998) studied the techniques for immobilizing algae cells for improved wastewater treatment performance and conducted an experiment using *Chlorella emersonii* for removal of phosphorous from wastewater. Chen (2003) investigated the marine microalgae *Isochrysis galbana* and successfully immobilised the algae to manage the water quality using clam cultures cultured in alginate beads for long-term storage. *Chlorella vulgaris* and the macrophyte *Lemnaminuscule* were used to clean wastewater containing high quantities of organic materials, such as ethanol and citric acid by-products (Valderrama et al., 2002). Photosynthetic bacteria and green microalgae have also been found to have the ability to remove nutrients. Under aerobic dark heterotrophic conditions, bacterial species, such as *Rhodobacter sphaeroides* and *Chlorella sorokiniana* were found to efficiently extract nutrients (Ogbonna et al., 2000). Andriana et al., (2012) cultivated freshwater and marine water algae to assess their potential for wastewater treatment and biofuel production and discovered that their growth rate is unrelated to the strain's nitrogen removal capacity. In 55 days, microalgae were able to remove 66.98 percent of chloride from chlorinated wastewater (Yang et al., 2016). The essential elements for the cultivation of microalgae for wastewater treatment, abiotic and biotic, operative conditions, and bioreactor design were examined by Jeon de la et al., (1992). The primary problems in successful waste remediation by algae are

how to preserve the mixed consortium's long-term effectiveness and homeostasis. (Goncalves et al., 2016).

Table.1.8 Algae reported for pollutant

Algal Species	Bioremediation targets	References
Prototheca zopfii	Crude oil	Walker et al.,1975
Diatoms	Naphtalene	Cerniglia et al., 1980
Phormidium, Oscillatoria, Nostoc and Synechococcus	Hydrocarbons	Abed et al.,2002
Algal mixed consortium *Chlorella* and *Scenedesmus*	N, P.	Shene et al.,2016
Spirulina	Domestic wastewater treatment	Laliberte et al., 1997
Phormidium sp.	N, Orthophoshphate	De la Nou et al., 1988
Coelastrum proboscideum	Pb (II)	Mac hardy et al., 1980
Cladophora glomerata	Zn	Vymazal et al., 1984
Dunaliella sp.	pyrethroid insecticide Deltamethrin	Baeza-Squiban et al., 1990
Chlamydomonas reinhardtii	Cu, Hg, Cd, Pb	Bayramo et al ., 2006
Monoraphidiumbraunii	bisphenol	Gattullo et al., 2012
Euglena, Oscillatoria, Chlamydomonas, Scenedesmus, Chlorella, Nitzschia and Navicula	Water pollution Indicator, Cr (IV) and Cl removal	Al-Homaidan et al., 2012; Alfonso., 2018

1.8. Bacterial Coagulants for Removal of Colloidal Turbidity of Wastewater

The bioremediation properties of several chemo heterotrophic bacteria were investigated. Bacterial colonies grow quickly and are easily genetically modified. Furthermore, extremophiles are important in decomposing dangerous substances or in killing other species in the environment. They are easily able to thrive in locations that are heavily polluted (Ahmad et al., 2012; Brim et al., 2000). *Pseudomonas*, *Rhodococcus*, and *Acinetobacter* are some of the bacterial genera used most often. (Shourian et al., 2009). Exopolymer binding has been shown to immobilize lead in a variety of bacteria, including *Staphylococcus aureus*, *Micrococcus luteus* and *Azotobacter species* (Maier et al., 2009). Many bacteria are lithotrophic in addition to adsorption of chemicals. They manufacture a variety of hazardous substances (Nakajima et al., 2008). Some bacteria enable for easy maintenance and collection of organisms due to their biofilm-forming capabilities (Radwan et al., 2002). The degradation or removal of wastewater constituents such as organic and inorganic components, such as ammonium and phosphorus, is a common subject of bacterial bioremediation research. The bacterial communities

operating in wastewater treatment plants, according to Wagner & Loy (2002), were linked with Betaproteobacteria, Alphaproteobacteria, Gammaproteobacteria, Bacteroidetes, and Actinobacteria. Ammonia oxidizers Betaproteo and Gammaproteo bacteria, in particular, are aerobic chemolithoautotrophs. In freshwater habitats, certain Alphaproteobacteria taxa, particularly *Nitrobacter* and *Nitrospira*, are known to oxidise nitrite to nitrate (Schmidt et al., 2003).

In order to eliminate colloidal turbidity from surface water, Wasify (2015) used *Bacillus licheniformis*, *Bacillus insolitus*, and *Bacillus alvei* bacterial species to create exopolysaccharides.

Bacterial exopolysaccharides, which operate as a natural coagulant, exhibited measurable efficacy in the treatment of surface water. *Bacillus sp*. exopolysaccharide was utilised as a flocculating agent (Kanmani., 2017). Heavy metal removal from wastewater has been studied using a *Klebsiella varricolla* bacterial strain isolated from the textile industry (Aslam et al., 2017). Peptidoglycan, carboxyl, amine, and phosphonates are components of bacterial cell walls that are thought to aid contaminant biosorption from wastewater (Vijay et al., 2004). Bacterial strains have been isolated from activated sludge from local hoggery displayed chromium removal and turbidity removal efficiency at 28% and 90% respectively (Zhang et al., 2012). Olanira (2009) studied bacterial strain coagulant property. Marine bacterial isolates assessed for turbidity removal of kaoline clay and reduction of COD of dairy industry flocculation efficiency have been observed as 99.7% and 93.9%, respectively (Onukwuli., 2012). *Rhodopsuedomonas sphaeroides was* used for the flocculation of coal slurry (Zhang et al., 2012). *Arthrobacter sp.* isolated from Tuyme river, South Africa was studied for flocculant production on lactose and urea as a sole source of nitrogen and carbon and showed flocculating activity of 75.4% and 83.4%, respectively (Mabinya et al., 2012). *Pseudomonas aeruginosa* and *Bacillus subtilis* showed great potential for heavy metal adsorption (Alencar., 2017). *Fusarium solani*, *Bacillus and Arthrobacter* have been used for the efficient treatment of mine tailing turbidity on a lab scale (Nazareth et al., 2001). The biosorption study conducted by Ansari et al., (2007) using isolates isolated from agriculture soil irrigated with industrial wastewater contaminated with toxic metals

showed resistance to metals. Under anaerobic conditions, *Desulfovibrio* spp., a sulfate reducing bacteria, can cause metal precipitation (Roane and Pepper, 2000).

Challenges were encountered during the larg scale production of bacterial strains, for effective biosorption or flocculation. Bacterial strains were found to be pH specific, and could not tolerate pH variation, osmoregulation and physical robustness (Keenan., 2000).

Table1.9 List of some Metal adsorbing Bacteria

Bacteria	**Heavy metal**	**References**
Aeromonas caviae	Cd, Cr (IV)	Loukido et al., 2004
Alcaligenes eutrophus	Cd	Mahvi et al., 2004
Aphanothece halophytica	Zn	Incharoensakdi et al., 2002
Bacillus firmus	Pb, Zn, Cu	Salehizadeh et al., 2003
Bacillus licheniformis, Escherichia coli, Pseudomonas fluorescens	Cd, Pb, Zn	Basha et al., 2014
Bacillus licheniformis	Cu, Cr, Fe	Samarth et al., 2012
Bacillus licheniformis	Cr (IV)	Zhou et al., 2007
Bacillus subtilis, Micrococcus	Cu	Nakajima et al., 2001
Bacillus thuringiensis	Cr (IV)	Sahin et al., 2005
Bacillus thuringiensis	Ni, Hg	Ozturk et al., 2007
Corynebacterium glutamicum	Pb	Choi et al., 2004
Nostoc muscorum	Cr (VI)	Gupta et al., 2008
Ochrobactrum anthropi	Cd	Ozdemiret al., 2003
Pseudomonas aeruginosa	Cu, Pb	Kazyet al., 2002; Lin et al., 2006
Pseudomonas cepacia	Cu	Savvaidis., et al., 2003
Pseudomonas putida	Pb, Cu	Pardo et al., 2003
Pseudomonas	As	Nitish et al., 2018
Sphaerotilus natans	Cu	Beolchini.,et al., 2006
Sphingomonas paucimobilis	Cd	Tangaromsuk., et al., 2002
Staphylococcus xylosus	Cd, Cr (IV)	Ziagovaet al., 2007
Streptomyces coelicolor	Cu	Ozturk et al., 2005
Streptomyces pimprina	Cd	Puraniket al., 1995
Streptomyces rimosus	Fe (III)	Selatniaet al., 2004

1.9. Fungi in Wastewater Treatment

Since the fungi kingdom is very diverse with both unicellular yeast and branching hyphae, it produces several remarkable reproductive structures. Fungal biomasses are reported highly efficient for the sequestration of Zn (II) and Pb (II) from wastewater (Aftab. et al., 2017). Much attention has been paid to the removal of heavy metal from wastewater via fungal strains as they have high percentage of cell wall materials, which increases the possibility of the presence of a number of functional groups involved in binding the metal from wastewater (Gadd. 1998; Dhankhar. 2011). They can be easily applied practically because a large number of fungal strains are reported as non-pathogenic (Dhankhar., 2011). Many fungal species such as *Rhizopus, Aspergillus, Streptoverticillum, Saccharomyces* etc. have been explored for biosorption of heavy metals (Volesky et al., 1990). *Trichoderma* sp. have been studied for the absorption of copper from wastewater (Saxena., 2006). Krauss (2011) reviewed the work done for mineralization of xenobiotic compounds to carbondioxide via fungal species. Fungal strains *Fomitopsis meliae*, *Trichoderma ghanense* and *Rhizopus microsporus* isolated from gold mining sites in South Africa, and studied for their metal tolerance efffciency were found to show remarkable tolerance in heavy metal rich media (Oluwatosi., 2016). Fungi have been studied for enzymatic treatment to remove chemicals from water (Becker. 2017). Various species of terrestrial, wood and soil basidiomycetes show outstanding mineralization of xenobiotics, while aquatic fungi have yet to be studied (Krauss et al., 2011). First report on fungal wastewater treatment had been reported by Curtis 1969. Yoshizawa in the end of 1970 drew attention for the use of yeast in wastewater treatment.

Furthermore, researchers have found yeasts produce lipids (Chung., 2016) glycolipids (Yang., 2013) and enzymes. Therefore, it is widely used in the treatment of high concentration organic wastewater, heavy metal ions wastewater and domestic sewage.

1.10. Challenges for implementation of fungal biomass at field level

Despite the fact that fungal biomasses have been investigated for the removal of heavy metals from wastewater, field application remains a barrier. The mechanism of bioremediation and technological constraints could be the major reasons behind this (Dhankhar et al., 2011).

Direct microbiological application is also tricky. As a result, several aspects of the implementation of fungal biotreatment of wastewater must be investigated. There are physiochemical aspects to be considered, such as pH, as well as the major difficulty of biomass immobilization in wastewater treatment plants. Because it creates a large volume of sludge, improvements in reuse methods should be investigated and optimized.

Table1.10 List of metal adsorbing Fungi

Fungi	Heavy Metals Resistant	References
Trichoderma atroviride	Cu, Zn, Cd	Lopez et al., 2013
Trichoderma atroviride, Mortierella exigua	Zn, Ba, Fe	Malina et al., 2005
Aspergillus niger, Trichoderma asperellum, Penicillum simplicissimum	Cu, Pb	Burton et al., 2003
Trichoderma, Aspergillus, Mortierella, Paecilomyces, Penicillium, Pythium and Rhizopus	Co	Ross et al.,1986
T. virens (PDR-28)	As, Cu, Cd, Ni, Pb, Zn	Babu et al., 1998
	Metal adsorbent	
Aspergillus brasiliensis, Penicillium	Cu, Mn, Zn	Pereira et al., 2014
Aspergillus niger	Pb, Cd, Cu, Ni	Kapoor et al., 1999

Fungi	Heavy Metals Resistant	References
Aspergillus niger, Aspergillus flavus,	Pb, Ni, Cr (V)	Dwivedi et al.,2012
Aspergillus terreus	Ni, Fe, Cr (V)	Dias et al., 2007
Aspergillus terreus	Pb, Cd	Joshi et al., 2011
Gliocladium roseum	Cd	Massaccesi et al., 2002
Mucor rouxii	Pb, Cd, Ni, Zn	Yan., 2008
Rhizopus arrhizus	Zn	Zhou 1999
Rhizopus delemar	Cu, Co, Fe	Tsekova et al, 2002
Rhizopus oligosporus	Cd	Aloysius et al., 1999
Saprolegnia delica, *Trichoderma viride*	Zn (II), Pb (II),	Ali et al., 2007
Talaromyces helicus	Cd	Massaccesi et al., 2002
Trichoderma longibrachiatum	Cr(V)	Joshi et al., 2011
Trichoderma viride	Cd	Joshi et al., 2011
Trichoderma asperellum	Pb	Zhu et al., 2018
Aspergillus niger, Rhizopus,Penicillium	Petroleum pollutant	Duniya et al., 2018

1.11. Yeast

Yeast is a polymorphic, unicellular creature. It keeps its round, ovoid, or cylindrical shape. It belongs to the ascomycetous and basidiomycetous fungal groups.

Yeast is divided into two types: active yeast and non-active yeast.

I. Fermented yeast (Baker's yeast, for example) is a type of yeast that can only ferment six-carbon sugar into alcohol and carbon dioxide (Mohmd., et al 2017). *Candida sp*. is an example of oxidised yeast, which has high oxidation ability. The chemical makeup of the cell wall changes as yeast develops (Lebrun et al., 2018). Mannan, glucan, and mannoproteins are thought to be the main components of *Saccharomyces cerevisiae*'s cell wall (Northocote et al., 1952). Calcium is essential for flocculine activation (Hattori et al., 1970). Older cell forms flocculate earlier and is stronger than younger cell flocculate, according to Herrere et al. (1991). Younger cells lack flocculin and have lower cell sizes.

1.11.1. Aquatic natural yeast

The biodiversity of aquatic environmental specifically freshwater yeast community haven't been well studied (Yorkov, et al 2017). A study by Libkind et al. (2017) looked at the diversity and ecology of yeasts in freshwater habitats (temperate and tropical rivers, lakes, and lagoons). In terms of conformity, yeast identification was mostly based on differences in morphological and physiological features. As such the identification, was difficult in several cases with inconclusive results (Kurtzman & Robnett.,1998). Morphological and physiological testing were introduced in 2001. After the use of the more trustworthy molecular data in identification was introduced, most studies focused on identifying yeasts (Libkind et al., 2017).

1.11.2. Yeasts in Rain Water

Microorganisms in the air, clouds, or precipitation have not yet been given a clear definition of their makeup. Despite the fact that there have been several studies on bacterial communities, bacterial strains from the clouds were isolated, and their role in shaping the atmosphere's chemical makeup was investigated (Tina et al, 2015). After expressing bacteria in an active form from an inactive state in a suitable environment, Morris et al. (2008) showed atmospheric dispersal may play a significant influence in bacterial biogeography patterns. Bacterial strains are shielded from UV radiation and given nutrients to ensure their survival in fog and clouds (Tina et al., 2015). Clouds facilitate long-distance microbial movement (Griffin 2006). In 2002, Baurer and colleagues recovered bacterial and fungal spores from cloud water.

1.11.3. Application of yeast for removal of colloidal turbidity

Yeasts are unicellular eukaryotic organisms but at times they form hyphae or pseudo-hyphae according to environmental conditions. Aquatic yeast abundance and diversity depend on the water reservoir whether it is fresh water or marine water source (Hagler et al., 1987). Yeasts are polyphyletic members of the kingdom fungi's phyla ascomycetes and basidiomycetes.

According to Mueller and Schimt (2007), as the kingdom fungus was identified only 40 years ago and its variety is understudied, only 7% of the total species have been

described, making it critical to document the undescribed, particularly aquatic species (Shearer., 2007).

Smirnov (1964) proposed introducing yeast into lakes as fungal propagules through inflowing stream rain water and wind. Freshwater yeast ecology, such as distribution patterns, diversity, and importance in aquatic systems, has yet to be well researched (Carlos et al., 2012).

Monika N. discovered black yeast in tap water in 2016. *Slovenian Exophiala sp*. and *C. parapsilosisa* white yeast. Garg discovered *Rhodotorula graminis*, a red coloured yeast, from fresh water flowing through the mining area of Goa in 2011. Chin (2015) isolated 43 species of yeast from the sea surface microlayer and underlying water on Taiwan's northern coast using a polyphasic molecular method. Currently there are approximately 12000 yeast species (Hawkworth 1991), among which few have been studied for the cleaning of wastewater. Many industries use full scale bioclarification wastewater treatment plants based on this process (Yoshizawa et al., 2014). Many studies of yeasts associated with polluted water have been performed eg. *Candida*, *Rhodotorula* screened to find out the pollution level (Slavík et al., 1998).

Niljana et al., (2017) extracted yeasts from pharma industry effluent to breakdown cefdinir. Candida spp. had the highest cefdinir degradation efficiency of 84 percent. Zhang Fan (2015) discovered that mixed strains of bacteria and yeast are more efficient than single strains of bacteria and yeast for wastewater treatment. Bread yeast was reported to be an efficient natural coagulant for removing 99.6% colloidal turbidity from synthetic turbid water (Northocote 1952). *Saccharomyces cerevisiae* and *Torula sporadel brueckii*, utilised to treat pharmaceutical effluents, showed the highest percentage reductions of 52.5, 52.5, and 58.7%, for BOD, COD, and nitrate respectively (Abiye et al., 2015). The yeast nanocomposite system was built utilising a mixture of hydrophilic and hydrophobic nano-silicates and yeast cell mixture to grow yeast cells in a hydrocarbon polluted aqueous medium to breakdown the hydrocarbon (Klymenko et al., 2017).

Candida tropicalis showed reduction of copper levels in the growth medium by nearly 80%. (Rahman et al., 2007). In studies involving *Fusarium solani* and *Bacillus arthrobacter* for efficient treatment of mine tailing on a lab scale at Goa University (Nazareth et al. 2001; Deshpande, 1990), fungal strains were found to be more efficient. Biosorption may be primarily a function of the binding of metal cations to chemical functional groups on the yeast cell wall via ionic and coordinate bonds (Brady et al.,1994). Particles having opposite charges were attached to the surface of microbial biomass (Len et al., 2006).

Soh (2022) studied dye removal approach using spent brewer yeast and concluded that adsorption was not affected by changing the operating temperature. Chaudhary et al., 2022 found an excellent removal of Zn, Pb, Cd and Ni with the help of fungal consortia i.e. 80%, 70%, 80% and 75% respectively.

Yeasts have been examined for a variety of purposes. For example, Emila (1979) used yeast to assess the nitrogen budget in estuarine sediments. *Rhodotorula* strains, isolated from soil, were studied for lead reduction efficiency with phosphate, reported a removal of 98% of lead cations (Tian et al., 2022). *Sterigmatomyces halophilus* reported 100% acid orange dye removal by (Ali et al., 2021). Aibeche et al., (2021) reported aquatic yeasts for removal of toxic lead. Artifon et al., (2022) used spent brewer yeast for removal of textile dyes and attained 80-90% dye removal efficiency.

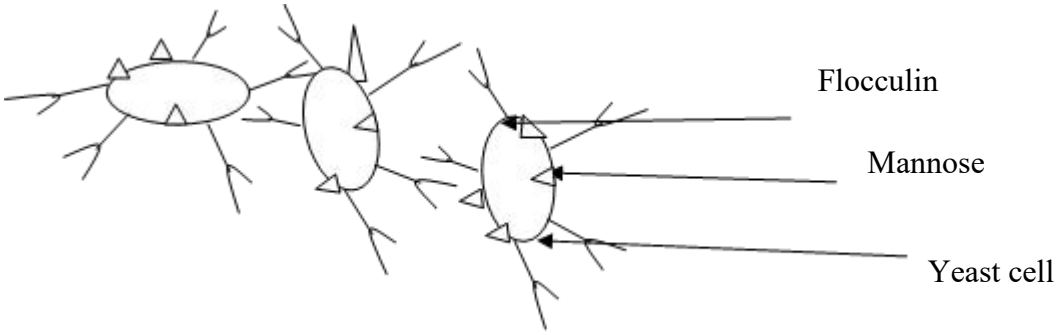


Figure.1.3: Mechanism of flocculation of cells

The primary characteristic of water clarifying by yeast is based on the unique structure of the microbial cell surface, morphology, and composition of the cell wall, all of which

must be carefully studied in order to achieve maximum performance (Verstrepen., 2004). Aside from cell-cell adhesion, yeast cell walls interact with their environment and have exceptional abiotic surface attachment abilities. This would reduce sludge generation, the treatment plant's ecological footprint, hydraulic retention time, and capital costs, as well as assist rural consumers. Yeast strains remove a lot of turbidity while reducing sludge generation.

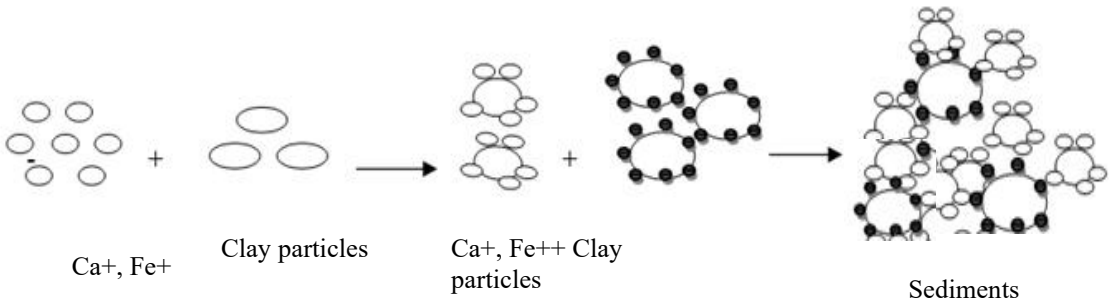


Figure.1.4: Mechanism of adhesion of clay minerals on microbial cell surface and sedimentation

According to Oh et al., (2009), the main source of metal biosorption is ionic interaction and complex formation between metal cations and acidic sites on the microorganism's surface. Because the potential of freshwater yeast in bioremediation has been underutilized, particularly in removing colloidal particles from turbid water, yeast's flocculating qualities may play a significant role in bioremediation. Millions of yeast cells are present in 1ml of yeast suspension ($1x10^7$ number of yeast cell in yeast suspension with absorbance1), are capable of binding clay colloids to its surface. Predicted mechanism of cell mediated biosedimentation is electrostatic force between anions and cations present on the surface of yeast and clay colloids respectively

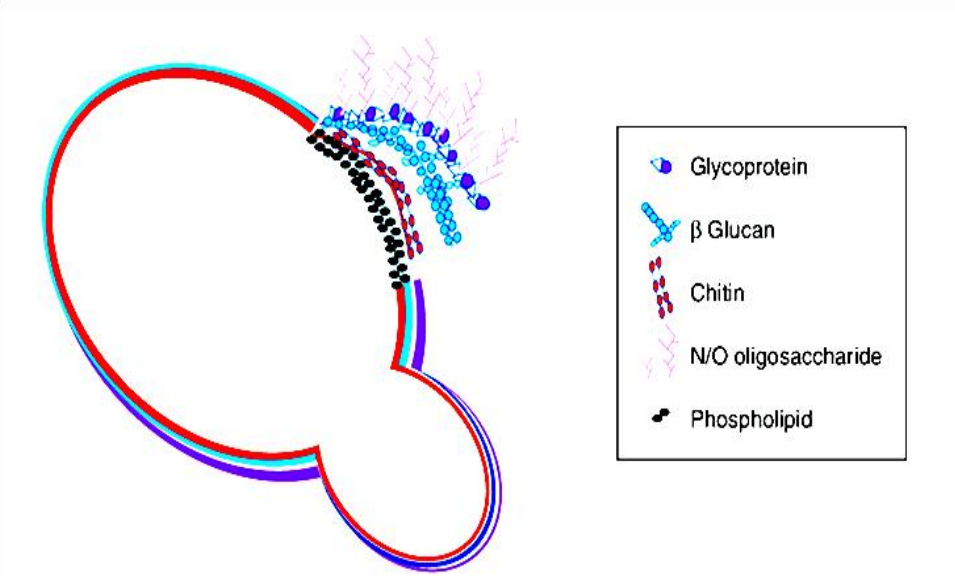


Figure.1. 5: Yeast wall composition demonstration

The fungal cell wall can compose approximately 40% of the total volume and range in thickness from 0.1µm to 1.0 µm. The main component of the cell wall of most fungi is chitin and β (1)-glucan are surrounded by a gel like substrate comprised primarily of α (1-)-glucans and galactomannan proteins (Hasim et al., 2019).

After thorough literature survey, it has been observed that yeasts were studied for removal of metals from water. For successful sedimentation of the colloidal particles, further removal of heavy metals of wastewater utilising freshwater yeast strains and its mechanism for particle adsorbent can be examined.

Turbidity caused by clay particles is a big issue in Goa's mining districts when it comes to providing potable water during the rainy season. The flocculation and sedimentation of colloidal turbidity present in water polluted by mine rejects are highly dependent on yeast adhesion properties.

Since chemical coagulants are not ecofriendly; use of plants generates sludge and bacterial strains have difficulty in large scale production for effective biosorption or flocculation, a study can be performed to evaluate yeast strains for effective bioflocculation. Yeasts could prove more advantageous as it can be produced on large scale with less effort and avoiding the production of secondary pollutants.

1.11. Present methods reported for the removal of turbidity

Method	Limitations
Chemical coagulant eg. alum salt	Non-biodegradable, Not ecofriendly,Chemical coagulant produces colours in effluent of treated water and forms fragile flocs difficult to settle. (Bhatti, Z. A 2009)
Physical	Long time required,Dissolved molecules not removed by conventional physical treatments.
Biologicaleg. Moringa	Produces huge sludge (Azni et al ., 2006)
Bacteria	Less efficient than fungi (Nazareth et al., 2001)

Biosedimentation is the process to achieve the destabilization of colloidal particle due to contact with biosedimentation to form large sediments that can be easily removed by from water. Particle are having opposite charges are attached to the surface of microbial biomass (Len et al., 2006). Clay minerals are having positive charge while microbial cell surface is having negative charge, clump together get settled down efficiently.

The literature, on the other hand, lacks a comprehensive assessment of the relative performance of yeast and water treatment systems for turbidity removal, as well as the consequences of integrating them in a continuous flow system.

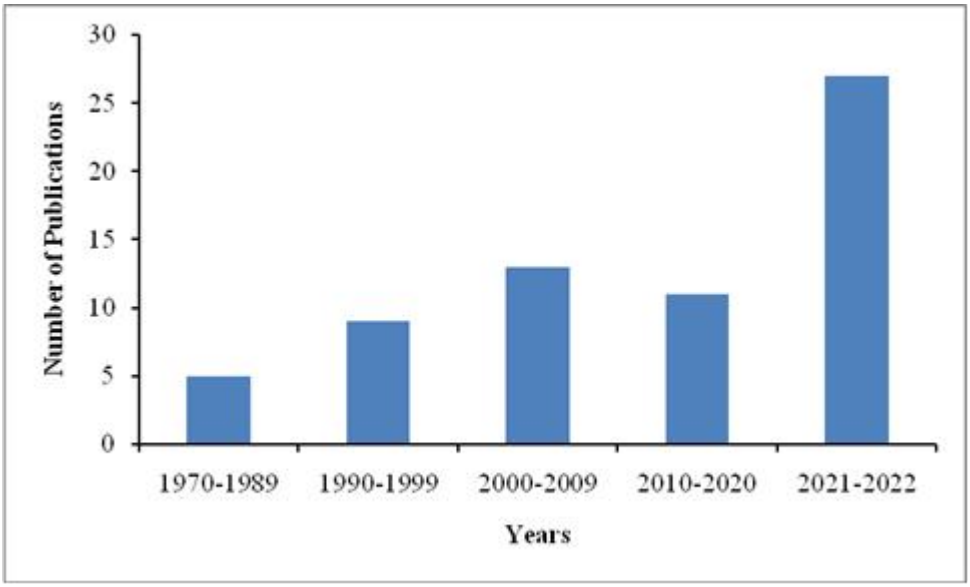


Figure.1.6: Number of publications for bioremediation of wastewater using yeasts

(Accessed from different search engines)

1.12. General mechanism of metal tolerance by resistant species

Several studies suggested that bacteria developed metal tolerance after being exposed to harmful metals shortly after life began, maybe as a result of the abundance of metal on the planet (Girno et al., 2002). Metal resistance in bacteria is assumed to have evolved as a result of recent exposure to metal pollution over the last 50 years. The current environmental degradation caused by anthropogenic metal activities has heightened the need for research into microbial metal resistance and treatment (Silva et al., 2012). Metals in the environment are directly influenced by microorganisms. During adaption, microorganisms have created innovative metal resistance and detoxifying methods. Plasmids can be used to codify the metal resistance of microbes (Galetti., 2019). It can be metal specific at times, or it can exhibit resistance to a variety of metals at other times. Metal resistance is caused by metals binding to extracellular materials, which immobilizes the metals. Cadmium, lead, zinc, and iron are a few of the cationic metals that can bind to anionic cell surfaces. Metals can bind strongly to carboxylic, amino, thio, hydroxo, and hydroxyl carboxylic phosphoryl groups on algal cell surfaces, while phospholipids in bacterial lipopolysaccharides in the outer membrane also interact strongly with cationic metals (Sathendra et al., 2018). The distribution of metals is influenced by cell surface binding, which is especially relevant in the aquatic environment. In practice, microorganisms are investigated for their ability to sorb metals for the aim of bioremediation, or the removal of metal pollutants from the environment.

1.12.1. Exopolymer binding

The most prevalent approach of lowering metal bioavailability is through this mechanism. Polysaccharides, carbohydrates, nucleic acids, and fatty acids make up the majority of exopolymeric compounds. Exopolysaccharides protect cells from desiccation, phagocytosis, and parasitism in addition to their role in heavy metal binding. The pH of the environment has a big impact on microbial exopolymers. Metal detoxification via EPSs causes metal immobilization and prevents metal entrance into the cell (Sathendra et al., 2018).

1.12.2. Siderophore complexation – Siderophores are low-molecular-weight organic compounds that chelate iron. Their biological role is to collect iron in low-concentration

environments and deliver it into the cell. Siderophores, on the other hand, may interact with metals that are chemically related to iron, such as aluminium, gallium, and chromium. Siderophores diminish metal bioavailability and thus metal toxicity by binding to metals. In cyanobacteria, siderophores, for example, minimise copper toxicity (Roane and Pepper, 2000).

1.12.3. Biosurfactants complexation – Several bacteria create this class of chemicals, which are expelled outside the cell. Biosurfactants have recently been studied for their role in the creation of complex metals such as cadmium, lead, and zinc (Miller, 1995). Metals become more soluble when they are complexed with biosurfactants, and the complexed metal is non-toxic to the cell. Metal reduction precipitation – Common metabolic by-products that result in metal reduction can affect metal bioavailability (Gupta et al., 1993). Soluble metals are converted to less soluble metal salts, such as sulfidic and phosphidic metal salts, in this situation. *Citrobacter spp*. can bind lead and copper in aerobic conditions due to phosphate synthesis by enzymes (Macaskie., et al 2000).

1.12.4. Metal dependent mechanism of metal resistance – The mechanisms of intracellular metal resistance in microbes are not well known. Metal binding and sequestration by metallothioneins or related proteins is perhaps the best-known process (Prasad et al., 2004).

When metal pollution is present in the environment, these ways are triggered in the bacterial system. Heavy metal metabolism and the management of various forms of stress are both aided by MTs. Plant phytochelations are comparable to metallothioneins in many aspects, including the high amount of cysteine residues in the protein and the fact that both are involved in heavy metal detoxification. Three traits are shared by all metallothioneiens - They are proteins with a low molecular weight. They are high in cysteine residue, high in metal content, and exhibit metal ion coordination in metal thiolate clusters. The metallothioneins can be categorised into two groups. All animal metallothioneins are classified as Class I, while those found in plants and other microbes are classified as Class II (Wilfried et al. 2000) The mammalian archetype is represented by the Class-I MTs, which have been fully described (Wilfried et al., 2000).

The thiol for mercaptide bonding in metal-thiolate clusters is provided by the invariant alignment of cys inside the protein. The alpha-domain in the craboxy terminal region of Cd and Zn is a metal cys cluster.

The metal 3-cys-9 cluster is found in the amino terminal beta-domain. 2-4 amino acids connect the two domains. At least 62 metallothioneins have been identified in mammals, fish, crabs, oysters, and mussels, among other animals (Fowler et al. 1987). *Synechcoccus spp*., *E. coli*, and *Pseudomonas putida* have all been found to produce metallothionein-like proteins (Gupta et al., 1993).

The efflux system is another metal-dependent mechanism of metal resistance. To remove metals from the cell, several microbes use plasmid-encoded energy-dependent metal efflux mechanisms. ATPases are used in some efflux systems, while chemiosmotic ion/proton pumps are used in others. These processes use active transport (ATPase pump) or diffusion (chemiosmotic ion/proton pump) to aggressively return harmful ions that have entered the cell out of the cell. The three metals most typically related with efflux resistance are arsenate, chromium, and cadmium (Nies., 2003).

1.12.5. Yeast in phosphate solubilization

As Phosphate is a important nutrient for plant growth, its solubility is slow in soil. Plant can take up phosphate only when it is in dissolved form. Many researchers have studied the adsorption of P from wastewater (Neal., 2001; Loganathan, 2014).

Phosphorus is an important component of plant growth, but excessive usage of phosphate fertilizers results in runoff and leaching during rainstorms, as well as overloading and eutrophication of aquatic habitats. Than. et al., (1973) studied yeast for the wastewater treatment and concluded, that water samples collected from different treatment sites, showed huge reduction in Phosphorous, ammonia and nitrogen after treatment with yeast. Aquatic hypomycetes *Tetracladium setigerum* studied for phosphate solubilization efficiency (Sati et al., 2018) found 3.15 mg/L phosphate soluble capability. Soil fungi were explored for phosphate solubilization by many researchers eg. *Trichoderma* studied for its phosphate solubilization efficiency and impact on growth rate of *Avicennia marina* (Rawat. et al., 2011). Sharma, (2011)

isolated soil fungi and studied it for phosphates solubilization efficiency in both solid and liquid medium and reported rock phosphate solubilization upto 61.6% gm.

Table.1.12 Reported technique for aquatic yeast isolation

Process	I	II	III		**References**
Name of sampler	Niskin	Van Dorn	Kemmerer	-	Jannasch et al., (1973)
Sampling	Deep sea	Surface	Near Shore	-	Reference/Remark (Jannasch et al., (1973)
Isolation	1.Water passing through membra ne filter 2.Filter kept on media.	Water sample plating directly, or after dilution	1. Water passing through membrane filter 2. Filter resuspended in sterile water for low density yeast population. 3. Water plated on media.	-	-
Medium	YPD Jannasch et al. (1973)	PDA	2% MEA	Y M	http://deskuenvis.nic.in/pdf/manual_for_yeast_work_sept 2008.pdf
Strain maintenan ce	MEA	-	-	-	http://deskuenvis.nic.in/pdf/manual_for_yeast_work_sept 2008.pdf

1.13.1. Fractal characters

Yeast are known to create cell wall polysaccharides that are useful in a variety of cell-cell and cell-to-inorganic particle interactions. Cell surface geometry and morphology, in addition to chemical appropriateness, influence the biosedimentation process.

The study of mycelial complex structures is difficult. Cultures were exhibiting fractal growth on the surface of their colony. Fractal geometry has made significant contributions to understanding of microbial growth patterns.

Fractal geometry allows the irregularity and complexity of mycelial surface structures to be measured in relation to growth, metabolic activity, enzyme synthesis, and coloration (Obert et al., 1990). The fractal dimension of mycelium can be related to its form and productivity, such as citric acid fermentation and different antibiotic fermentations (Papagianni., 2004).

Fractal growth patterns can also be used to evaluate the availability of nutrients.
Low nutritional environments result in low hyphal mass, while rich nutrient environments result in high hyphal mass (Ritz et al., 2001). Branches begin to develop in diverse directions once microbial mycelia have begun to proliferate.

Mandelbrot's fractal theory presented a novel technique to defining the geometry of systems with no specified geometry. Puchkov (2016) has provided examples of using image analysis in the study of both the macroscopic and the microscopic microbiological objects obtained by various imaging techniques.
The fractal dimension is the most essential numerical parameter in calculating the fractal of any mass. The number of independent quantities required to specify the location or arrangement of points on an item is referred to as fractal dimension (Shirali 2014). For calculating the fractal dimension of any fractal mass, a variety of concepts have been offered.

Cluster to cluster collision results in the creation of aggregates with a low fractal dimension. Yan L. X (2005) devised a new method for calculating the fractal dimension of bio flocs in activated sludge suspensions. The fractal dimension was used to distinguish between the flocs and granule particles generated in an anaerobic digester used for water treatment (Bellouti. et al., 1997). Using fractal geometry theory, Saiedi (2017) investigated temporal variation in aggregate stabilization, carbon content and microbial respiration. Balaban (2018) investigated the evolution of spatial arrangement in *Enterobacter cloacae* aggregates using multifractal analysis to evaluate its radial growth on semi-solid substrates.

The type of microorganisms and the method employed to aggregate them affect the fractal behaviour of microbial aggregation (Logan., 1991). Because colony and density

heterogeneities, as well as vacuoles within mycelia, are difficult to quantify, averages are assessed using image-based approaches (Boddy, 1999). Mycelia are also fractal in nature, like other irregular structures. Fractal geometry and fractal dimension may be a useful tool for determining the mycelial development pattern or extent in space because it involves their space filing capacity. According to Barry (2009), filamentous bacteria fermentation process is influenced by their size and morphological behavior.

Because traditional methods such as projected area, length, and perimeter are limited, fractal dimensions can be used to assess morphological development productivity, which is connected with morphological adaptability and can be determined by fractal dimensions (Barry 2009). Computer models have been created to replicate fungal colonies, which have been researched for the nature of hyphal branching points, growth, and next-door neighbour characteristics, as well as growth factors in relation to fractal dimension (Obert.,1993). The diameter and division of single yeast cells were measured using fractal dimensions (Tomankova., 2006; Vesela., 2001).

Woriax (2009) used the fractal dimension of bacterial colonies to compare biochemical and physiochemical changes in bacterial colonies as well as how they responded to environmental changes.
Achlya bisexualis morphology studied, the fractal dimension of the response to heavy metal concentration decreases as concentration rises (Lundy 2001). Gonzalez (2016) used polymerosomes to mimic biofilm formation and the aggregation process was investigated using a fractal framework, as well as antibacterial properties. Fractal dimensions can be a useful tool for analysing aggregates since they allow for quantitative evaluation of aggregate shape (Logan., 1991). However, due to their fragile nature, flocs cannot be investigated. Particle aggregates formed during the wastewater treatment process have fractal characteristics (Ganczarczy., 1989).

The fractal dimension tool was used to characterise *Trichiurus haumela* in a frozen state. During storage, it was discovered that when the temperature dropped, the quality (hardness, springiness, and fractal dimension) of the product diminished (Luan et al., 2018).

The whey protein gel structure and visco elasticity have been described using the fractal dimension idea. The fractal dimension has been used to investigate soil characteristics. Different forms of aggregates form as a result of soil particle association, mineral and organic materials, and soil structure. Using fractal dimensions, the water retention of soil particles was compared. The soil bulk density and clay silt content were found to be connected to the fractal dimensions of the soil water retention curve (Pazira et al., 2018). Lestrai (2018) and Malekanis (1996) evaluated fractal analysis techniques for determining clay material fractal dimensions.

The fractal dimension of flocs changes as the method of floc creation changes. The geometric characteristics of flocs can be understood using the fractal analysis theory (Li., 2006).

The lowest fractal dimensions were detected in biological flocs and the flocculation process due to bridging the aggregates, while larger fractal dimensions were observed in aggregates created by sweep substrate transfer. The fractal dimensions of flocs are also determined by hydraulic conditions (Sun, 2013).

Furthermore, after churning, the fractal dimension initially grows and subsequently declines. There are several methods for calculating fractal dimensions, but all of them are based on the power law connection (Kramer., 2004). The irregularity and complexity of mycelia surface structures are related to growth, metabolic activity, enzyme synthesis, and coloration, according to fractal geometry (Martin et al., 1990).
Hypothesis was made to determine whether the fractal dimensions of yeast colony edges could serve and assist in screening and selecting strains capable of bioclarification of turbidity.

1.13.2. Fractality Index

Fractality index is a four digit number obtained by multiplying the score produced by the fractal analysis software JFRAD by 1000 with the last number being rounded up. (Kamat., 2019).

1.14. Drinking water status in mining areas of Goa

Goa is blessed with a plethora of freshwater bodies. In Goa, various surface water reservoirs are used as a source of drinking water. The physiochemical characteristics of water are a critical aspect in determining the water quality for various applications. Analyzing the physiochemical properties of water aids in the understanding of water quality and the development of techniques to address problems. Water quality is well understood to be as vital as its quantity. As a result, an examination of the physiochemical properties of water bodies was required.

Manderker et al. (2012) assessed ground and surface water in Goa's mining sites and found that all water parameters were within acceptable limits.
Moreover, they also discovered that the pH of ground water in mining regions was somewhat acidic. Further, Krishan et al. (2016) investigated the quality of Goa's groundwater and created a water quality index tool that combines data from various water quality criteria into a single number. According to them, these indices are temporal and spatial based and may vary depending on local factors. Their research indicated that the water quality was good and safe to drink. The quality of Goa's coastal water was also assessed (Sampath et al., 2018).

(Sawaiker. et al., 2016) investigated the lake's physiochemical characteristics as well as its phytoplankton diversity, concluding that seasonal fluctuations in the lakes' physiochemical characteristics were detected. The physiochemical parameters of tap and filter water were studied by Singh et al., (2015). While tap water had a pH that was acceptable, filter water had a pH that was less than ideal. Both tap and filter water had alkalinity and total hardness within acceptable limits. Sawaiker et al., (2017) assessed water quality using microbes as a bio monitor and found that diatoms are excellent indicators of water contamination. Sreevnivasa. et al., (2013) investigated the chemical properties of water samples obtained from a bore well and an open well in Bicholim, Goa. The majority of the parameters in the water sample were found to be within acceptable limits. Furthermore, bicarbonate levels were found to be over 1000 ppm in several areas, such as Shikheri and Gaonkarwada0. The water quality of four beaches was studied by Saxena et al., (2018). Because the urban discharge point was closer to this location, beaches had lower water quality and a higher quantity of coliforms.

Figure.1.7: Water tank at Morlem

1.15. Bioflocculation test need at laboratory scale

The purposes of these laboratory-scale biosedimentation tests are to assess the viability of wastewater treatment using SMTTW and to look into the characteristics of the process, including the microscopic mechanism and parameters influencing the process. Cultures are first screened as bioflocculants. Second, the goal is to identify any differences in treatment intensity across the different characteristics of the investigated cultures, such as biomass dose, age, and pH of wastewater. Procedures can be optimised by testing at various scales.

1.15.1 Rapid mixing

The quick mixing stage is perhaps the most critical point of the coagulation-flocculation/biosedimentation process because it is here that destabilizing reactions take place and where primary floc particles are generated, the features of which have a significant impact on later flocculation kinetics.

1.16. Molecular Study of yeast strains

Several yeast species from various environments have yet to be identified and used for commercial purposes. The majority of therapeutically or medically important yeasts are identified using molecular sequencing. Most species found in the aquatic environment are still unidentified (some species are included in the table 1.13) in the soil, and in the

air. After yeast has been identified, it can be classified and graded in comparison to other species. Some yeast species have antifungal properties, meaning they are resistant to fungus, whereas others have particular colours or can be fatal. As a result, adequate management is essential to keep the yeast culture in line with their nonviolent property. Butler et al. (2009) sequenced the genome of *C. tropicalis*, a diploid yeast with 12 chromosomes per cell, a genomic size of 14.5 Mb, 6,258 genes producing proteins, and a guanine-cytosine concentration of 33.1 percent, according to Doi et al. (1992). The number of chromosomes is not precisely known. *C. tropicalis* was formerly thought to be an asexual yeast. Some research has suggested that tetraploid cells can be produced by mating between diploid cells and (Porman et al., 2011; Xie et al., 2012; Seervai et al., 2013). The cause of cells turning from white to opaque, according to the researchers, could be mating, which is controlled by colony phenotypic flipping. According to Seervai et al. (2013), *C. tropicalis* strains can be made to go through a parasexual cycle without meiotic reduction from a tetraploid state polyploidy, which affects gene expression and protein production in the cells (Morrow, 2013). *C. tropicalis* showed reduction in ploidy and considered a mechanism of adaptation and may be associated with cell stress (Bermanet et al., 2012). *C.tropicalis* has showed huge genetic similarity with *C. albicans* to other *Candida* species. (Butler et al., 2009). This minute different can be noticed with the help of molecular identification.

Table.1.13 Aquatic yeast reported from different regions

Name of Aquatic yeast	Source	Place	Reference
Aureobasidium pullulans	Sea water Lake River	Bohai Sea, Nahuel Huapi Lake Argentina Brazil	Zhou et al., 1991 Carlos et al., 2010 Adriana et al., 2012
Candida azyma	Sea water	Bohai Sea	Zhou et al., 1991
Candida curvata	Sea water	Bohai Sea	Zhou et al., 1991
Candida famata	Sea water	Bohai Sea	Harrison et al., 2017
Candida guilliermondii	Sea water	Bohai Sea	Guerra et al., 1938
Candida humicola	Sea water	Bohai Sea	Zhou et al., 1991
Candida insectorum	Sea water	Bohai Sea	Zhou et al., 1991
Candida krusei	Sea water	Bohai Sea	Zhou et al., 1991

Candida multis-gemmis	Sea water	Bohai Sea	Zhou et al., 1991
Candida parapsilosis	Sea water	Bohai Sea	Zhou et al., 1991
Candida pelliculosa Redaelli	Sea water	Bohai Sea	Zhou et al., 1991
Candida sake	Sea water	Bohai Sea	Zhou et al., 1991
Candida terebra	Sea water	Bohai Sea	Zhou et al., 1991
Candida tropicalis	Sea water	Bohai Sea	Zhou et al., 1991
Candida valdiviana	Sea water	Bohai Sea	Zhou et al., 1991
Cryptococcus albidus	Sea water	Bohai Sea	Zhou et al., 1991
Cryptococcus ater C	Sea water	Bohai Sea	Zhou et al., 1991
Cryptococcus hungaricus	Sea water	Bohai Sea	Zhou et al., 1991
Cryptococcus infirmominiatus	Sea water	Bohai Sea	Zhou et al., 1991
Cryptococcus laurentii	Sea water	Bohai Sea	Zhou et al., 1991
Cryptococcus luteolus	Sea water	Bohai Sea	Zhou et al., 1991
Cryptococcus magnus	Sea water	Bohai Sea	Zhou et al., 1991
Cryptococcus uniguttulatus	Sea water	Bohai Sea	Zhou et al., 1991
Debaryomyces hansenii	Sea water	Bohai Sea	Zhou et al., 1991
Debaryomyces polymorphus	Sea water	Bohai Sea	Zhou et al., 1991
Pichia burtonii	Sea water	Bohai Sea	Zhou et al., 1991
Pichia carsonii	Sea water	Bohai Sea	Zhou et al., 1991
Pichia etchellsii	Sea water	Bohai Sea	Zhou et al., 1991
Pichia guilliermondii	Sea water	Bohai Sea	Zhou et al., 1991
Pichia heimii	Sea water	Bohai Sea	Zhou et al., 1991
Pichia ohmeri	Sea water	Bohai Sea	Zhou et al., 1991
Pichia philogaea	Sea water	Bohai Sea	Zhou et al., 1991
Pichia scolyti	Sea water	Bohai Sea	Zhou et al., 1991
Rhodotorula aurantiaca	Sea water	Bohai Sea	Zhou et al., 1991
Rhodotorula glutinis	Sea water	Bohai Sea	Zhou et al., 1991
Rhodotorula graminis	Sea water	Bohai Sea	Zhou et al., 1991
Rhodotorula minuta	Sea water	Bohai Sea	Zhou et al., 1991
Rhodotorula rubra	Sea water	Bohai Sea	Zhou et al., 1991
Saccharomyces cerevisiae	Sea water	Bohai Sea	Zhou et al., 1991
Saccharomyces kluyver	Sea water	Bohai Sea	Zhou et al., 1991
Torulaspora delbrueckii	Sea water	Bohai Sea	Zhou et al.,1991
Candida austromarina	Sea water	-	Yarrow et al., 1978
Candida maritima	Seawater	Australia.	Zhou et al., 1991
Leucosporidium antarcticum	Antarctic sea water	Antarctic	Fell et al., 1970

Metschnikowia bicuspidata	Sea Water	USA	Zhouet al., 1991
Metschnikowia krissii Uden	Seawater,	USA.	Zhou., et al 1991
Metschnikowia zobellii	Seawater,	USA.	Zhou et al., 1991
Rhodosporidium dacryoideum	Seawater	Antarctic	Fell et al.,1991
Metschowinkia australis	Sea Water	Antarctic	Zhou., et al 1991
Rhodosporidium sphaerocarpum	Seawater	Antarctic	Zhou., et al 1991
Candida Mycoderma	Atlantice ocean	-	Zhou., et al 1991
C. membranaefaciens	Arbian Sea	India	Gupta., 1990
Ambrosiozyma platypodis	River	South Santiam, USA	Zhou et al., 1991
Candida methanosorbosa	River mud	Japan.	Zhou et al., 1991
Kurtzmanomyces tardus Gimenez-Jurado & van Uden	Contaminated demineralized water	Portugal	Zhou et al., 1991
Lindnera sargentensis	Fresh Water	USA	Zhou et al., 1991
Metschnikowia bicuspidata var. chathamia	Fresh water of lake,	New Zealand	Zhou et al., 1991
Sporopachydermia lactativora	Seawater	Antarctic	Zhou et al., 1991
Sterigmatomyces halophilus	Seawater.	-	Zhou et al.,1991
Ogataea minuta	Water	USA	Zhou et al., 1991
Ogataea minuta var. nonfermentans	Water	USA	Zhou et al.,1991
Pseudozyma antarctica	Sediment of Lake Vanda	Antarctica	Zhou et al., 1991
Sympodiomyces parvus	Seawater	Antarctic	Zhou et al.,1991
Debaryomyces	Central Pacific Ocean	-	Zhou et al., 1991
Candida torulopsis	Pacific Ocean	-	Zhou et al., 1991
Torula	Atlantice Ocean	-	Zhou et al.,1991

Debaryomyces fabryi	Sea	Arabian, Bay of Bengal	Kuriokose., et al 2012
Debaryomyces nepalensis	Sea	Arabian, Bay of Bengal	Kuriokose ., et al 2012
Debaryomyces subglobosus	Sea	Arabian, Bay ofBengal	Kuriokose., et al 2012
Rhodotorula ferulica	Polluted river water,	Portugal	Zhou et al., 1991
Trichosporon coremiiforme	Water bug,	Thailand	Zhou et al., 1991
Candida fukuyamanensis	Pond	Japan	Zhou et al., 1991
Rhodotorula mucilaginosa	River	-	The yeasts : A Taxonomic Study. (1952)
Candida krusei	River,	Brazil	Adriana et al., 2012
Candida parapsilosis	River, lake	Brazil USA	Adriana et al., 2012 Den et al., 1963
Cryptococcus laurentii	River	Brazil	Silva et al., 2012
Cryptococcus luteolus	River	Brazil	Silva et al., 2012
Cryptococcus hungaricus	River	Brazil	Silva et al., 2012
Cryptococcus albidus	River	Brazil	Silva et al., 2012
Kloeckera japonica	River	Brazil	Adriana et al., 2012
Kodmaeae	River	Brazil	Adriana et al., 2012
Metschnikowia	River	Brazil	Adriana et al., 2012
Pichia	River	Brazil	Adriana et al., 2012
Rhodotorula	River	Brazil	Adriana et al., 2012
Issatchenkia sp.	lake	Brazil	Silva et al., 2012
Candida diversa	lake	Brazil	Silva et al., 2012
Torulaspora pretoriensis	lake	Brazil	Silva et al., 2012
Candida pseudolambica	lake	Brazil	Silva et al., 2012
Cryptococcus podzolicus	lake	Brazil	Silva et al., 2012
Hanseniaspora uvarum	lake	Brazil	Silva et al., 2012
Trichosporon jirovecii	lake	Brazil	Silva et al., 2012
Williopsis saturnus	lake	Brazil	Silva et al., 2012
Hanseniaspora thailandica	lake	Brazil	Silva et al., 2012
Trichosporon laibachii	lake	Brazil	Silva et al., 2012
Bullera sp.	lake	Brazil	Silva et al., 2012
Cryptococcus rajasthanensis sp.	lake	Brazil	Silva et al., 2012

Cryptococcus podzolicus	lake	Colombian, Brazil	Silva et al., 2012
Candida lipolytica	Guanabara Bay	Brazil	Hagler et al., 1979
Sporobolomyces	Lakes	Argentina	Libkind et al., 2004
Cryptococcus agrionensis	River Agario	Argentina	Libkind et al., 2014
Rhodotorula mucilaginosa	River Agario, Lake	Argentina	Libkind et al., 2014 Carlos et al., 2014
Holtermanniella festucosa	River Agario	Argentina	Libkind et al., 2014
Cystofilobasidium capitatum	River Agario	Argentina	Libkind et al., 2014
Cystofilobasidium. macerans	River Agario	Argentina	Libkind et al., 2014
Cryptococcus albidus	River Agario	Argentina	Libkind et al., 2014
Cryptococcus antarcticus	River Agario	Argentina	Libkind et al., 2014
Cryptococcus cylindricus	River Agario	Argentina	Libkind., et al 2014
Cryptococcus laurentii	River Agario, River Suwanee	Argentina, Florida	Libkind et al., 2014 Lazarus et., al 1974
Cryptococcus victoriae	River Agario	Argentina	Libkind et al., 2014
Rhodotorula colostri	River Agario	Argentina	Libkind et al., 2014
Rhodosporidium babjevae	River Agario	Argentina	Libkind et al., 2014
Rhodosporidium toruloides	River Agario	Argentina	Libkind et al., 2014
Holtermanniella festucosa	River Agario	Argentina	Libkind et al., 2014
Cystofilobasidium capitatum	River Agario	Argentina	Libkind et al., 2014
Cystofilobasidium macerans	River Agario	Argentina	Libkind et al., 2014
Candida austromarina	River Agario	Argentina	Libkind et al., 2014
Bauerago sp	River Agario	Argentina	Libkind et al., 2014
Sporobolomyces roseus	River Agario	Argentina	Libkind et al., 2014
Cryptococcus agrionensis	River Agario	Argentina	Russo et al., 2009
Delphinella strobiligena	Lake	Nahuel Huapi lake Argentina	Carlos et al., 2010
Dioszegia hungarica	Lake	Nahuel Huapi lake Argentina	Carlos et al., 2010
Candida.sp.	Lake	Nahuel Huapi lake Argentina	Carlos et al., 2010
Cystofilobasidium capitatum	Lake	Nahuel Huapi lake Argentina	Carlos et al., 2010

Cystofilobasidium. Infirmominiatum	Lake	Nahuel Huapi lake Argentina	Carlos et al., 2010Libkind., et al 2014
R. colostri	Lake	Nahuel Huapi lake Argentina	Carlos et al., 2010
Rhodosporidium diobovatum	Lake	Nahuel Huapi lake Argentina	Carlos., et al 2010
Bullera dendrophila	Lake	Nahuel Huapi lake Argentina	Carlos et al., 2010
Cryptococcus adeliensis	Lake	Nahuel Huapi lake Argentina	Carlos et al., 2010
C. diffluens	Lake	Nahuel Huapi ake Argentina	Carlos et al., 2010
C. festucosus	Lake	Nahuel Huapi ake Argentina	Carlos et al., 2010
C. heveanensis	Lake	Nahuel Huapi lake Argentina	Carlos et al., 2010
Cryptococcu magnus	Lake	Nahuel Huapi lake Argentina	Carlos et al., 2010
C. saitoi	Lake	Nahuel Huapi lake Argentina	Carlos et al., 2010
C. stepposus	Lake	Nahuel Huapi ake Argentina	Carlos et al., 2010
C. wieringae	Lake	Nahuel Huapi lake Argentina	Carlos et al., 2010
Guehomyces pullulans	Lake	Nahuel Huapi lake Argentina	Carlos et al., 2010
Candida parapsilosis	Lake	Nahuel Huapi lake Argentina	Carlos et al., 2010
C. railenensis	Lake	Nahuel Huapi lake Argentina	Carlos et al., 2010
C. sake	Lake	Nahuel Huapi lake Argentina	Carlos et al., 2010
C. carnescens	Lake	Nahuel Huapi lake Argentina	Carlos et al., 2010
Cryptococcus tephrensis	Lake	Nahuel Huapi lake Argentina	Carlos et al., 2010 2010
Debaryomyces hansenii	Lake	Nahuel Huapi lake Argentina	Carlos et al., 2010

Hanseniaspora uvarum	Lake	Nahuel Huapi lake Argentina	Carlos et al., 2010
Pichia fermentans	Lake	Nahuel Huapi lake Argentina	Carlos et al., 2010
Candida famata			Harrison et al., 1961
C. glabrata	Coastal water	Taiwan	Sheng ., 2012
Saccharomyces yakushimaensis	Coastal water	Taiwan	Sheng ., 2012
Kazachstania jiainicus	Coastal water	Taiwan	Sheng ., 2012
Kodamaea ohmeri	Coastal water	Taiwan	Sheng ., 2012
Pichia anomala	Coastal water	Taiwan	Sheng., 2012
Issatchenkia orientalis	Coastal water	Taiwan	Sheng., 2012
Hanseniaspora uvarum	Coastal water	Taiwan	Sheng 2012
Rhodotorula pacifica	Deep sea	Pacific ocean	Nagahama et al., 2006
Kluyveromyces nonfermentans	Deep sea	Pacific ocean	Nagahama et al., 1999
Cryptococcus surugaensis	Suruga Bay	Japan	Nagahama et al .,2003
Cryptococcus	Sea	Antarctic	Vishniac., et al 1979
Candida pulcherrima	lake	USA	Den. et al., 1963
Cryptococcus albidus	lake	USA	Den. et al., 1963
Cr. diffluens	lake	USA	Den. et al., 1963
Cr. gastricus	lake	USA	Den. et al.,1963
Cr. laurentii	lake	USA	Den. et al 1963
Rhodotorula glutinis	lake	USA	Den. et al 1963
R. pilimanae	lake	USA	Den. et al 1963
R. rubra	lake	USA	Den. et al 1963
Trichosporon cutaneum	lake	USA	Den. et al 1963
Candida sp.	Marine	Nicobar Islands	Rao et al., 2011

Candida albicans, Candida tropicalis, Debaryomyces hansenii, Geotrichum sp., Pichia capsulata, Pichia fermentans, Pichia salicaria, Rhodotorula minuta, Cryptococcus dimennae and Yarrowia lipolylica	Mangrove sediment	South east cost, India	Saravanakumar et al., 2013
Candida spp, Pichia spp., Cyberlinera spp. , Meyerozyma spp.,Clavispora spp.,Saccharomyces spp.,Kluyveromyces spp.,Yamadazyma spp. ,Trichosporon spp. and Wickerhamomyces spp.	River	South Africa	Monapathi et al., 2020
Torula , Pichia Wickeramomyces, Candida	River	Japan	Naito et al., 2019
Yamadazym, Candida atmosphaerica, C. spencermartinsiae, C. atlantica, C. oceani and C. taylorii	Mid-Atlantic Ridge ocean wate	Atlantic	Burgaud et al., 2016
Rhodotorula mucilaginosa, *Clavispora lusitaniae*, *Wickerhamomyces anomalus*	Ghellaze lake	Algeria	Aibeche et al., 2021

Considering the gap in knowledge regarding the use of freshwater yeast for the removal of colloidal turbidity from waste water, the present work aimed to fill the gaps by targeting freshwater yeasts as biofloccuants for wastewater treatment. The lead of work was obtained by Kamat et al., (2010) during the dissertation work of Post graduate students.

The main aim of this work was to test this hypothesis **"Whether freshwater yeast cultures could effectively reduce turbidity in freshwater, polluted due to entry of mine tailing water."**

The specific objectives of the work were formulated:

OBJECTIVES OF THE PRESENT STUDY

1. Survey of Freshwater habitats from mining and non mining areas of Goa and isolation of natural aquatic yeasts culture.
2. Systematic screening, detection and evaluation of promising yeast strains capable of bioflocculation of colloidal turbidity.
3. Assessment of drinking water quality of mining areas of Goa.
4. Lab scale bioflocculation using natural yeast isolates upto a scale of 10-100 L.
5. Standardization of bioflocculation process for field level application scale 500 L-1000 L
6. Molecular study of only the selected strain.

CHAPTER II
MATERIALS AND METHODS

2.1. Literature surveyed

Fresh water yeast and wastewater treatment using yeast, related scientific literature was surveyed using a variety of online querying techniques, with the search terms bioclarification, biosedimentation, and turbidity reduction used in various databases, including general search engines, academic / institutional databases, specific journal search, patent websites, culture collections, biological, and chemical databases. Google. (https://scholar.google.co.in) Scholar's growth rate of online sources relevant to particular search phrases till August 2022.

2.1.1. Freshwater habitats surveyed

Water habitats were selected based on their location, availability and water use or source. The main source of freshwater reservoirs was rainwater. Lake, temple tank, water fall's stream were among them. Water tanks were built in rectangular and in square shapes. Some of them were transparent and some of them were greenish in appearance (Fig.2.3, karapur Pond).

The seasonal pond in the Goa university campus showed macrophyte rich environment, at their surroundings. The pond near SafaMaszid had large number of fish population.

The Arvalem water fall's stream was turbid as human activities were going on, while the waterfall at chorla ghat and Netravali were clean and very less turbid. Mayem and Carombolim lakes were chosen as sampling sites as they are well known freshwater bodies in Goa's mining area. Macrophytes were absent near the Mayem lake Carombolim showed sufficient population of macrophytes. Both lakes had approximately identical turbidity

2.1.2. Water Sampling

Freshwater bodies in Goa, including mining and non-mining areas, were chosen for water sample collection for the freshwater yeast isolation. As previously stated, the study locations were included temple tank, stream of water fall, and lake. Water samples were collected approximately 20 cm below surface in 1-L pre-sterile plastic bottles (Himedia). At the sampling sites, water parameters such as temperature (Multi Thermometer), electrical conductivity (Aquaro digital water conductivity meter), and total soluble solid (HM Digital TDS-3 meter) were measured. Freshwater samples were

taken in fifteen distinct locations from six talukas of Goa for the period of 2016 to 2018 monsoon season. Samples of water were brought to the laboratory, analysed and processed within 2-24 hrs of the collection of water samples in order to obtain yeast strains. Moreover, no preservative were used for the storage of the water samples.

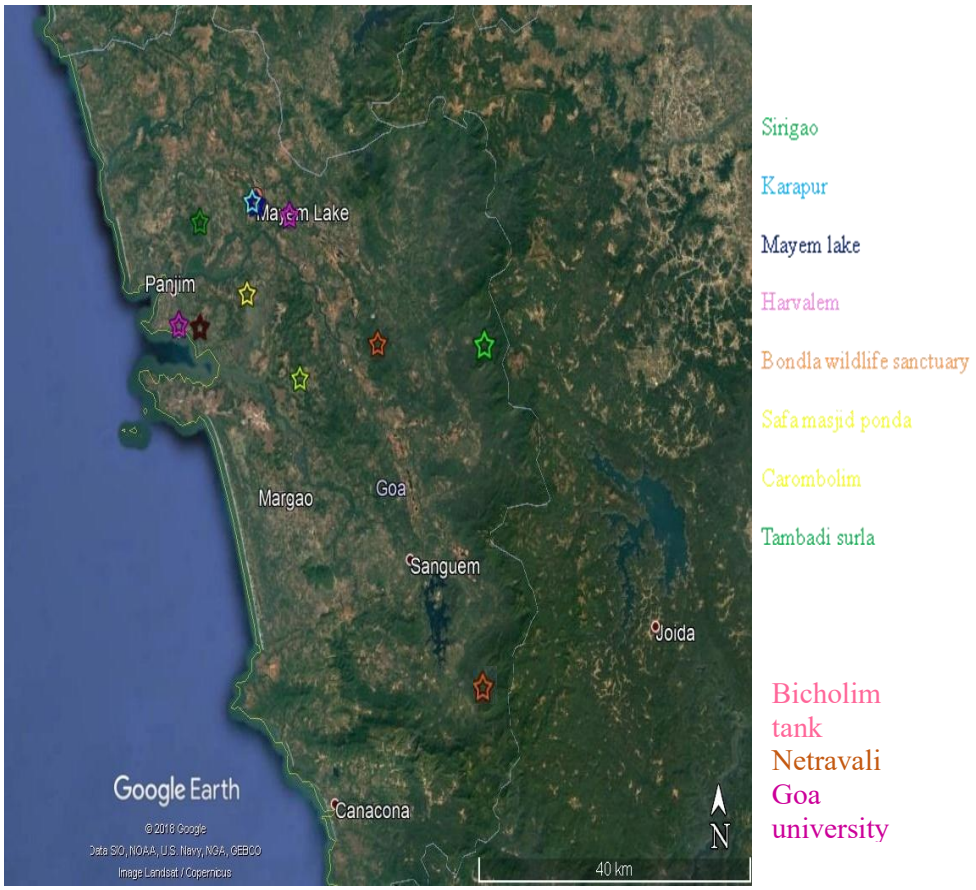


Figure. 2.1: Google locations of water sampling sites

Figure.2.2: Water sampling locations (Created with SimpleMappr, https://www.simplemappr.net)

Table.2.1 Water sampling sites

Water Body	**Location (Talluka)**	**Latitude**	**Longitude**
Bicholim tank	Bicholim	15 °36 ´6.7500“N	73 °57 16. 66 96”E
Bondla stream	Ponda	15°23'56.87"N	74° 0'44.59"E
Carambolim lake	Tiswadi	15.4888° N	73.9279° E
Chorla water fall's stream	Sattari	15°35'45.18"N	74° 3'33.79"E
Harvalem water fall stream	Sanquelim	15°33'29.24"N	74° 0'44.84"E
Karapur tank	Bicholim	15°33'23"N	73°58'35"E
Mayem lake	Bicholim	15.5760° N	73.9400° E
Netravali water fall	Sanguem	15.0955° N	74.2178° E
Netravali bubbling lake	Sanguem	15.0942°N	74.20919°E
Safamasjid pond	Ponda	15.4066° N	74.0000° E
Sirigao tank	Bicholim	15.6099° N	73.9036° E
Tambdi surla stream	Sanguem	15°12'38.96"N	74° 9'1.51"E
Goa University campus (pond 1,2,3,	Tiswadi	15.4588° N	73.8342° E

Figure.2.3: Water bodies(a) Safamasjid Pond (b) Sirigao Pond

Figure.2.4: (a) Bicholim temple tank, (b) Karapur tank, (c, d) Goa University seasonal pond (d) Bubbling Lake, (e) Netravali water fall

2.2. Isolation of yeast cultures using membrane filter technique

Water samples were collected in 1000 ml pre-sterilized plastic bottles. 500ml water sample (in triplicates) was passed through a sterilized membrane filter (0. 22 µm pore size and 47 mm diameter, Millipore) in a sterilized membrane filter assembly, set up in the laminar air flow. After filtration the Membrane filter was removed and eluted in sterile 3ml water to remove the trapped material. 1 ml water sample was spread plated over MEA (2% w/v) media (incorporated with antibiotics 0.1mg/ml) and incubated at 24 ± 2°C. (Kamat et al., 2010). Plates were observed for microbial growth, and morphological characteristics studied under the microscope, dissimilar colonies were picked from plates. Cultures were purified and maintained on malt extract agar slants.

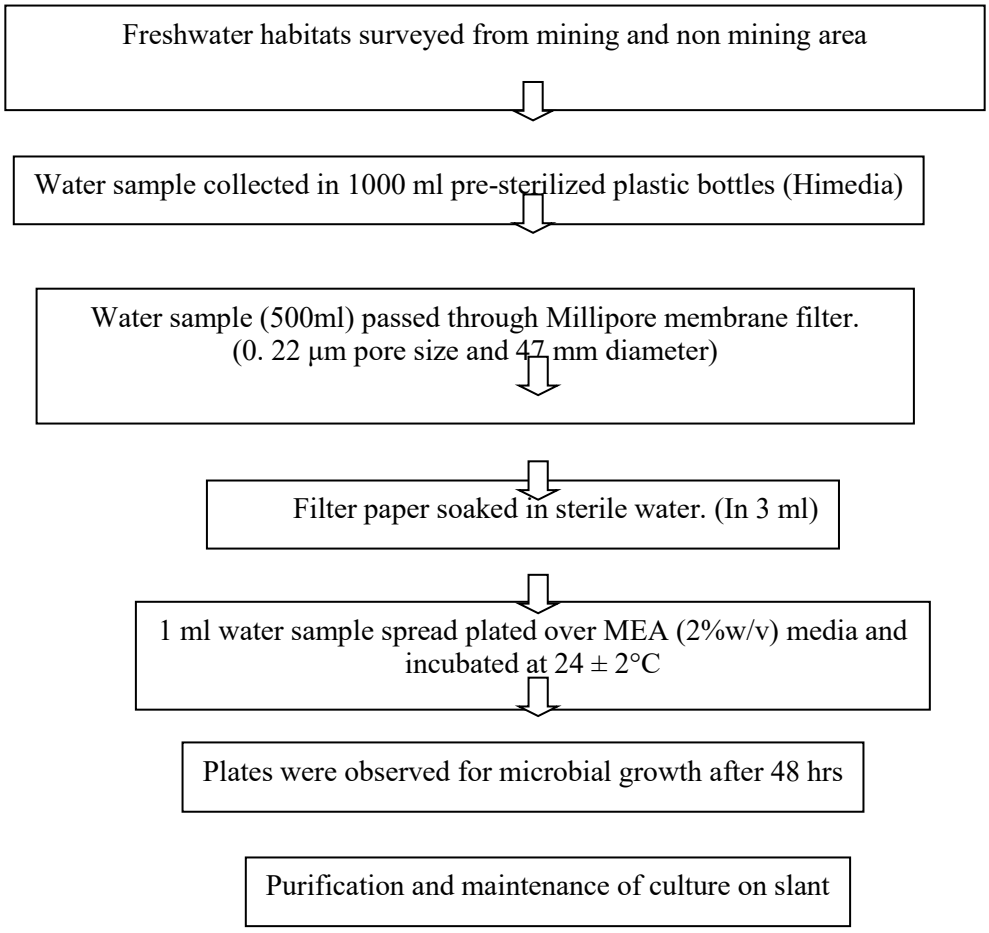


Figure.2.5: Scheme for freshwater yeast isolation

2.2.1. Rainwater sample collection

This work was done to check whether any rainwater yeast could enter the surface water body.

2.2.2. Assembly of sterile, dust- and contamination-free PVC containers

Pre-sterile plastic bottles were used to collect the rain water. Six times in every monsoon from 2016 to 2018.

2.2.3. Selection of clear vegetation-free open spaces

Water was collected at Goa University campus at the same place during every sampling. The area was vegetation free and open in order to avoid contamination.

2.2.4. Isolation of yeast, purification and maintenance

Rain water samples were spread on MEA plates (containing 0.1mg/ml antibiotics) which were incubated for 48 hrs at 25°C. The plates were observed under a microscope for the growth of yeast and colony morphology.

2.2.5. Control plates

Plates with nutrient agar and MEA (2%, with 0.1mg/ml antibiotics) were kept in the open air for one hour at the rain water collection site at the Goa university campus. Other plates were spread plated with water, which was used for rinsing the rainwater sampling apparatus. One plate with MEA (2%) was kept without spread plating rain water.

2.3. Taxonomic identification of the cultures tentatively

Standard procedure for identification of yeast were employed and the yeast cultures were identified on the basis of their cultural and morphological characteristics.

2.3.1. Morphological study of the cultures

Strains were studied for its morphology under stereo microscope (Olympus SZ51, Tokyo, Japan). Micromorphology of cultures was studied under light microscope (Nikon Eclipse E200) by monochrome gram staining and photographed with NIS element microscope imaging software and confirmed the presence of yeast.

2.3.2. Biochemical tests

The yeast strains were identified on the basis of sugar fermentation, assimilation, ascospore production, formation of true mycelium test (Yarrow., 1998; Bhargva et al., 2015). Cultures tentatively identified upto the Genus level. (All chemical used, were of HiMedia Chemicals Ltd., India)

2.3.2.1. Standard protocol for carbohydrate fermentation test(Dunford et al., 1999)

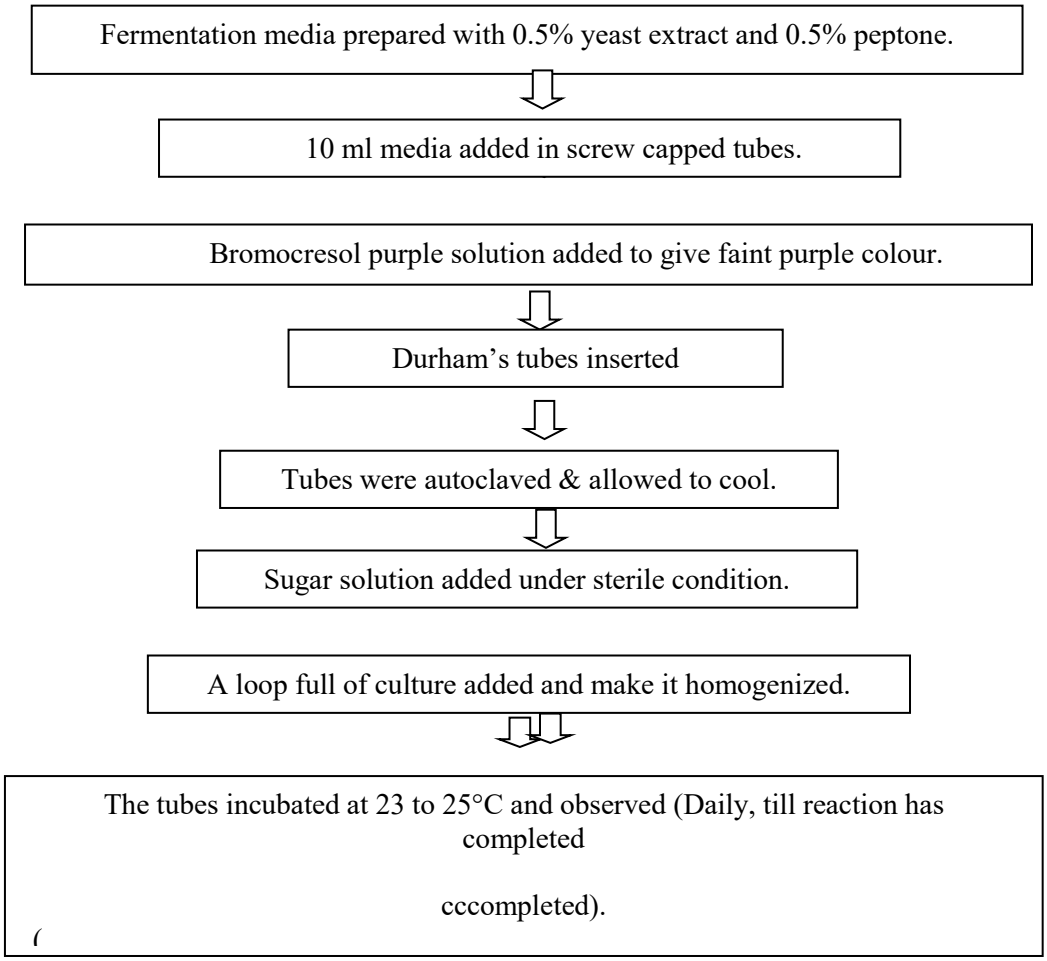


Figure.2.6: Scheme of the of sugar test

2.3.2.2. Observed assay

(a) Acid production as change in color of medium for purple to yellow.

(b) Gas detection as bubbles of gas in durham tube.

2.3.2.3. Study of cultures on Candida differential agar

Candida Differential Agar is a selective and differential medium that enables for quick isolation of yeasts from mixed cultures as well as distinguishing Candida species such as *C.albicans*, *C.krusei*, *C.tropicalis*, and *C.glabrata* based on colony form and coloration. Results are obtained in 48 hours, making it ideal for the quick and presumptive identification of common yeasts in the mycology and clinical microbiology laboratories.

Nitrogenous, carbonaceous chemicals, and other critical growth nutrients are provided by peptone special and yeast extract. Phosphate acts as a medium buffer. Chloramphenicol lowers the bacterial flora in its immediate environment. *C.albicans* appears as light green smooth colonies, whereas *C.tropicalis* appear as blue to metallic blue elevated colonies. *C.glabrata* colonies have a cream to white smooth appearance, but *C.krusei* colonies have a purple fuzzy appearance.(Himedia manual information). (Bardkar et al., 2010)

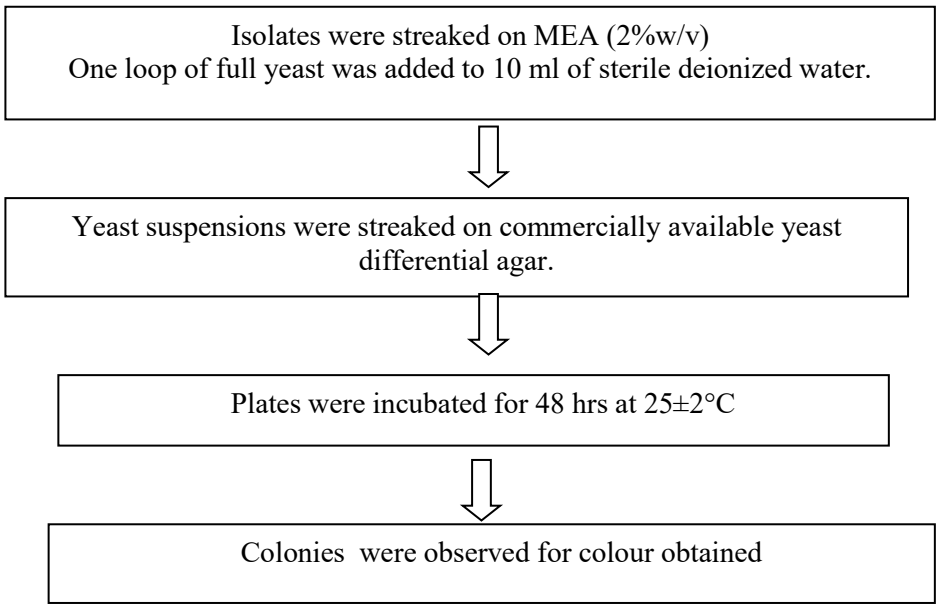


Figure.2.7: Scheme for cultures study on differential agar media

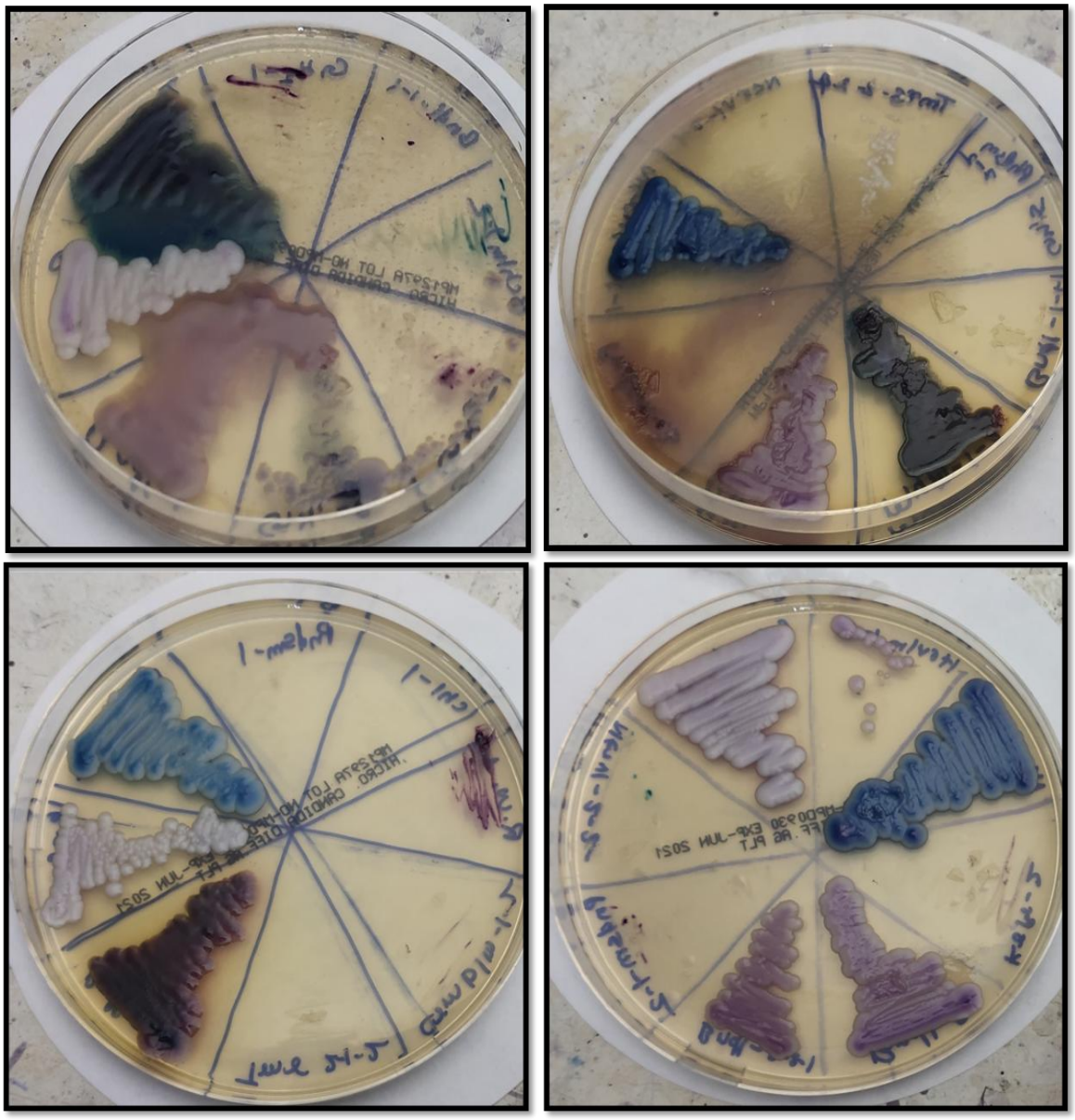


Figure. 2.8.: Cultures grown on differential agar media

2.3.2.4. Organic acid production

During alcoholic fermentation, several important organic acids, such as oxlate, succinic, pyruvic, lactic and acetic acid, are produced by yeast. So test yeast evaluated for its organic acid production. (Campbell et al., 1996)

2.3.2.5. Standard protocol for organic acid production

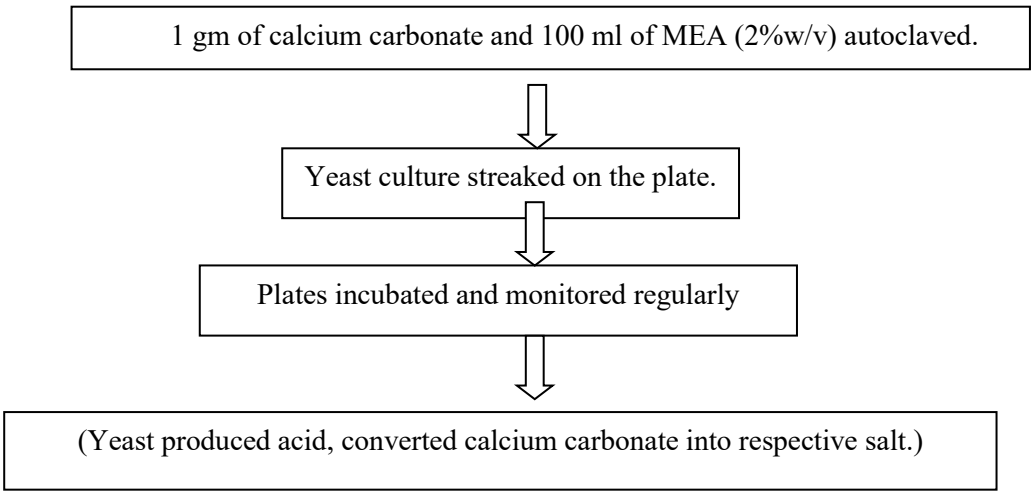


Figure.2.9: Scheme of acid production test

2.4. Catalase production test

This test is based on the principle that yeast has high-powered peroxidases, such as catalase. Peroxidases are enzymes that act as catalysts in the oxidation of specific substances in living systems. Anaerobes do not produce catalase. (Dunford et al., 1999).

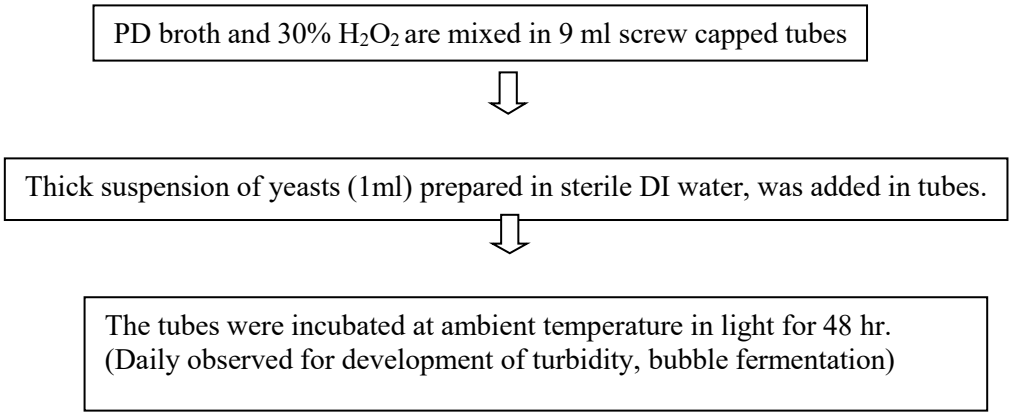


Figure.2.10: Scheme of the catalase production test

2.4.1. Morphological study of promising yeast on modified media

Most promising strain was streaked on MEA media's plat and the plate prepared by sterile mine tailing soil and agar. Plates were incubated at 25±2°C for 48 hrs. Yeast grown on modified media were restreaked on MEA (2%w/v) media (commercially available) and observed under microscope.

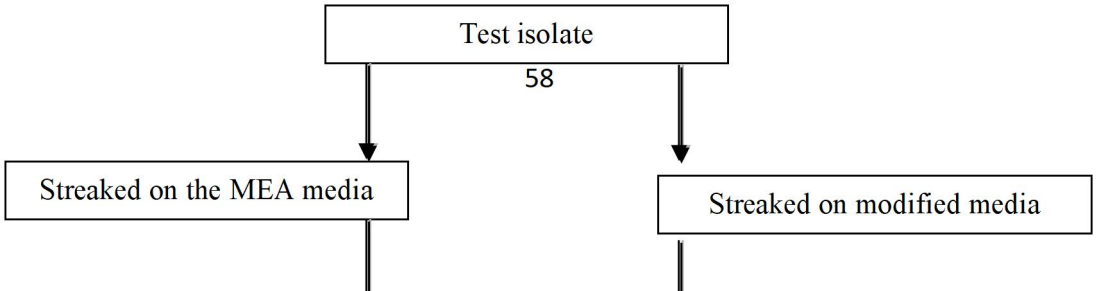


Figure. 2.11.1: Scheme for promising strains's study in simulated environment

2.4.2. Phosphate solubilization test

Phosphate exists in both organic as well as inorganic forms in soil. Organic matter derived from dead and decaying plant debris is rich in organic sources of phosphorus. However, plants are able to utilize phosphorus from soil only in the free available form. Soil phosphates are rendered available either by plant roots or by soil microorganisms. Therefore, phosphate-dissolving soil organisms play a part in correcting phosphorus deficiency of crop plants. Turbidity is also caused by insoluble phosphate colloids including particle colloids.

The potential of wild ascomycetes yeast strains from polluted water bodies in the mining area to solubilize phosphate was tested.

Phosphate solubilization efficiency of selected cultures

Yeast culture streaked on the pikovskaya agar plates (Nautiyal., 1999) on predetermined pattern and incubate for 2-3 days at ambient temp (25-30°C). Colony growth and the phosphate solubilization zone were monitored on a daily basis.

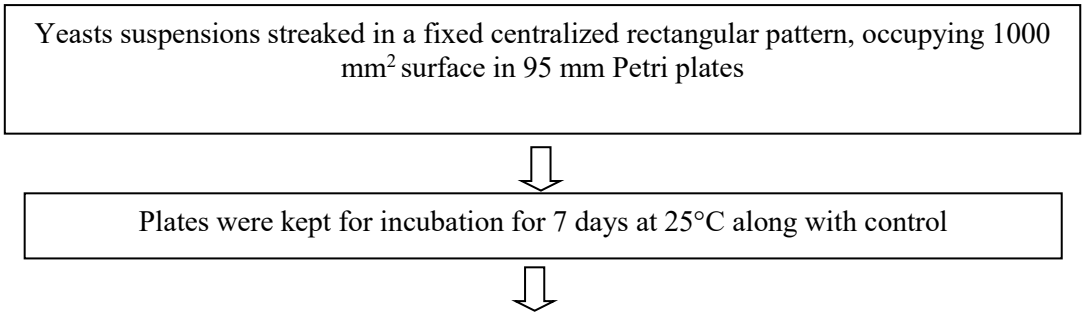


Figure .2.11.2: Scheme for phosphate solubilization efficiency test

$$PSE=[(A_{BB}+A_{OB})/7089]X100$$

PSE= Phosphate solubilization efficiency

A_{BB}=Area below biofilms

A_{OB} =Area outside biofilm

2.5. Screening of promising strain for efficient turbidity removal capacity

During cultures's morphological study it has been observed that some strains were showed simple colony margins while some were complex colony margins. So, cultures were classified on the basis of their colony margins.

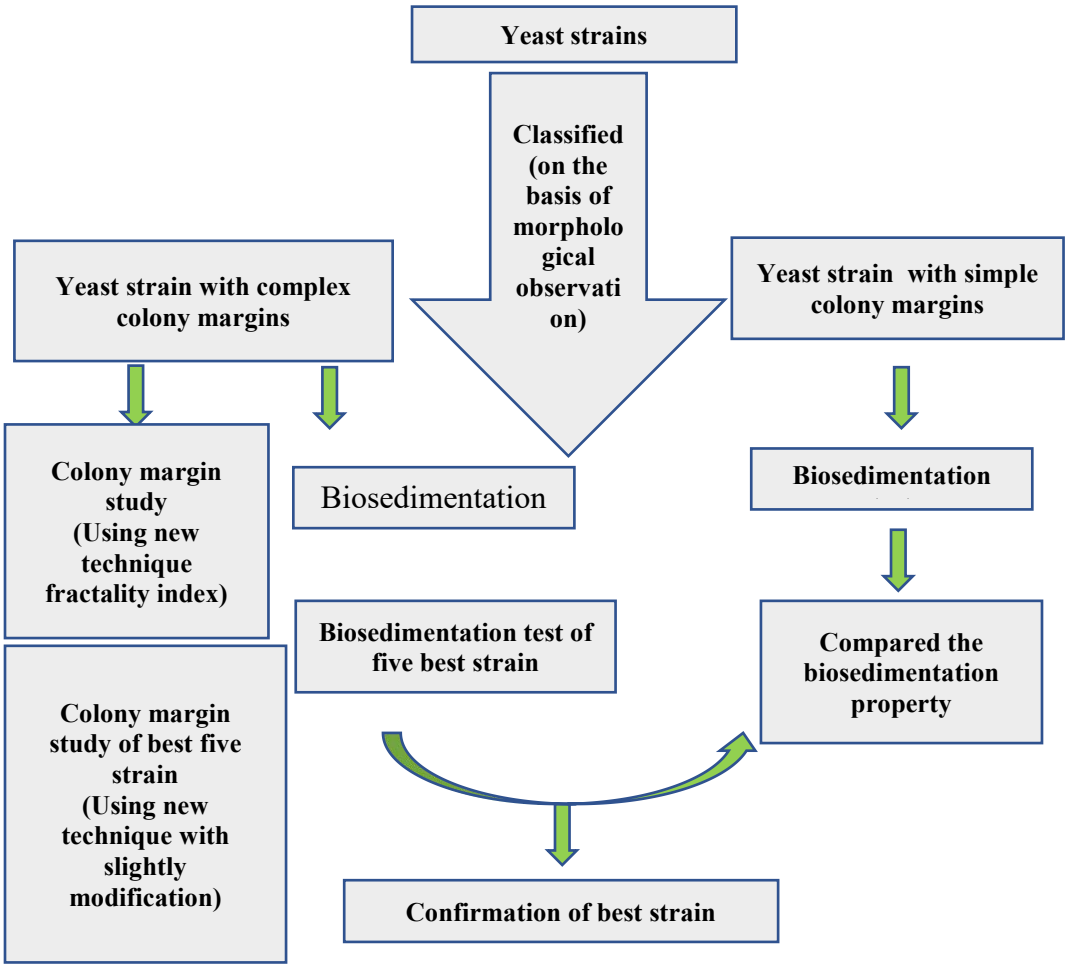


Figure. 2.12: Scheme strategies for screening of promising strains for biosedimentation of SMTTW

Table.2.2 List of cultures with simple and complex colony margins

Cultures with complex colony margins	Cultures with simple colony margins	
Bchlm-1	Bchlm-2	Sc-1
Bchlm-1-2	Srg-2-2	Srg-2-1
Gh1-1	Bndl1-1	Tmrs-2-2-3
Gh2-1	Pndsm-3	Pnd-2-2
Gh1-3	Gh1-2	Srg-1
Mym-1-2	Gh1-5	Tmrs-2-3
Pnd-2	Chl-1	Ntrvl-1-1-1
Pnd-3	Krpr-1	Ntrvl-1-2
Pndsm-1-2	Krpr-2	Ntrvl-2-2
Pndsm2-2	Bndl-1-1-1	Ntrvl 2-3
Tmrs-2-2-4	Bndl-1	-
Crmblm-2	Bnd-2-2	-
Gh1-4	Crblm-1	-
Hrvlm-1-2	Srg-3-3	-
Bndl-3	Krpr-3-3	-

2.5.1. Screening of Strains

Cultures were evaluated for their biosedimentation efficiency on the basis of visibility of tubes, biosediment compactness and sattleability in10 ml screw caped tubes by mixing 1 ml yeast cultures suspensions (same absorbance) and 9 ml simulated mine tailing water and scored according to table 2.3. Simple colony margins and complex colony margins key was used to classify strains

(A) Visual screening

All cultures were screened for efficient biosedimentation of clay colloids in turbid water by manual observation using biosedimentation efficiency scoring scheme as described in table 2.3.

Freshwater yeast strains were tested to see whether they could bind clay colloids found in SMTTW in 10ml screw test tubes and compared.

Table.2.3. Scoring scheme for efficient biosedimentation

A	Water column very clear no deposits on inner wall	100
	Water column very clear, some deposits on inner wall	80
	Water column partially clear no deposit on inner wall	50
	Water column Partially clear some deposits on inner wall	25
	Water column turbid	0
B	Sediment very compact	100
	Sediment just about compact	80
	Sediment loose	50
	Sediment very loose creates instant turbidity	25
C	After agitation of sediments	
	It breaks into fine particles takes long time to rain down to reformsediment	0
	Not as above breaks into large sediments and settles rapidly	100

2.5.2. (B) Screening of cultures by digital image analysis of colony margins

Further, cultures with complex colony margins were screened with the help of JFRAD software, image analysis based technique. Puchkov (2016) has provided examples of using image analysis in the studies of both the macroscopic and the microscopic microbiological objects obtained by various imaging techniques. Fractality index of colonies margins was investigated, and an attempt was made to link the fractality index and biosedimentation efficiency. Those cultures with complex margins have been investigated for colony margin characteristics as a preliminary step and with modified method.

All cultures showing highest fractality index of colony margins were studied for bioclarification ability, sediments formation time and morphology of sediments were recorded and scored.

2.5.3. Fractality index

It is a four-digit number obtained by multiplying the score produced by the fractal analysis software like JFRAD by 1000 with last number being rounded up (Kamat, 2019). The detailed description of method is given below

2.5.4. Primilary study of fractality Index of colon margins

Colonies were grown on 2% MEA in a square pattern to produce defined colonies (Fig.2.13), viewed under a 10X objective lense (Nikon microscope), and photographed the outer and inner colony margins (at same point) with NIS Element software up to 7 days, after the inoculation of strains on media (9 c.m. petriplates). Representative images were imported and processed to compute fractal dimension employing CMEIASJFrad version 1.0 software freely available at http://cme.msu.edu/cmeias/ (Ji et al., 2015).

The output data of yeast colony fractal dimensions were saved as *csv files and analyzed statistically using the SYSTAT 13. Fractality index of the margins were calculated and compared.

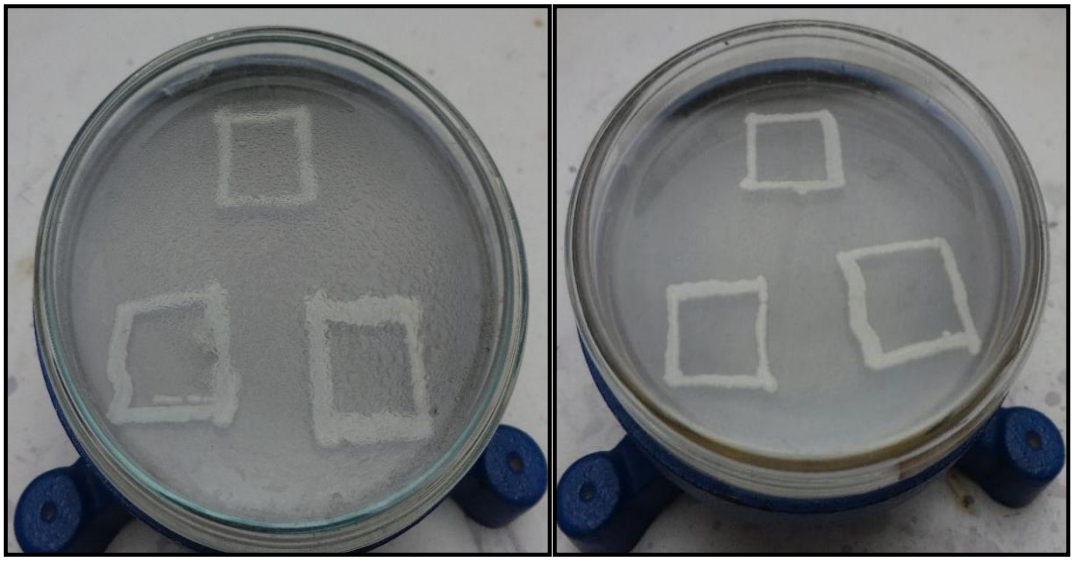


Figure. 2.13: Streaking pattern of cultures for preliminary study

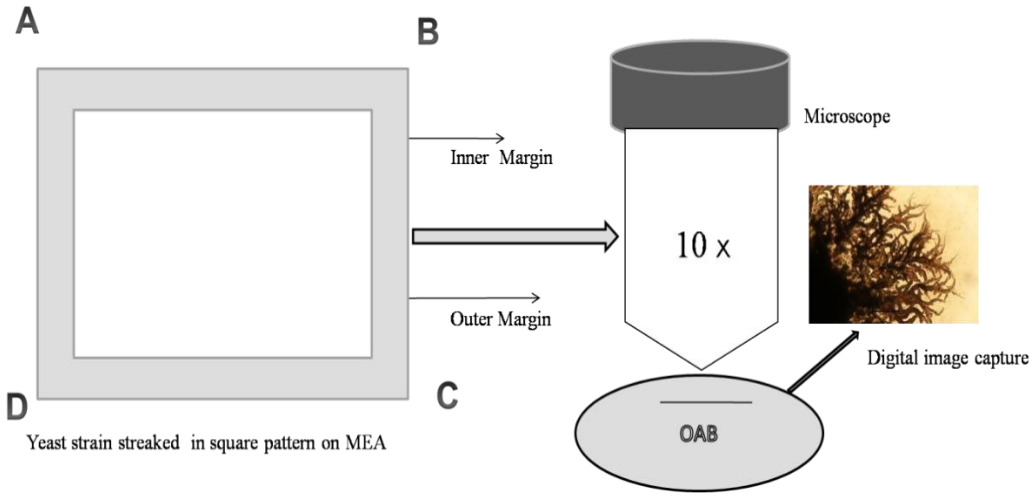


Figure.2.14: Scheme for colony image capturing

2.5.5. Screening of cultures with complex colony margins for turbidity reduction

Colonies of selected cultures with complex colony margins were taken by nichrome loop, mixed to10 ml of sterilised deionized water to make a yeast suspension with the same absorbance. In screw-capped tubes,1ml yeast suspension was introduced to 9 ml SMTTW.

The fractality Index of biosediment has also been investigated, with the goal of correlating it with the fractality Index of strains colony margin for optimal biosedimentation of suspended solid in mine tailing water.

2.5.6. Study of fractality Index of colony margins with modified method

After a little change in approach, the best five strains with complex colony margin (among all preliminary tested strain) were studied again for colony features.Yeast strains were streaked on solid media in a nested colony square pattern (in 9cm Petri plates), allowing colonies to be observed under the microscope easily (Fig 2.16).

Images of colony margins were processed using CmeisJFrad software to determine the colony margins' Fractal dimension and fractality index calculated as described above.

Nested colony patterns allow for simultaneous observation of colony margin interaction on the same media on which cultures are injected. Furthermore, it has been discovered that the same method may be used to study strains adaptation to inadequate nutritional conditions.

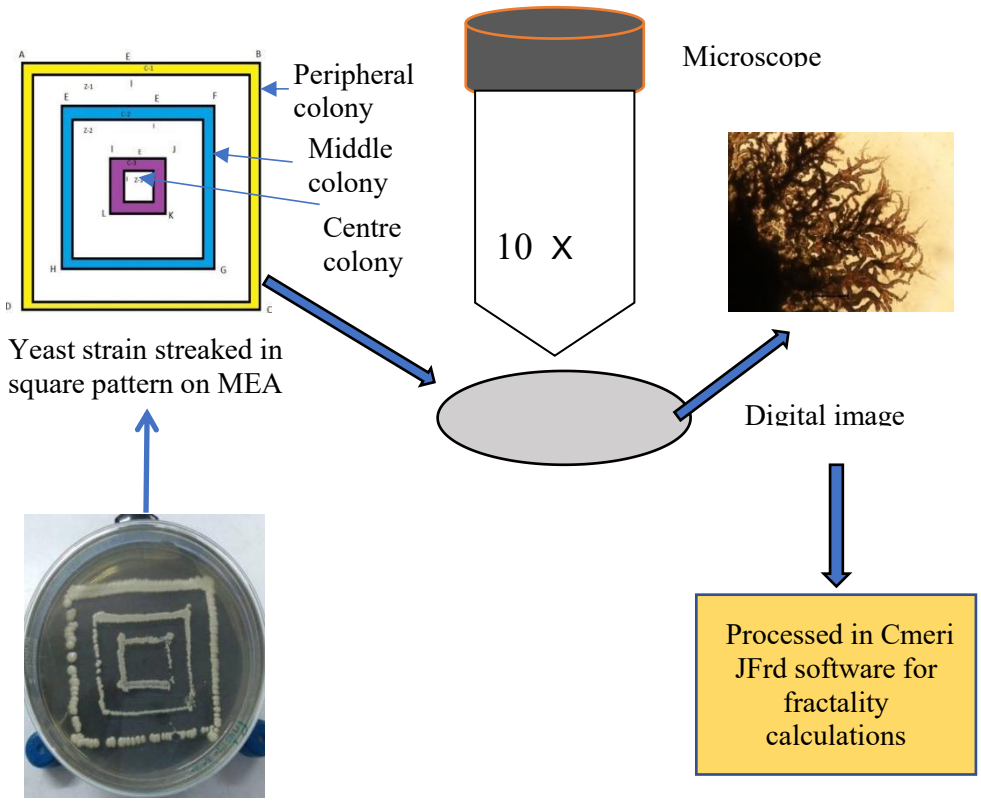


Fig.2.15: Scheme of colony image capturing after slightly modification

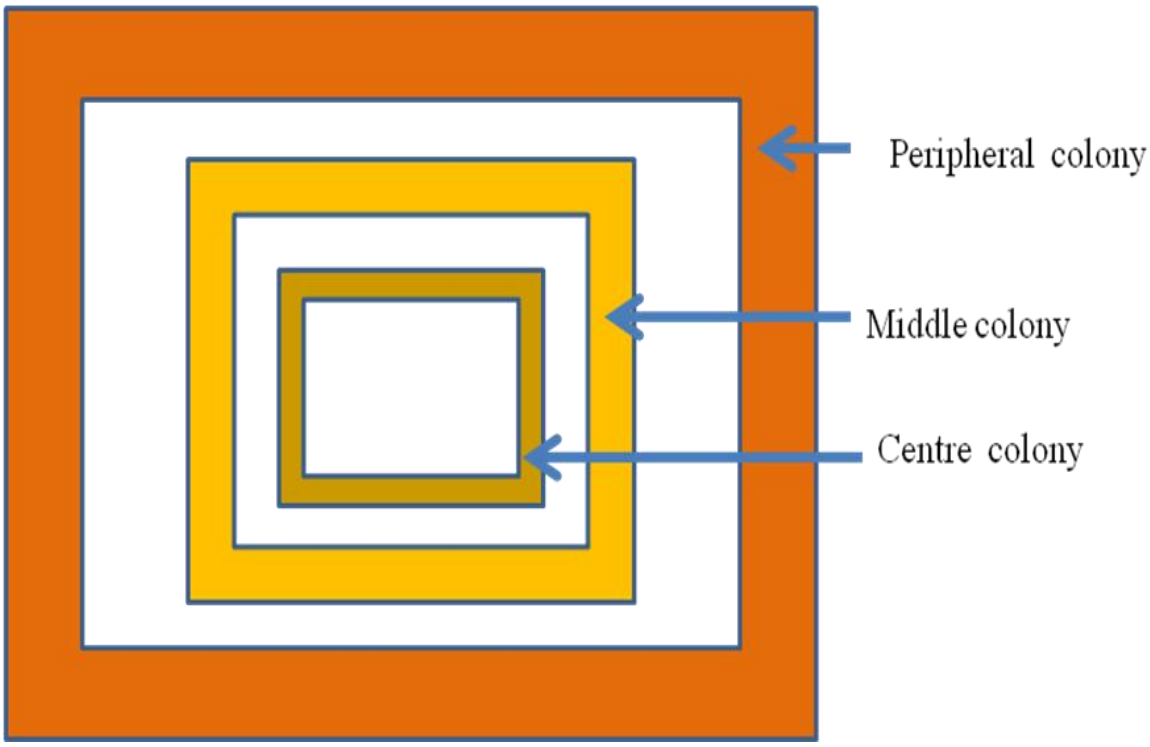


Figure.2.16: Predetermined pattern to obtain colonies amenable to digital analysis

2.6. Assessment of drinking water quality of mining areas of Goa

The sampling locations were chosen based on their location and intended purpose. Sampling was carried out on 6 stations during the monsoon and non-monsoon seasons to assess the quality of fresh surface water bodies. Water samples were taken and analysed for two seasons in a row. Surface water was sampled at every location. Furthermore, several of them were using for drinking purposes. A portable temperature and TDS meter was used to measure the temperature (Multi thermometer) and TDS (HM Digital TDS-3 meter) in the field. The physiochemical parameters of water samples were investigated.

The goal of this study was to determine the physicochemical parameters of surface water in the Goa mining areas.

This research will include data on the quality of water bodies as well as their environmental status. The correlation coefficient between water quality metrics has been calculated in a systematic manner.

The APHA's (1998) standard prescribed protocol, which is a standard protocol for water quality characterization all around the world, was used to develop the methodology for water sample characterization. Plastic canes were used to collect the water samples. During the sampling, grab sampling was commonly used.

Himedia India procured analytical-grade chemicals for the current study were used. All of the glassware were standard, and it was made by Borosil India Ltd. Standard procedures were used to conduct the analysis.

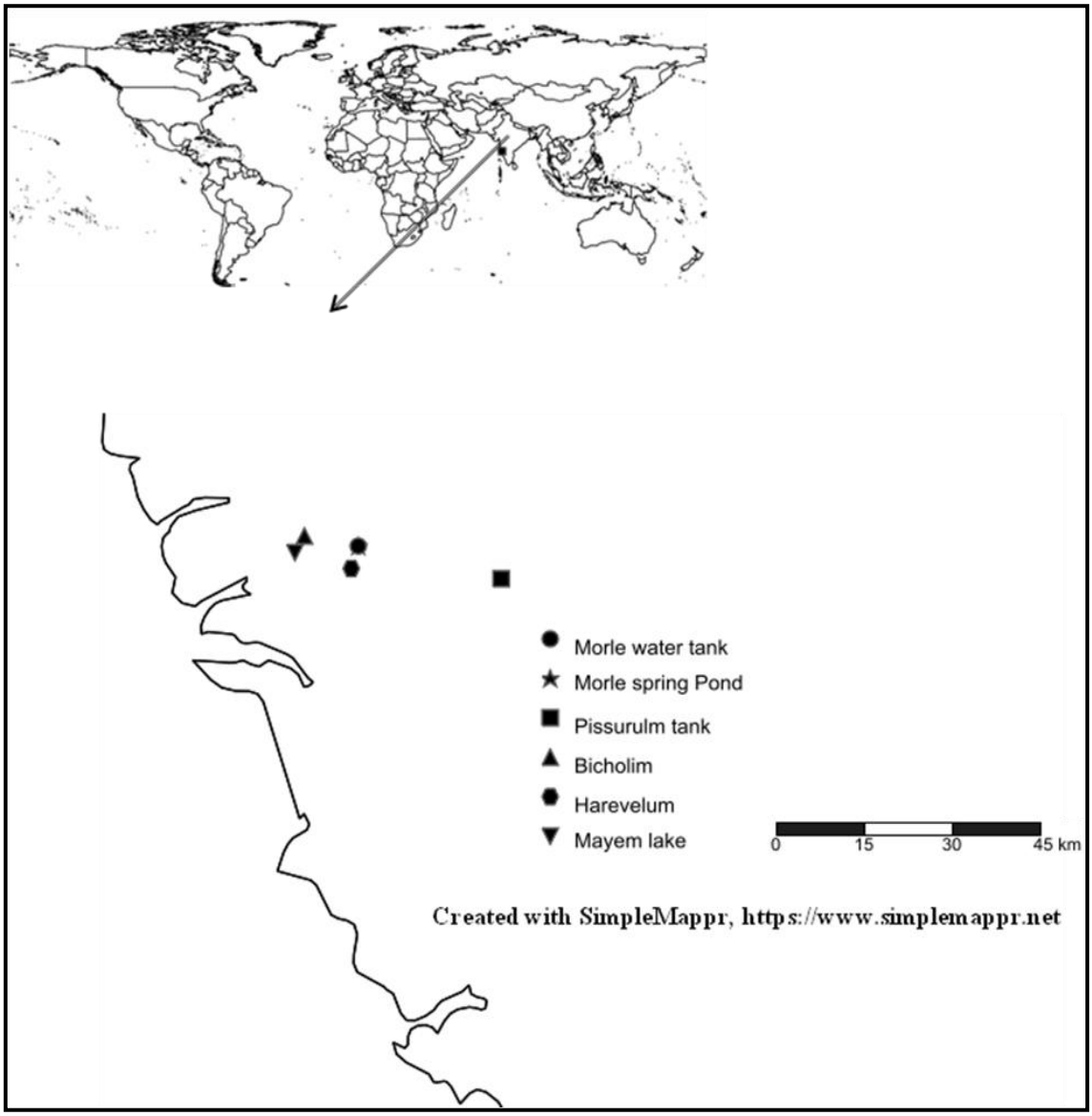


Figure.2.17: Water sampling sites

Table.2.4. Geographical co-ordinates of locations

Place	**Longitude/ Latituted**
MorlemWater tank	15.58611 °N,74.0375 °E
Morlemspring pond	15.5861 °N,74.0375° E
Pissurlem tank	15.5351 °N,74.2574 °E
Bicholim tank	15.6018 °N, 73.9546°E
Arvalem fall	15.5511° N, 74.0267° E
Mayem lake	15.5760° N, 73.9400° E

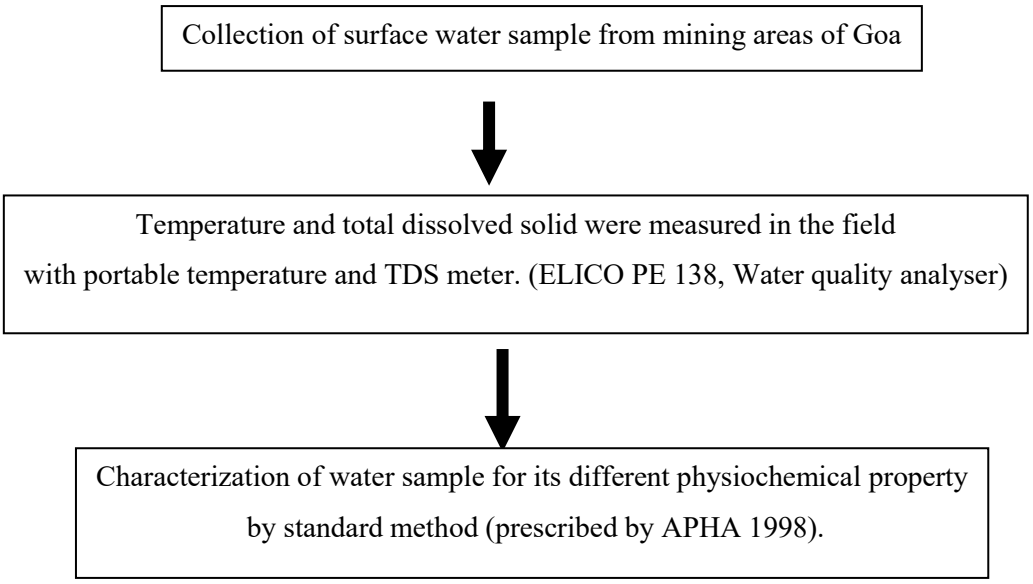


Figure.2.18: Scheme for water analysis

Table.2.5 Water analysis methods and instruments

Water Parameters	**Instrument/ Methodology**	**Water Parameters**	**Instrument /Methodology**
Turbidity	Thermo scientific EU Tech TN 100	Cl	Iodometric
TDS	HM Digital TDS meter	No^-_3	UV -100 SHIMAZDU
EC	ELICOPE138	So^-_4	UV -100 SHIMAZDU
pH	pH meter	Po^-_4	UV -100 SHIMAZDU
Temperature	Multi-thermometer	Alkalinity	Titration
DO	ELICOPE138	Total hardness	Titration
Na	Flame photometer	K	Flame photometer

2.7. Biosedimentation test using promising strain

Following the screening of the most promising strain for biosedimentation of clay colloids in SMTTW, the strain was further investigated for its turbidity reduction quality on a wide scale using various experiments. Furthermore, prior to the large-scale trial, the biosedimentation technique was standardized to improve strain efficiency for larger-scale biosedimentation tests.

Process and conditions were optimized for the efficient biosedimentaion of clay colloids. SMTTW was prepared by adding the mine tailing soil in distilled water. Yeast was grown in MEA broth and yeast suspension was prepared in sterile de-ionized water (all chemicals used were of HiMedia Chemicals Ltd., India).

Biomass's dose, age and pH of turbid water (4 to 9) estimation was performed for efficient biosedimentation, using sedimentation efficiency scored scheme (Table.2.3). Further, biosedimentation study was performed in the laboratory at the scale of 10 ml, 1L, 20L, 100 L. Simulated turbid water and yeast suspension (with desired concentration) were mixed in the ratio of 1:9 respectively at every scale. Water column stirred vigorously at 50 rpm for 1mins smaller scale, 10 mins higher scale and water samples withdrawn with glass pipette at different time intervals (t_1=0 min, t_2=10 min, t_3=20 min, n=120 min. Turbidity were measured of withdrawn samples. (Thermo scientific EU Tech TN 100).

Biosedimentation scoring scheme is independable for screw capped tube and Imhoff cone.
Concentration of yeast in yeast suspension – 0.005gm/l Three yeast suspensions with 0.005gm/l were prepared.
Ratio of yeast suspension and SMTTW 1:9. (to attain more water treatment using less yeast biomass.)

2.7.1. Preparation of log phase Yeast suspensions by both method on plate and broth.

During various trial experiments, standardized yeast suspensions of known concentration were generated in the laboratory. Diluted MEA broth (0.4%w/v) in deionized water was used to create yeast inoculums in Erlenmeyer flask. And then put it on the rotator shaker for 48 hrs at 25 ºC and 150 revolutions per minute (Fang.T.J. et a.,l 1996) (Scigenics Biotech orbitek). The inoculums were centrifuged for 10 minutes at 10,000 rpm. In the supernatant, sterilised water was added and centrifuged twice under the same conditions. Furthermore, yeast colonies were grown in bulk on MEA media in Petri plates incubated for 48 hr at 25±2°C, scratched with a sterile blade, and mixed with sterile deionized water to prepare a yeast suspension of the desired concentration.

Because this weight was specified for quick biosedimentation in previous investigations, yeast suspension (0.005gm/l) was made by dilution with sterilised water.
Turbidity reductions were investigated using cultures harvested using both procedures, and the turbidity reductions achieved by both yeast suspensions (cultivated by different methods) were compared .

2.7.2. Characterization of SMTTW.

SMTTW has been characterized for its pH, temperature (Multi thermometer), SPM by standard method, electrical conductance, (Aquaro digital water conductivity meter), and TDS (HM Digital TDS-3 meter).

2.7.3. Addition of yeast in SMTTW and observation of the reduction in turbidity

0.005gm/l (dry weight) of yeast suspensions was mixed into simulated mine tailings turbid water (1:9). Whole water was stirred with the help of glass rod. Water samples were withdrawn at different interval of time to measure the turbidity with turbidity meter (Thermo scientific EU Tech TN 100).

Moreover, biosedimentation procedure has been evaluated by observation of complex sediments formation and its rapid sedimentation. Further biosedimentation of clay colloids using yeast had been compared to control, (in which yeast suspension were not added in turbid water) under the laboratory condition.

2.7.4.1. Biosedimentation of SMTTW using heat treated yeast cells

The composition of the yeast cell wall is responsible for the attachment or adsorption of components. Clay colloids cause colloidal turbidity in mine water, and this notion has been utilised to remove it. As in a prior experiment, viable cells were used to reduce colloidal turbidity Yeast suspensions (0.005gm/l dry weight) were heated with a sprit lamp for various periods of time and then tested for sedimentation properties in screw caped tubes and the Imhoff cone. The biosedimentation patterns of live yeast and heat-treated yeast were also compared.

2.7.4.2. Heat treatment of cells

The most promising strain, Bchlm-1-2 suspension with known concentration, was heated with a sprit lamp for 60 seconds before being introduced to 900 ml of SMTTW in an Imhoff cone.

2.7.4.3. Strains combination study for efficient biosedimentaion

Strains were studied for their combination to reduce turbidity of SMTTW. 0.5 ml of each strain suspension were mixed with same absorbance and added in 9ml SMTTW, scored for its biosedimentation efficiency according to table 2.25.

2.8. Biosedimentation test upto 10 ml to 1L

Mine tailing soil brought from mining area were dried in room temperature crushed properly using ceramic mortal pestle. Soil were sieved using sieve with different mesh size.0.57 um was the last sieved. 5.6 gm of soil was added in 1000 ml of distilled water and stirred vigoursly (50 rpm for 10min). After 45 min decanted soil water was used for biosedimentation study. Yeast suspension and SMTTW with desired concentration were used in 1: 9 ratio to maintain the less amount of yeast biomass. Compactness of sediment observed (according to table 2.25) and turbidity were measured in 1L of imhoff cone. Turbidity reduction in test cone were compared with control cone.

2.8.1. Turbidity removal efficiency

The data acquired after executing the biosedimentation test up to the scale 1 L and 1000 L were used to compute the adsorption efficiency of turbidity reduction of SMTTW. The adsorption efficiency of the biosedimentation test was calculated using the NTU reduction values of the control and test's reactors.

$$\text{Turbidity removal efficiency} = \frac{C_f - C_o}{C_o} X100$$

where Co is the initial turbidity of the water sample (NTU) and C_f is the final turbidity of the water sample (NTU). The experimental studies were repeated thrice to check its repeatability and the average values only were discussed in the report.

Co =initial turbidity V= Volume in L

Cf= Final turbidity m= mass in m

(Kumar P.S. et al 2016, Nirmala rani 2010)

2.8.2. Surface charges of Yeast cells

Alcian blue is a positively charged phthalocyanine complex that is absorbed by negatively charged yeast cell surfaces, particularly the mannosylphosphate. This dye has

been used to calculate changes in the charge on the cell surface. The degree of this dye's adsorption is proportional to the negative charge on the cell surface.

Yeast cells at a concentration 5×10^7 ml^{-1} were washed twice in phosphate buffer (pH = 7.0) and harvested by centrifugation at 143Xg for 5 min at 4° C(Research centrifuge TC 4100F). Then yeast was suspended in 0.02 mol L^{-1} sodium acetate buffer (pH = 4.0) and washed twice with the same buffer. Yeast was incubated with 1.8 ml of Alcian blue tetrakis-chloride solution (50 mg L-^{1} in the buffer for 30 min at 25°C. After centrifugation at 10000 for 10 min at 20°C (Research centrifuge TC 4100F) the supernatant was decanted and its absorbance was measured at 615 nm (UV-100 SHIMAZDU) using uv-sphectrometer. The Alcian blue retention ratio (ABR) was calculated according to the following formula, The ABR was expressed as the mean of three experiments. (Fukudome et al., 2003).

ABR= (A_{AB}solution-Asupernatant) X 100 / A_{AB}solution

2.8.3.SEM EDX FTIR of sediments

Scanning Electron Microscopy (SEM) was used to analyse the surface morphological structure of purified yeast, control sediment, and test sediment (Evo-18 Carls-Zeiss, India). EDS recorded with a JOEL-JSM 5800 LV was used to determine the elemental composition of the samples.

Cells were suspended in osmium tetroxide (2 %, w/v), incubated statically for 60 min at room temperature, and harvested by centrifugation (13000 r.p.m. for 1 min). The fixed and stained cells were dehydrated by 10 min incubations with gentle agitation in a graded ethanol series of 10, 20, 30, 50, 70, 100 and 100 % dried absolute ethanol. Cells and sediment were prepared for electron microscopy by air drying for 24 hrs room temperature. Sample were processed after coating, mounted on SEM sample stub, with double sided sticky tape (Barker and Smart, 1996, C.D. Powell et al. 2003, Das et al., 2016).

Control sediment and test sediment was subjected to Fourier Transform Infrared (FTIR) spectral characterization using, Shimadzu with FTIR grade KBr powder (Reddy et al, 2018).

2.8.4. Hydrophobicity

Hydrophobicity was determined using a solvent approach described in a prior study (Solvent method) (Amar et al., 2006). The microbial cell adhesion to solvent test compares microbial cell affinity for a polar and nonpolar solvents. Baker yeast used as control.

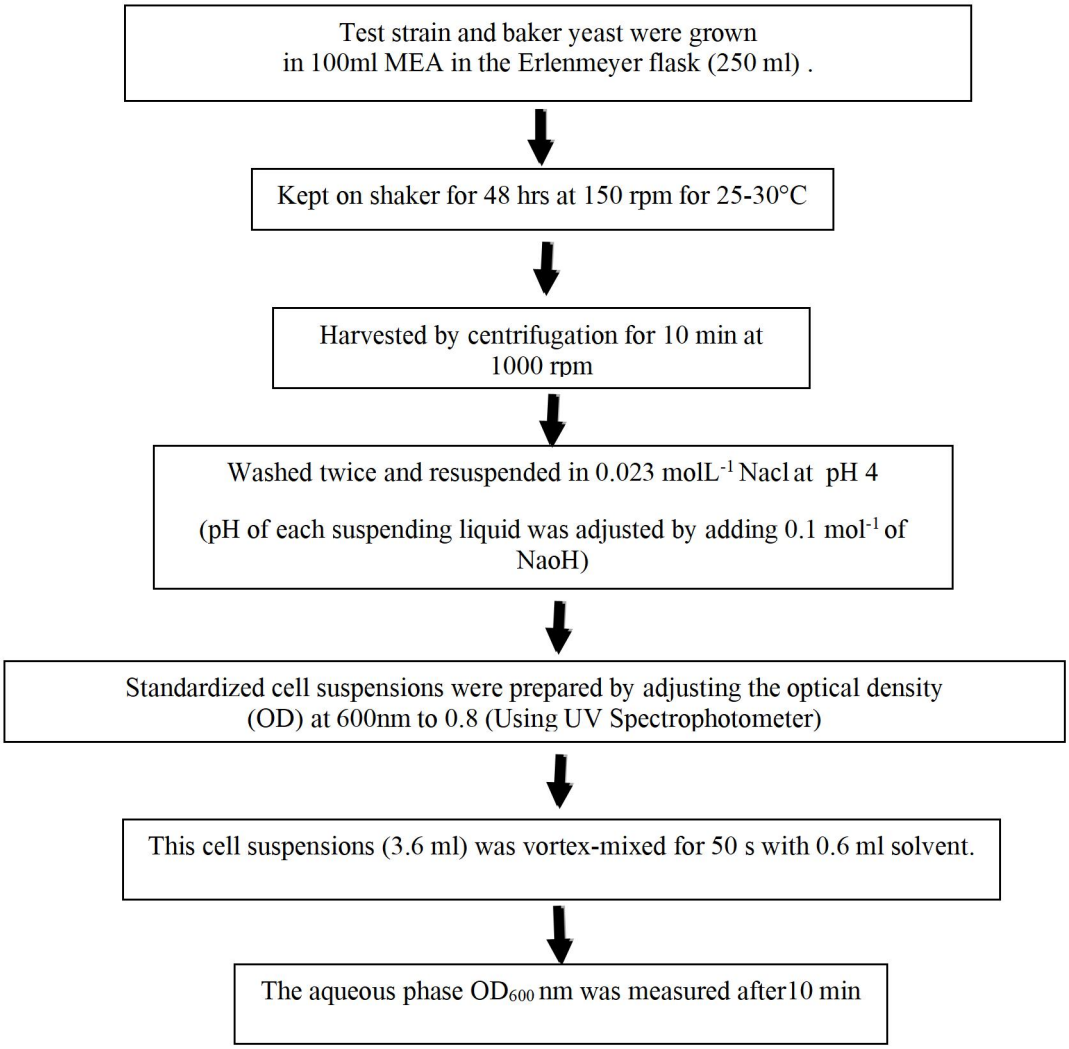


Figure.2.19: Scheme to calculate cell surface hydrophobicity

Results are given as, where *A*0 and *A* are OD600 of the aqueous microbial suspension before and after mixing, respectively.The hydrophobicity in this method is defined as the cell adhesion to hexadecane.

(Amaral et al., 2006).

Percentages of bound cells: % adhesion = 1 − *A/A*0,

2.9. Lab scale biosedimentation using natural yeast cultures upto scale of 1 to100L

Any treatment technique's progress or dependability can be tested on a bench scale in the laboratory. In the laboratory, the removal of turbidity from SMTTW using yeast cultures has been tested up to a size of 1-100 L. As a laboratory test, it may be used to optimize the treatment process in order to facilitate the treatment procedure on a wider scale. It saves money and time, and it can assist in the design of the treatment plant. Many biological methods for wastewater treatment have been effectively investigated at bench scale (Sheoran et al., 2005). The quality of SMTTW was measured before and after it was treated with yeast for turbidity removal. The results were compared in order to assess the biosedimentation capacity of the yeast strain.

The lab scale study provided insight on how to improve the method on a larger scale. The general goal of this research was to optimize the biosedimentation technique for application on a larger scale, as well as its behavior, such as sedimentation rate, HRT, sediment formation, and sediment wall deposition.

With the results of this research, a plan or approach for removing turbidity from SMTTW using yeast at a larger scale can be developed.

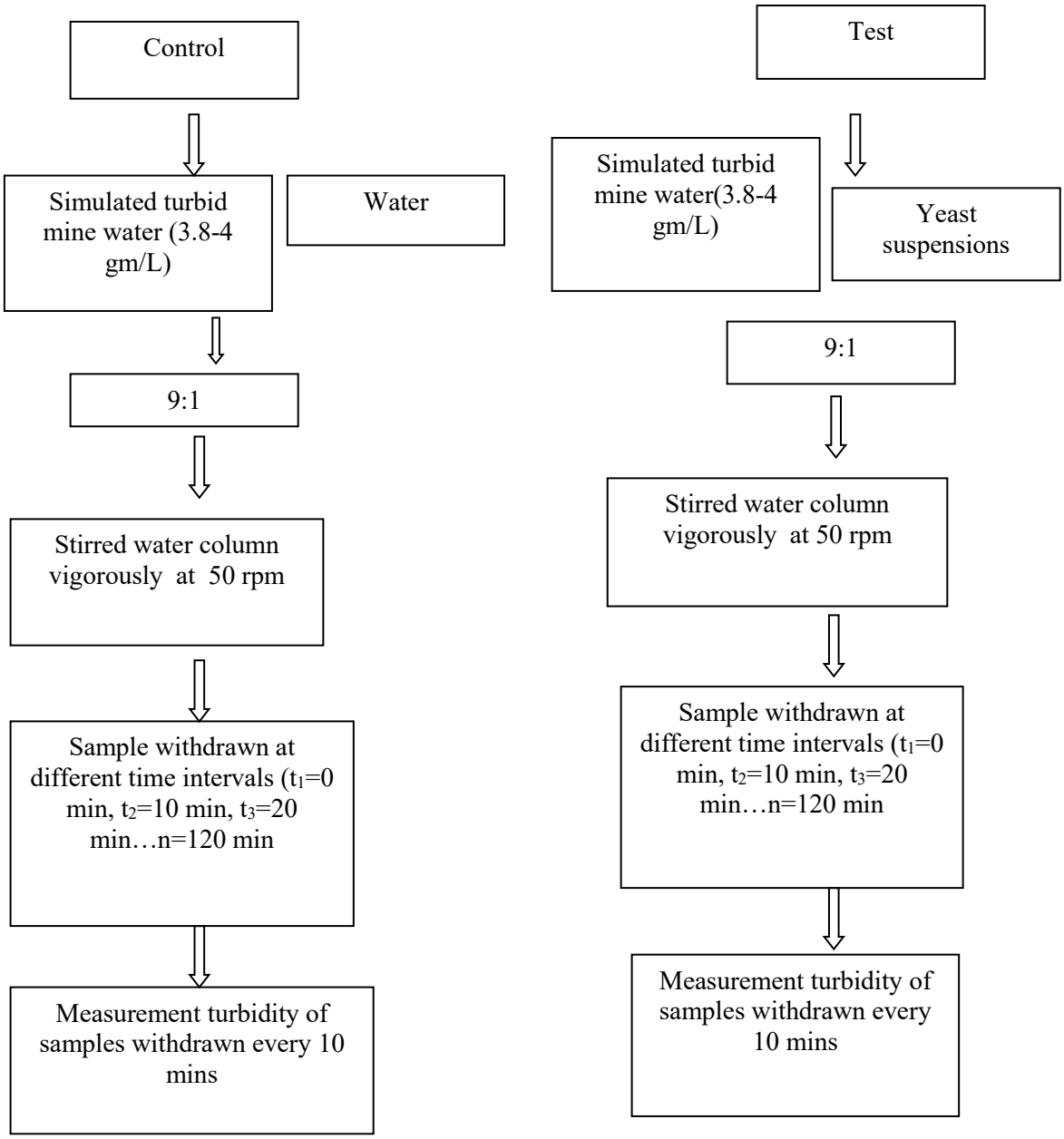


Figure.2.20: Scheme for the biosedimentation test

2.9.1. Turbidity reduction study of SMTTW in Bell jar using test cultures

Biosedimentation of clay colloids in SMTTW by yeast cultures was done and standardized at a lower scale in prior research. Biosedimentation tests on a larger scale, ranging from 14 to 100 L, were carried out in a bell jar and a columnar plastic tank. Yeast were cultivated as discussed previous section, Yeast culture was employed after the fourth day of inoculation because this harvesting time had been standardised in prior experimental studies. And standardised yeast suspension was used. Experiments were carried out at room temperature with a pH of 6.8 in the medium throughout.

13500 ml SMTTW was placed to a 20 L bell jar, and 1500 ml yeast suspensions (0.005 dry weight) were added to the SMTTW (temperature 26.9 °C pH 6.4). Water samples were withdrawn with a glass pipette at varied time intervals and from different depths of the bell jar. The turbidity of the removed water sample was determined. The biosedimentation pattern and reduction in turbidity with time were investigated in a control bell jar in which yeast suspension was not added. The turbidity of water samples taken from the control tank at various depths was also assessed. Mean of the turbidity of both control and test jar were compared.

2.9.2. Microscopy of aliquots

Microscopy of aliquots collected from different depth of bell jar was performed under 10x objective lense using light microscope.

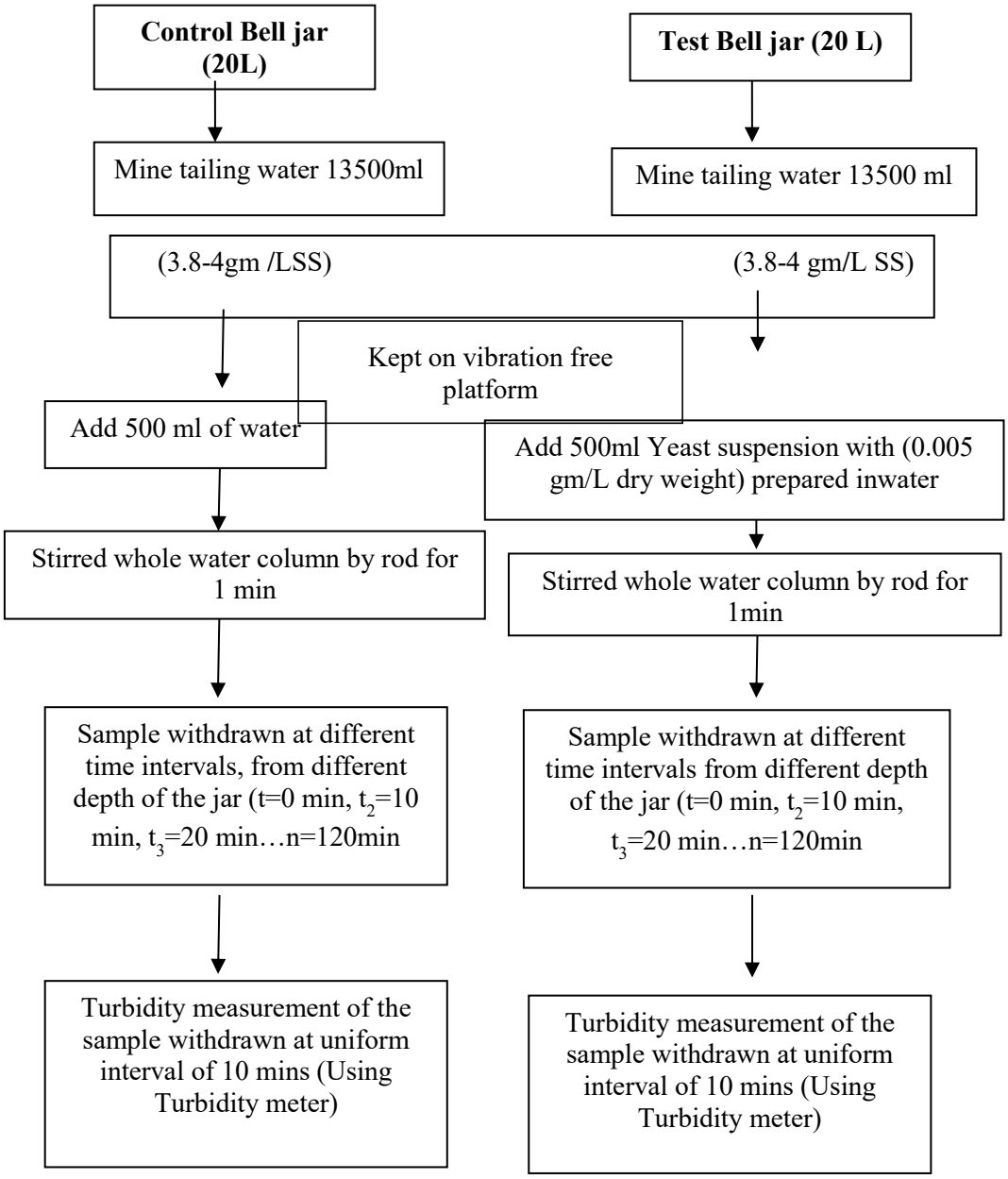


Figure.2.21: Biosedimentation test in the Bell jar

2.9.3. Biosedimentation test in 100 Litre of tank

In the tank, 90 litres of mine tailing water (prepared in reverse osmosis water (Suspended solild 3.8) was added, along with 10 litres of yeast suspension with desired concentration. Furthermore, the turbidity reduction of a control tank in which yeast suspension was not added was investigated over time at various depths.

Whole suspensions were agitated thoroughly with a rod, and samples were taken from various depths in the tank, and turbidity was assessed across time intervals.

After two hours, the entire medium was agitated for two minutes and the sample was collected once more. Turbidity was also assessed in water samples collected at various depths in the tank and at varied time intervals.

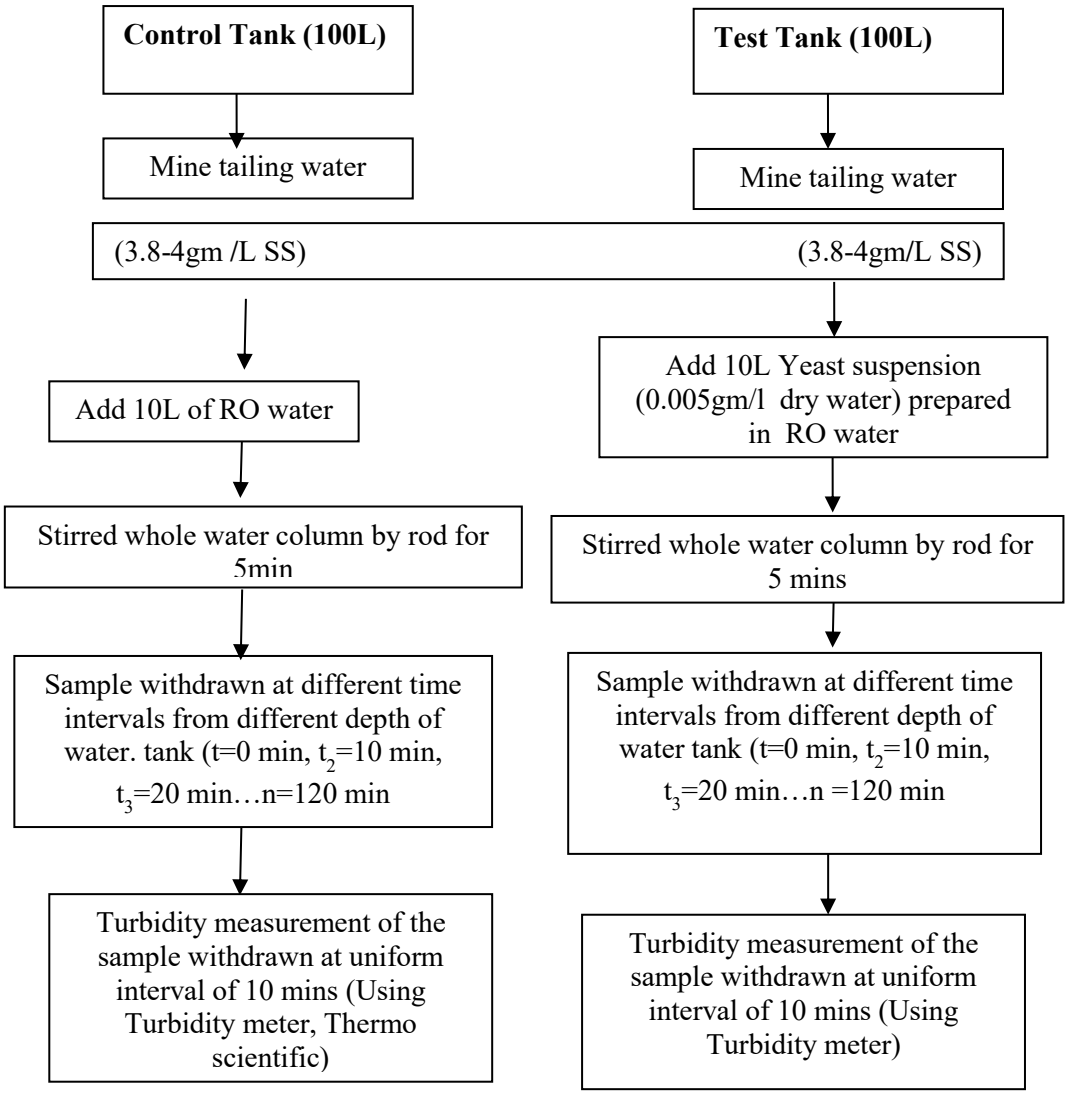


Figure.2.22 Biosedimentation test in 100 L tank

2.9.4. Sand bed filtration test at laboratory condition

The sand bed filtration technique is a common method for removing turbidity from water. It usually reduces turbidity by 0.1-1 NTU. It does, however, necessitate regular cleaning or backwashing, as well as pump operation. The sand bed filtration procedure necessitates both pre-treatment (coagulations) and post-treatment (biosedimentation) (e.g. Disinfection chlorinations.). It works by percolating water via a sand bed. It is not suited for cleaning extremely turbid water directly; however, it can be utilised after the coagulation, biosedimentation, and sedimentation processes**.**

2.9.5. Design of sand bed filter in laboratory

A 0.60 m glass column with a 0.004 m diameter was filled with 1.47g/cm^3 sand. The sand bed column was allowed to pass treated water with NTU 190. Using a peristaltic pump, the flow rate was kept at 5 ml/min. In order to maintain constant turbidity and avoid particle sedimentation owing to gravity, the reservoir water was agitated continually using a magnetic stirrer set to 70 rpm.

2.9.6. SMTTW treatment using Sand bed filter

A sand bed filter with a flow rate of 5ml/min was used to filter mine tailings and treated water. To keep the turbidity of the entire medium constant, water was kept on a magnetic stirrer. The turbidity was measured at different rpms to normalize it. At 70 rpm, the turbidity of the entire medium remained constant. At various intervals of time, samples were collected and analyses for their properties. To observe microbial growth, samples were plated on malt extract agar (2%) and nutrient agar. The effluent of sand bed filtration was also tested for turbidity reduction, pH, Fe and Mn concentration, and other water parameters.

Figure.2.23: Sand bed setup at lab scale

Figure.2.24: Petriplates spread plated with Sand bed effluent

(a) Nutrient agar (b) Malt extract agar

2.9.7. Standardization of biosedimentation process for field level application scale 500 -1000 litres

The observed laboratory results were encouraging and have inspired to scale up the volumes for relatively high-volume experimentation beyond laboratory scales. The strategy adopted was gradual scaling up. Hence initially a reactor volume of 500 liters was considered for experiment and it was tried for three times. Subsequently, the volume of 1000 litres were considered for reactor. The other benefit of this scaling up is that it may provide us with the possible deviation factor when scaled up.

The process of natural rate of biosedimentation of SMTTW's suspended solid under ambient conditions can be enhanced by using yeast as biosedimentaion agent to enhance the rate of biosedimentation and general properties of sediments.

2.9.7.1. Environmental concerns

The general environmental concern for any additives in reactors is that the product or additive should not leave behind residues in the stream especially when the life cycle assessment has not been carried out. Hence the microbial growth was observed in the outlets of control as well as reactor outlets and their respective sand filter outlets. These results are also encouraging.

Effective actual availability of yeast per gm or per ml of turbid water will definitely differ due to the hindrance of the particle co-hesive adhesive forces within liquid phase or the other chemical and physical compatibilities forces between the particles due to heterogeneity.

Area selected for performing the pilot study is based on representative condition. DM's college is located at plateau, not very far from mining area. Tailing soil sample is collected from Codlem mines of Vedanta Resources located in Dharbandora Taluka, was a part of representative sample collected.

Sediment concentrations cannot be determined easily or quickly in the field, and transportation to a laboratory for analysis is time-consuming and can be costly (Thackston and Palermo, 2000). As a result, these traditional methods are increasingly being replaced in favor of accurate, continuously-collected surrogate data for quantification of suspended solid that may be safer and (or) less expensive to obtain, such as turbidity measurements.

2.9.7.2. Preparation of SMTTW

Figure.2.25: Mine tailing soil samples

Total amount of soil was arranged in circular heap and divided into 4 parts (Fig.2.25). Soil samples were taken from the top, middle & bottom parts of the heap and mixed properly. Composite mine tailing soil was grinded with the help of ceramic mortar to get homogenous mixture of representative soil sample. 10 kg of mine tailing soil sample was added in the 100L water tank to prepare slurry. This suspension was stirred for ten min and left for 24 hr. After 24 hr suspension was restirred.

After 10 min of stirring, soil suspension was added in 800 L of water in the 1000 L of tank. (Turbidity was measured 860 (mean) and suspended solid (3.8-4 gm/L) of SMTTW). The total volume of 900 L was attained.

2.9.8. Preparation of Yeast Suspension (0.005mg/ml, Dry weight)

- Since 0.005mg/ml yeast suspension was required for turbidity reduction. (Based on standardization in previous experiment in lab)
- For 1000 L mine tailing (reactor volume) 100 L of yeast suspension required.
- Since, the desired strength of yeast is 0.005 mg per ml it will become 0.005gm in 1 Liter, and 0.5 gms in 100 L.
- So, from 20 gm /L(Dry weight) stock of yeast, actual volume required to be taken was calculated as below

$$\frac{1000}{20} \text{ X } 0.5 = 25 \text{ ml}$$

- Hence, it comes out to 25ml of yeast suspension in 99.975 L of water.

- 100 L suspension of yeast in water as prepared above was added in the 900L of turbid water. Making up total volume of reactor to 1000 liters.

(1 ml of antibiotics added in 1 L of yeast suspension to avoid bacterial contamination – the effective antibiotics concentration desired is 0.001 % - as standardized in previous experimental work)

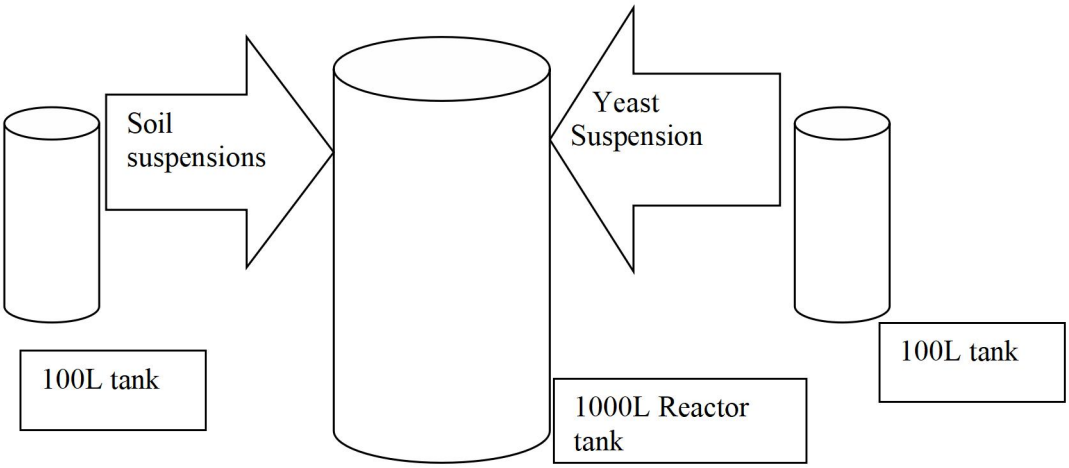


Figure. 2.26: Scheme for assay preparation

2.9.9. Sand bed treatment (From mid-point of the tank height)

After 60 min of yeast addition in reactor tank & control tank and stirring, water sample was collected from both the tanks.

Sample from reactor tank was studied for physicochemical parameter of water – sample referred as (**A**)

Water sample collected from the outlet of sand bed was also studied for physicochemical parameter of water – sample referred as (**B**).

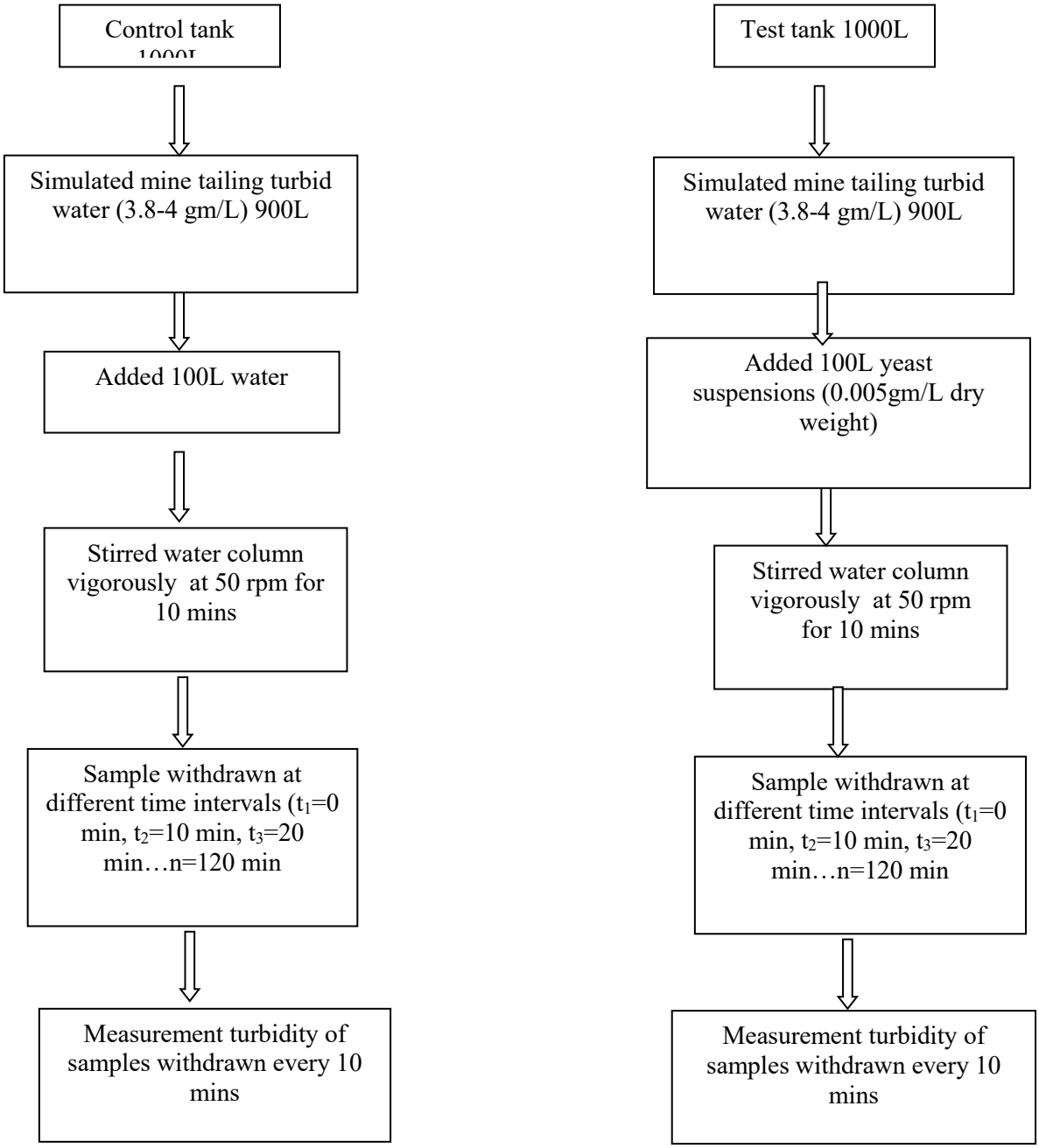


Figure.2.27: Biosedimentation test in 1000 L tank

Figure. 2.28: Experimental setup for 1000 L test

2.9.10. Sedimentation kinetic modeling aspects involved in the experiment

It is well known that the fundamentals of the rate of aggregation started from the classic work of Smoluchowski (1917). To describe the aggregation rate of particle count and sedimentation based on the Brownian controlled and Stock's law, the general differential equation can be shown below

$dc/dt = kc^{\alpha}$ …..eq.1

where α is the order of coagulation process, k is the coagulation rate constant in (L/mg-min), c is the total concentration of constituent particles in (m) at time t (min).

To simplify and solve the Eq. (1), the theoretical values of the order of coagulation or sedimentation process are in the range of ($1 < \alpha > 2$) (Chukwudi et al. 2009; WST 2003). Elimelech et al. (1995) proposed that the aggregation phenomenon followed second order by which the collision is proportional to the product of concentrations of two colliding species. Moreover, in real and empirical practice, extensive studies used ($\alpha = 2$) (Ani et al., 2012) and found that it was more logical in representing primarily the aggregation rate of particles depend on both colloids and coagulants concentrations.

Based on the pervious information substituting ($\alpha = 2$) in the Eq. (1) and integrating it with the following conditions, at initial condition ($t = 0$, $c = c_0$) and at final condition ($t = t_c = c$) to obtain Eq. (2):

$1/c = (k_2 t + 1)/co$ ……eq.2

Rearranging the eq. (2) which is used to calculate the values of (k_2) mathematically in the current research:

$k_2 = (1/c - 1/co)/t$ eq.3

Where k_2 is the second order coagulation rate constant. The total concentration suspended constituent particles of three levels of synthetic turbid water as (blank) before treatment (c_0) and after treatment (c) used in eq. (3) could be expressed in r (m) and the unit of (k_2) would be (L/mg-min) (WST 2003; Niam et al. 2007) or the unit of (k_2) would be ($NTU^{-1}\ min^{-1}$), (NTU) as turbidity reading.

2.10. Preparation of immobilized yeast in agar beads

Yeast suspension with known concentration was pipetted into micro wells of molten agar and allowed to cool before collecting beads. Under a microscope, beads were examined to see if yeast had been trapped. The control beads were made using the same

technique but without the addition of yeast. To increase the frequency of collisions between the beads and clay colloids, beads were placed in turbid water with known NTU and shaken at 100 rpm. After the experiment, the turbidity of the water was measured, and the clay colloids content of the beads
was investigated.

Figure.2.29: Beads preparation in microwells

Table.2.9. Yeast immobilization study

Test	**Positive control**	**Negative control**
120 ml turbid water and beads with yeast.(20 ml yeast suspension with known absorbance+180 ml agar beads)	180 ml Turbid water	120 ml turbid water and beads without yeast
pH, 6.4, Tem (°C) 25.4, NTU 780 of SMTTW		

In 9 ml turbid water with known NTU, add 0.5 ml of the most promising yeast suspensions (known absorbance). In separate tubes, add 0.5 ml of each strains with the same absorbance, followed by 0.5 ml of Bchlm-1-2 suspensions. Observed and scored tubes for the removal of clay colloids, as it had been investigated in prior research. The sediment generation of the two most promising combinations was also investigated using the same methods up to a scale of 10-300 ml

2.10.1 Turbidity reduction study of natural mine tailing turbid water

Natural SMTTW from mining area of Goa have been brought to the laboratory and characterized for its different physiochemical properties. Biosedimentation test of clay colloid using yeast cultures have been performed. Further biosedimentation fashions have been compared with mine simulated water

.

2.10.2 Characterization of natural mine tailing turbid water

Natural mine tailing turbid water was characterize in terms of turbidity and pH.

2.10.3 Biosedimentaion test of natural mine tailing turbid water using yeast

In 900 ml of natural mine water, 100 ml of yeast suspension (0.005 mg/ml, dry weight) was mixed. For 60 seconds, the mixture was vigorously stirred (50 rpm). Water samples were taken from different depths of the Imhoff cone at different intervals of time to measure turbidity with a turbidity meter. Complex sediments generation and quick sedimentation were used to evaluate the biosedimentation efficiency, which was compared to turbidity reduction in a control cone (which yeast were not added, under laboratory condition)

Figure.2.30: Water body in mining area, turbid due to runoff of the mine tailing soil

2.11.1. Molecular identification of most promising cultures for biosedimentation:

Culture were identified by PCR method, (Triyat Scientific Company (Nagpur) provided this service)

2.11.2. DNA Extraction ⟶ PCR ⟶ Purification of PCR Product ⟶ Sequencing ⟶ Bioinformatics

2.11.3. DNA Extraction

Cells grown in monolayer lysed by suspending 1-3 colonies aseptically and mixed with 450 µl of "B Cube" lysis buffer in a 2 ml micro centrifuge tube and lyse the cells by repeated pipetting.

2. Added 4 µl of RNAse A and 250 µl of "B Cube" neutralization buffer.

3. Vortexed the content and incubate the tubes for 30 minutes at 65˚C in water bath. To minimize shearing the DNA molecules, mix DNA solutions by inversion.

4. Centrifuged the tubes for 20 minutes at 14,000 rpm at 10 ˚C.

5. Following centrifugation, transfer the resulting viscous supernatant into a fresh 2 ml micro centrifuge tube without disturbing the pellet.

6. Added 600 µl of "B Cube" binding buffer to the content and mix thoroughly by pipetting and incubate the content at room temperature for 5 minutes.

7. Transferred 600 µl of the contents to a spin column placed in 2 ml collection tube.

8. Centrifuged for 2 minutes at 14,000 rpm and discard flow-through.

9. Reassembled the spin column and the collection tube then transfer the remaining 600 µl of the lysate.

10. Centrifuge for 2 minutes at 14,000 rpm and discard flow-through.

11. Add 500 µL "B Cube" washing buffer I to the spin column. Centrifuge at 14,000 rpm for 2 mins and discard flow-through

12. Reassemble the spin column and add 500 µl "B Cube" washing buffer II and Centrifuge at 14,000 rpm for 2 mins and discard flow-through.

13. Transfer the spin column to a sterile 1.5-ml micro centrifuge tube

14. Add 100 µl of "B Cube" Elution buffer at the middle of spin column. Care should be taken to avoid touch with the filter.

15. Incubate the tubes for 5 minutes at room temperature and Centrifuge at 6000 rpm for 1 min.

16. Repeat the above mentioned step 14 and 15 for complete elution. The buffer in the microcentrifuge tube contains the DNA.

17. DNA concentrations were measured by running aliquots on 1% agarose gel.
18. The DNA samples were stored at -20°C until further use.

2.11.4 Polymerase Chain Reaction

(PCR) is a method for amplifying certain cloned or genomic DNA sequences using primers and a special enzyme. On a single-stranded DNA template, the enzyme DNA polymerase guides the synthesis of DNA from deoxynucleotide substrates. When a custom-designed oligonucleotide is annealed to a longer template DNA, DNA polymerase inserts nucleotides to the 3' end. When a synthetic oligonucleotide is annealed to a single-stranded template with a region complementary to the oligonucleotide, DNA polymerase can use the oligonucleotide as a primer and elongate its 3' end to form a double-stranded region.

2.11.5. Purification of PCR Production

Montage PCR Clean up kit was used to remove unincorporated PCR primers and NTPs from PCR products (Millipore). The primers were used to sequence the PCR result. ABI PRISM BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq DNA polymerase (FS enzyme) were used to execute the sequencing procedures (Applied Biosystems).

2.11.6. Sequencing protocol

Using 26s rRNA universal primers, single-pass sequencing was done on each template. Using an ethanol precipitation technique, the fluorescent-labeled fragments were separated from the unincorporated terminators. The samples were resuspended in distilled water and run through an ABI 3730xl sequencer for electrophoresis (Applied Biosystems).

CHAPTER III
RESULTS

In this chapter results of the performed experiment is presented, as the methodology presented in previous chapter. Freshwater sampling sites and water sample's physiochemical characters are reported. Furthermore, isolated cultures`s biochemical characteristics is shown. After screening of the most promising yeast cultures biosedimentation kinetics of simulated mine tailing water using yeast, is presented. Furthermore, results of the experiment performed for the validation of biosedimentation experiment is presented eg.

1. Biosedimentation of natural mine tailing water using yeast culture.
3. Adsorption efficiency and biosedimentation or bioflocculation rate constant.
4. Cultures combination study for efficient turbidity reduction is presented.
5. Study of presence of the microbial in treated and nontreated water is presented.
6. Besides turbidity reduction other water parameter reduction result is presented.

3.1. Freshwater habitat from mining and non mining areas of Goa Surveyed

The physico-chemical parameters of all under study waterbodies depicted in table 3.1Temperature in selected water habitats from various locations ranged from 24.5 to 30 °C. The pH level of selected sampling sites ranged from 6.3 to 7.8. Netravali water bodies showed high pH due to ongoing activity. Karapur pond and Netravali recorded the highest electrical conductivity 140 µs. Each sample's TDS were observed within a permissible level. As per Data on NTU of under study water sample locations ranged between 2 and 15 NTU.

Table.3.1 Water sampling sites and sample characteristics

Mining Area					
Name	**Temp °C**	**pH**	**E.C. µs**	**TDS ppm**	**Turbidity NTU**
Sirigao tank	28.9	6.8	64	20	ND
Mayem lake	29	6.3	44	15	8
Bicholim tank	28	7.33-6.7	90	40	ND
Karapur tank	28	6.87	140	64	ND
Aravelum Water fall's stream	28	7.2	49	27	15
Tambdi surla stream	24.5	6.5	-	-	ND
Bondla stream	25.5	6.4	-	-	ND
Chorla stream	25.5	6.5	50	37	ND
Netravali waterfall'stream	26	7.47	40	15	2.35
Netravali bubbling lake	27	7.8	140	89	8.59

Non Mining Area					
Name	**Temp °C**	**pH**	**E.C. µs**	**TDS ppm**	**Turbidity NTU**
Safa masjid Pond	30	6.7	103	76	ND
Carambolim lake	29	6.9	128	48	ND
Goa University campus (Talegao plateu)					
Pond-1	25	6.9	102.5	51	6.2
Pond-2	25	6.9	-	-	6.1
Pond-3	25	6.33	92	45	6.2

3.2. Isolation of yeasts

A total 40 yeast colonies were isolated from various freshwater bodies of Goa. Control plates, on which water samples were not plated, no microbial growth were observed. On MEA plates without antibiotics, both bacteria and yeast cultures were appeared. On the antibiotic incorporated test plates, only yeast growth was observed.

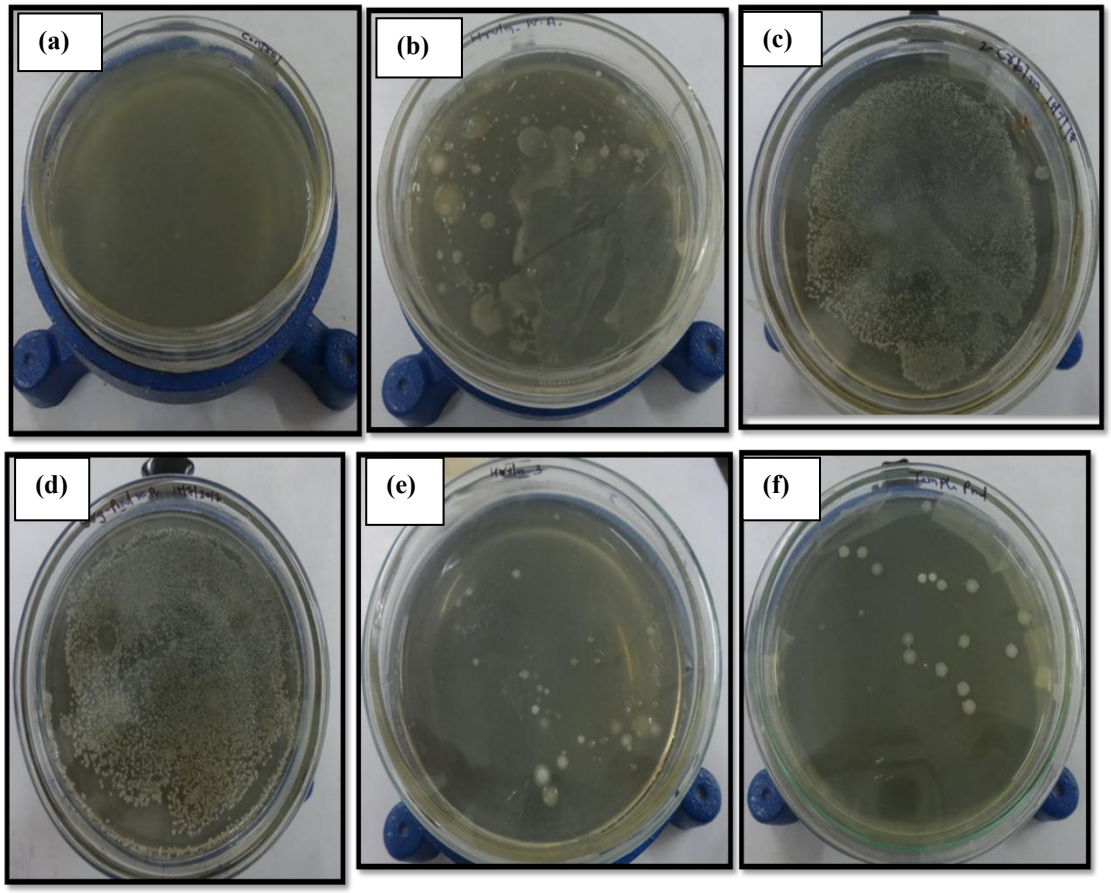


Figure.3.1: Representative isolation plates, (a) Control plate, microbial growth was not observed (b) Plate without antibiotics, mix culture observed. (c), Isolation plate carambolim, small tiny colony growth observed (d) isolation plate ,Sirigao, small tiny colony growth observed. Isolation plate of (e) Aravelum, two large dissimilar colonies observed (f) Isolation plate of temple pond, two dissimilar morphological colonies were observed.

3.2.2. Colony Characteristics on the isolation plates

On a regular basis, isolation plates were observed for the microbial colony growth, and the following microbial growth pattern characteristics were observed (Table3.2). The colony of the some isolate had a waxy texture, some of them were shiny and smooth. The isolation plates have showed mixture of flat and elevated, elevations of the colonies. The colonies were white, beige, and cream colour in nature. Single colony showed pink colour Pnd-2-2. Moreover, plates without antibiotics showed mixed growth of microbial colonies.

Table.3.2: Growth rate and colony morphology of cultures

Strain Designation	Colour	Colony shape	Surface	Elevation	Texture
Bchlm-1	White	Circular	Shiny	Flat	Smooth
Bchlm-1-2	White	Circular	Shiny	Flat	Smooth
Bndl-2-2	Beige	Granular	opaque	Raised	Smooth
Bndl-1-1-1	Creamish	Circular	Shiny	Flat	Smooth
Bh-1	White	Circular	Shiny	Flat	Smooth
Bnd-1	Creamish	Circular	Shiny	Flat	Rough
Bndl-3	Creamish	Circular	Shiny	Flat	Smooth
Bndl-2-1	Creamish	Circular	Shiny	Flat	Smooth
Chl-1	Beige	Granular	opaque	Raised	Smooth
Crmblm-1-2	White	Circular	Shiny	Raised	Smooth
Gh1-1	White	Circular	Shiny	Flate	Rough
Gh2-1	White	Circular	Shiny	Raised	Rough
Gh1-3	White	Circular	Shiny	Raised	Rough
Gh-4	White	Circular	Shiny	Raised	Rough
Hrvlm1-2	White	Circular	Shiny	Flate	Smooth
Krpr-1	White	Circular	Shiny	Flate	Smooth
Krpr-2	White	Circular	Shiny	Flate	Smooth
Mym-1-2	Beige	Circular	Shiny	Flate	Smooth
Ntrvl-1-1-1	Beige	Circular	Waxy	Raised	Smooth
Ntrvl-1-2	White	Circular	Shiny	Raised	Smooth
Ntrvl-2-2	Beige	Circular	Waxy	Raised	Smooth
Ntrvl-2-3	White	Circular	Shiny	Raised	Smooth
Pnd-2	Pinkish	Granular	Shiny	Flat	Smooth
Pnd-3	Beige	Circular	Shiny	Raised	Smooth
Pndsm-1	White	Circular	Shiny	Flat	Smooth

Strain Designation	Colour	Colony shape	Surface	Elevation	Texture
Pndsm-1-2	Beige	Circular	Shiny	Flat	Smooth
Pndsm-2-2	Pinkish	Granular	Shiny	Flat	Smooth
Pndsm-3	White	Circular	0paque	Flat	Rough
Pnd-2-2	White	Circular	Shiny	Raised	Smooth
Pnd-3	Beige	Circular	Shiny	Raised	Smooth
Sc-1	White	Circular	Shiny	Raised	Smooth
Rw.a	White	Circular	Shiny	Flat	Smooth
Srg-3-3	White	Circular	Shiny	Flat	Smooth
Srg-1	White	Circular	Shiny	Flat	Smooth
Srg-2-1	Cream	Grannular	Shiny	Flat	Smooth
Tmrs-1-2-4	White	Circular	Shiny	Flat	Smooth
Tmrs-2-2	Beige	Circular	Waxy	Flat	Smooth
Tmrs-2-2-4	Beige	Grannular	opaque	Raised	Smooth
Tmrs-2-3	Beige	Circular	Waxy	Flat	Smooth

3.2.3. Micromorphology of the cultures

Cultures were studied under the microscope; mix culture were grown on the plates which were not incorporated with antibiotics, bacterial cells and yeast cells were associated as typical bacilli and yeast. Most important point can be concluded is all yeast cultures are antibiotics resistance as they have grown in antibiotic incorporated media. Pure cultures have a variety of morphologies, as illustrated in the morphologyof the cultures **(**Fig 3.2**)**. Some of the cultures are spherical, few of them showed oval cell shape. Some of culture were in cylindrical shape and have pseudomycilieum. Dense colony cells were compactly bounded to each other and strongly stained with crystal violet in the strain designated as Ntvrl-2-1, Pnd-3, and Ntrvl-1-1-1. The cells of the cultures Ntrvl-1-1, Ntrvl 2-3, showed scattered single cells and stained strongly with crystal violet. Tmrs-2-2-4 showed polymorphism as some cells were elongated, oval and spindle in shape, when the culture's smear stained in the crystal violet stain. Moreover, most of the culture's cells with different morphology formed pseudomycielium. The Pnd-2-2 cells were lightly stained in the crystal violet stain and showed small free cells. Chl-1 cells were small in size and highly stained in crystal violet. Some of the cultures showed single- budding independent cells eg. Bchlm1-2, Ntrvl-2-2. Short branched chains were observed in many colonies eg. Gh-1-3 and long chains were observed in Ch-1 and Bnd-1-1-1.

3.2.3. Morphology of cultures in monochrome staining

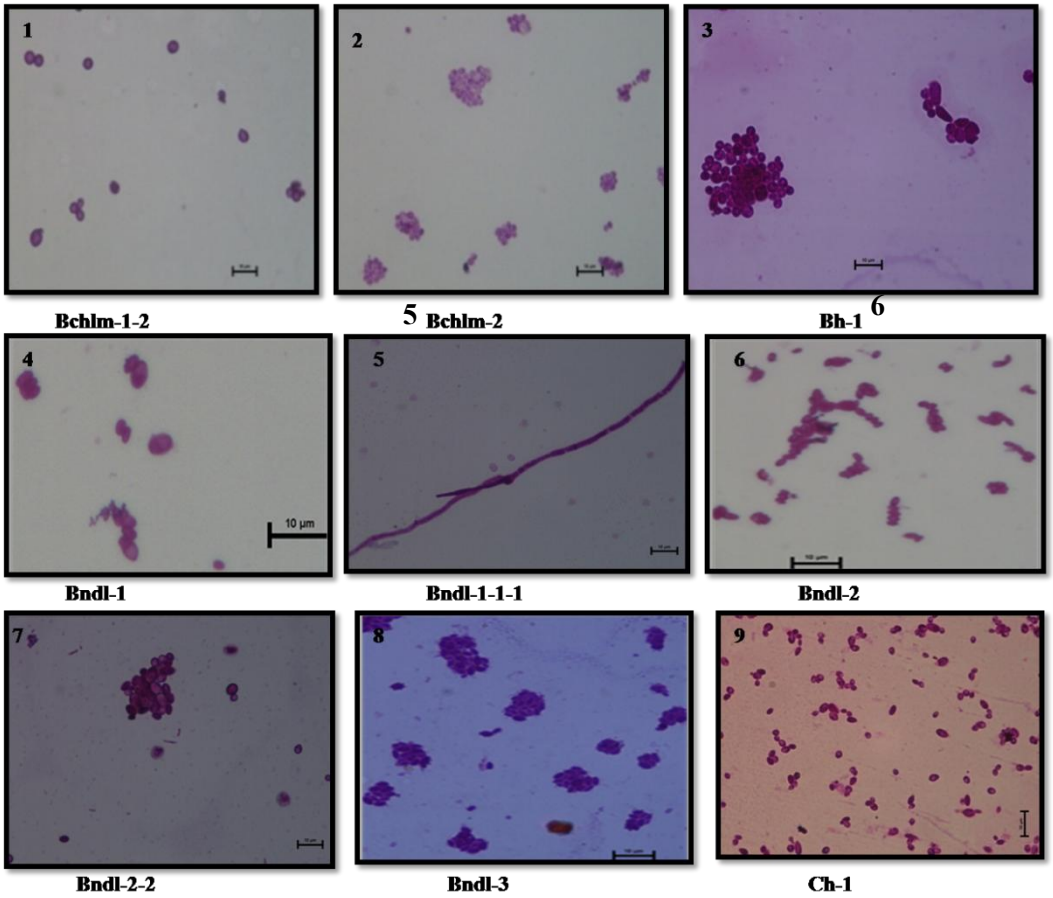


Figure.3.2: (1-9) Monochrome stained cells of yeasts

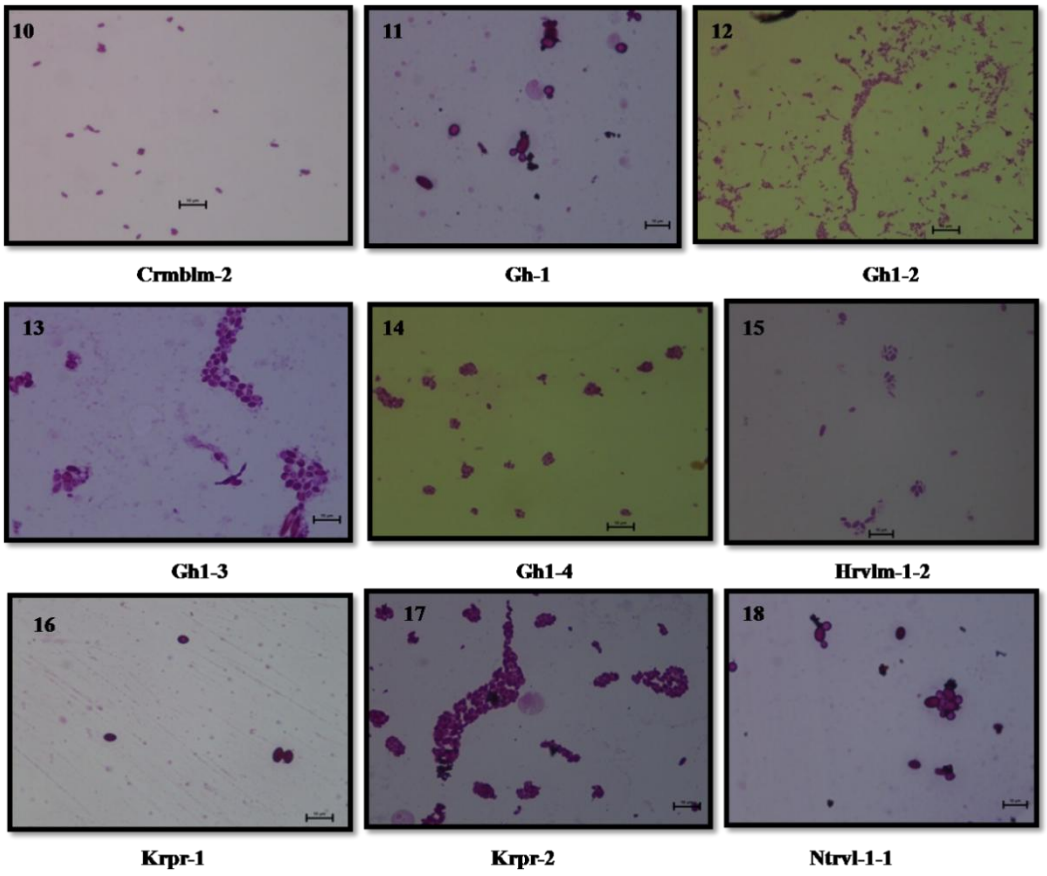


Figure.3.3: (10-18) Monochrome stained cells of yeasts

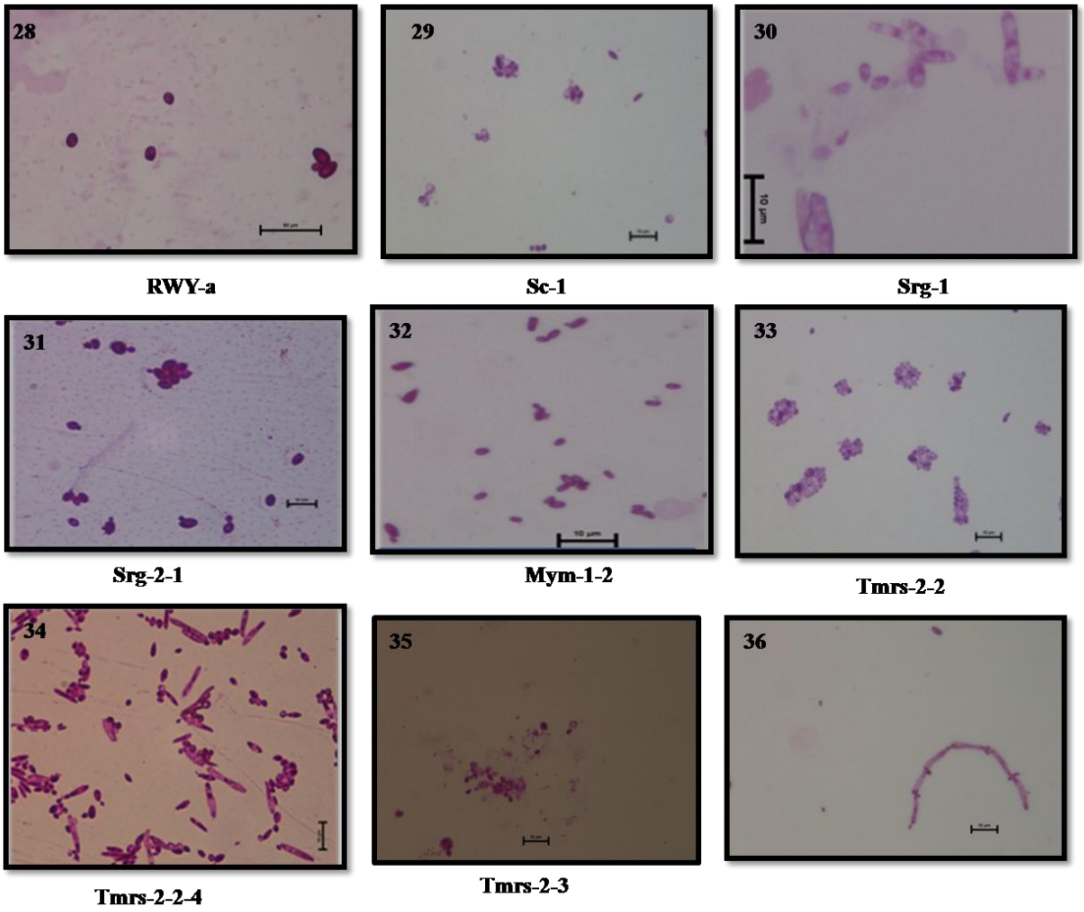


Figure.3.4: (28-36) Monochrome stained cells of yeasts

3.2. 4. Biochemical tests

Sugar fermentation tests of the cultures revealed a change in the medium colour from blue to purple pink, yellow, indicating the formation of acid (fig3.5). The reaction with sugar of cultures is showed in the table 3.3. Most of the strain showed positive test with glucose except Bchl1m-1, Crmblm-2, Chrl-1, Hrvlm1-2, krpr-2, Mym-1-2. Ntrvl-2-3, Pnd-3. On the other hand Bchlm-1, pnd-3, Pndsm-1, and Tmrs-2-3, did not ferment sugars at any stage. Bndl-1-1-1, Gh-4, showed positive test with glucose only. Some of the strain showed negative test with lactose and some of the strain showed negative result with maltose.

Yeast cells showed various sizes and shapes. Pseudomycilium and ascospores were examined on corn meal agar media and sodium acetate agar (Fig.3.10). The colour of the colonies and texture remained same on corn meal agar as on the isolation plate with MEA media and after streaking on the slant.

Bndl-1-1-1, Bndl-1-1, krpr-2, GH_1-5,Pnd-2, Pndsm-1, Mym-1-2, Ntrvl-1-1-1 did not displayed the presence of mycelium in their colony after grown on specified media.
Moreover, after growing on Nitrate agar, strain designated as, Bchlm-1-2, BH-1, Bndl-1-1-1, Crmblm-2, Gh1-4, Hrvlm-1-2, Mym-1-2, Ntrvl-1-2, Ntrvl-2-3, Pndsm-1-2, showed positive growth.

On the sodium acetate agar Bchlm-1-2, BH_1, Bndl-1, Bndl-1-1-1, Chr-1, Hrvlm-1-2, Mym-1-2, Ntrvl-1-1-1, Ntrvl-1-2, Ntrvl-2-2, Pnd-2, Pnd-3, Pndsm-1, Pndsm-1-2, Pndsm-2-2, Srg-1 showed positive result (Table. 3.4).

Culture streaked on the *Candida* differential agar media were showed different colour of the colony (Table. 3.4). Many strains did not grow on differential agar media can be predicted as belongs to saccharomyces group.

On the basis of colour development in the colonies on *Candida* differential agar, and using morphological features and biochemical tests, strains were tentatively identified, as (Table.3.5).

Figure.3.5: Sugar fermentation test

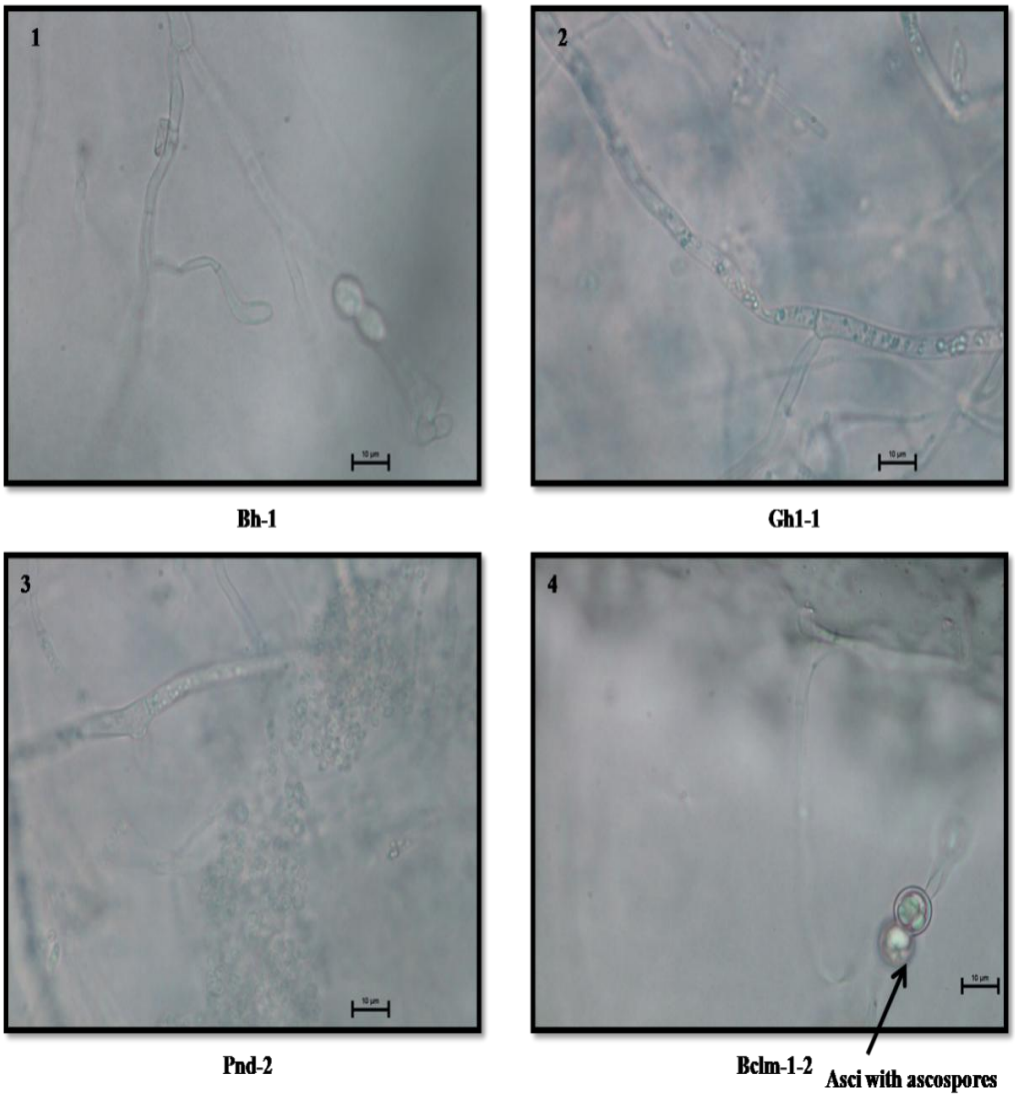


Figure.3.6: (1-4) Various form of ascospores

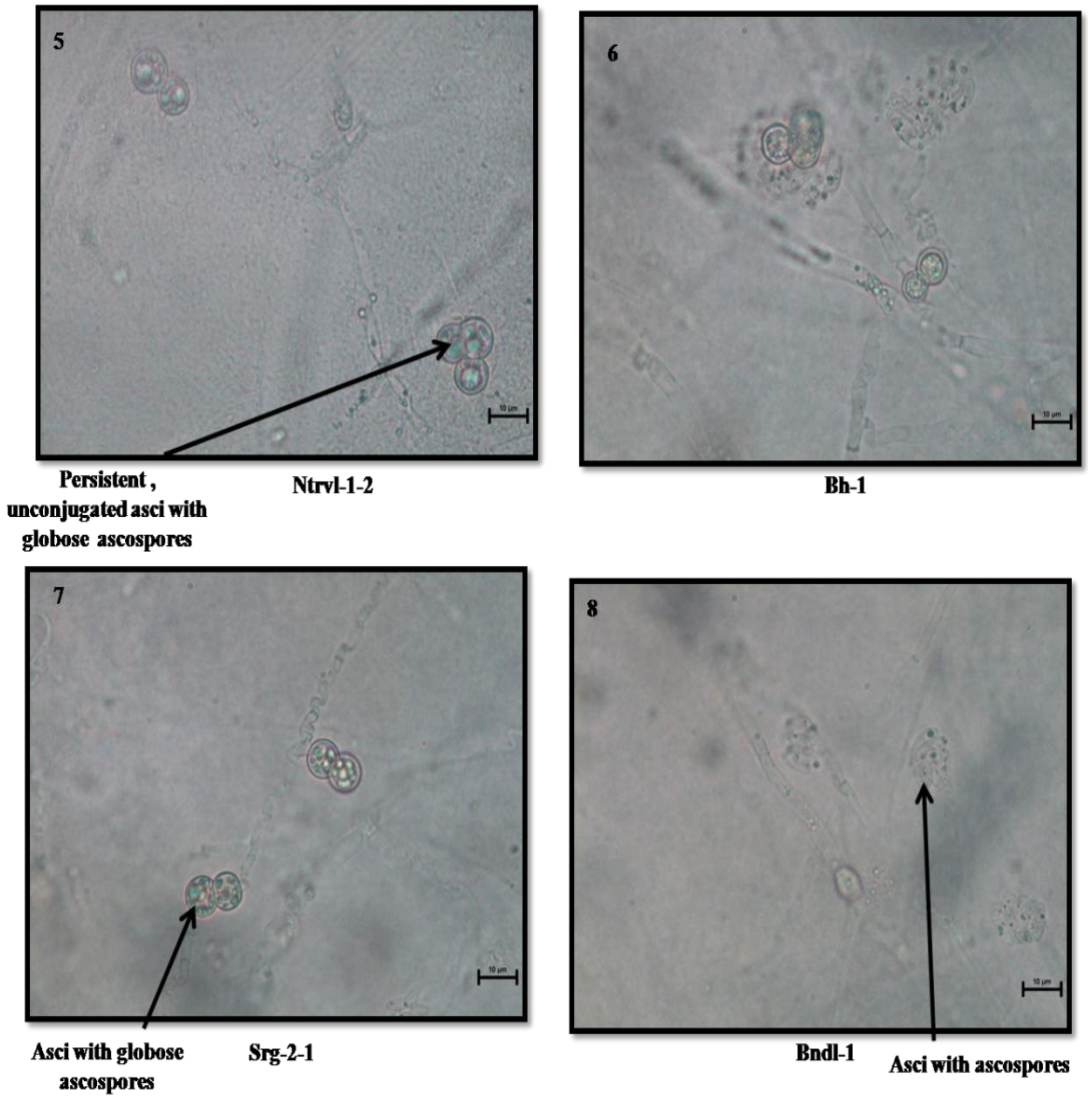


Figure.3.7: (5-8) Various form of ascospores

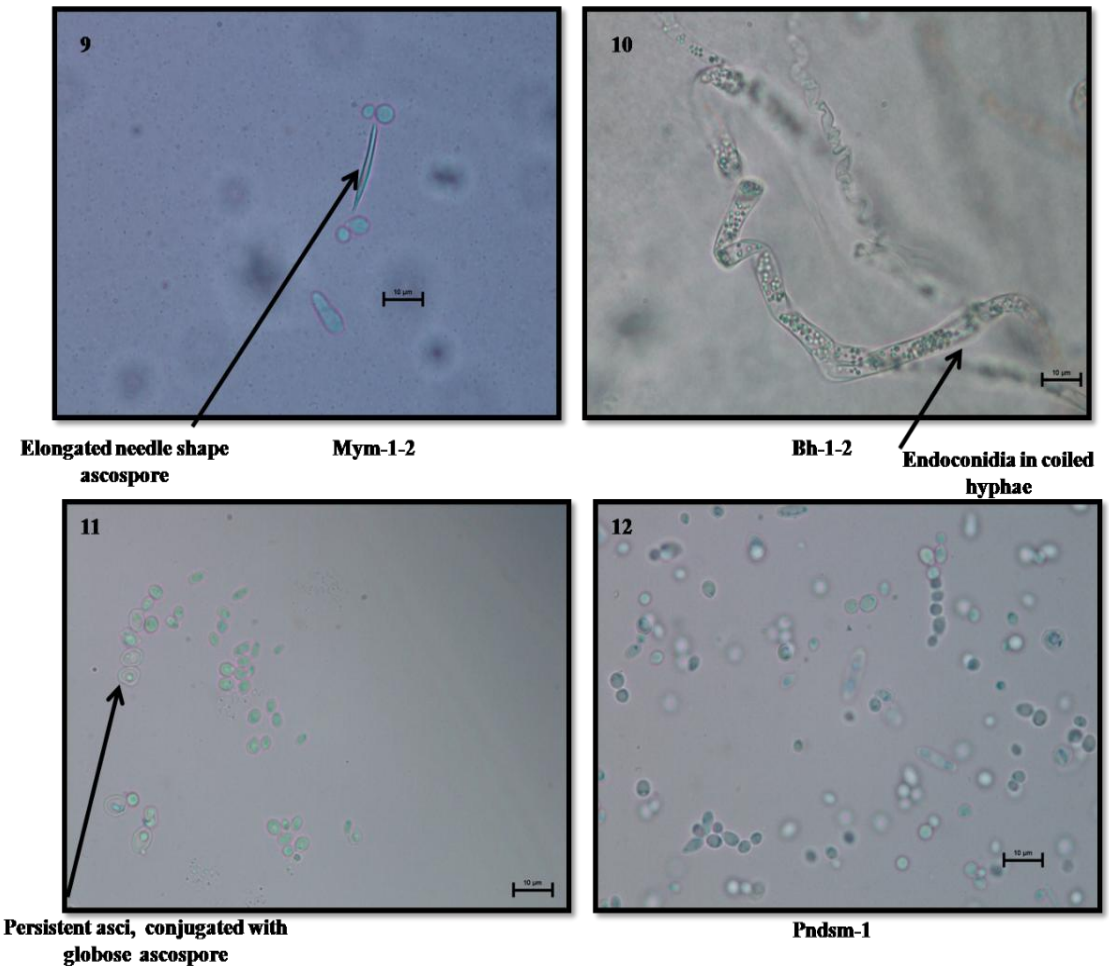


Figure.3.8: (9-12) Various form of ascospores

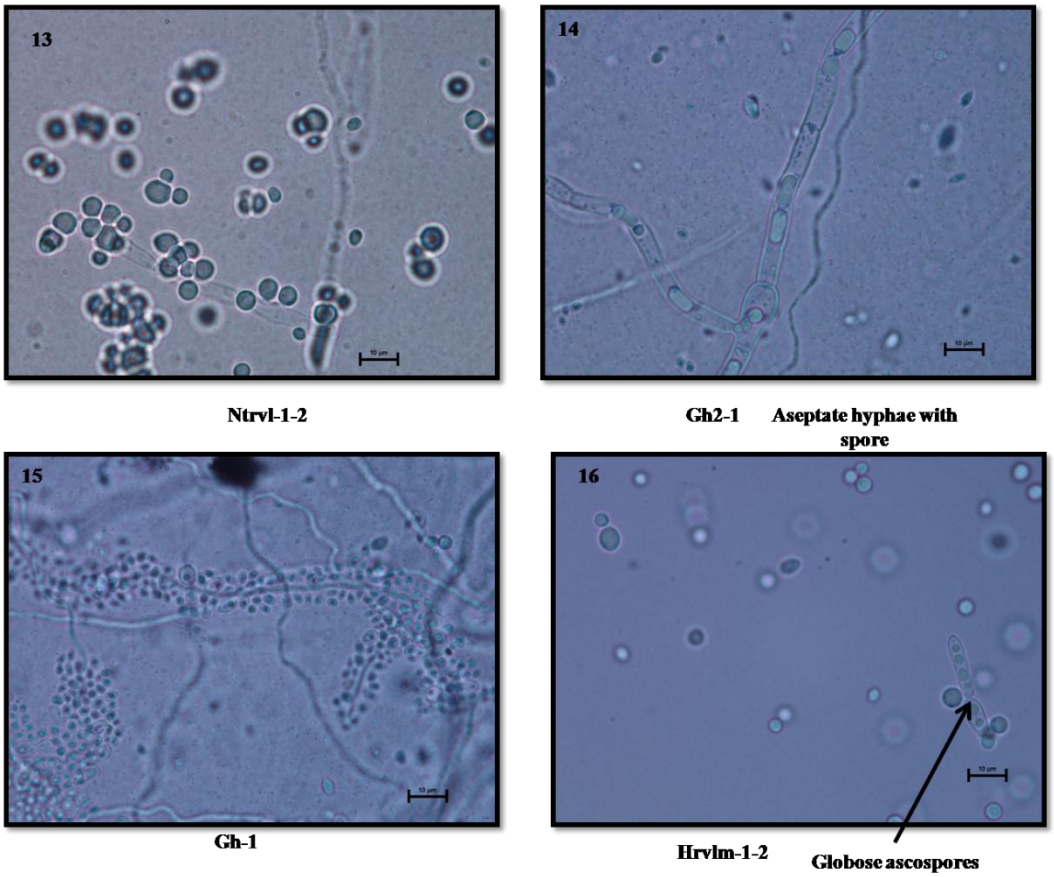


Figure.3.9: (13-16) Various form of ascospores

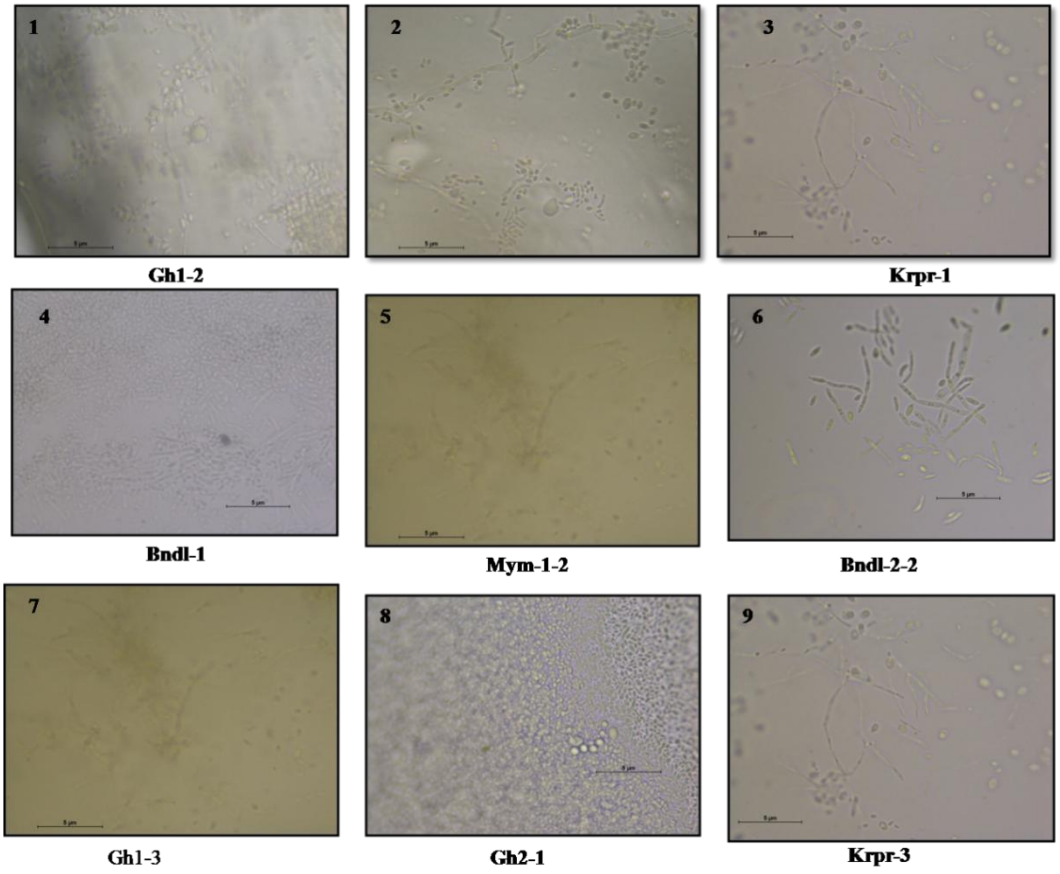


Figure.3.10: (1-9) Various form of pseudomycelium and non mycelia cells

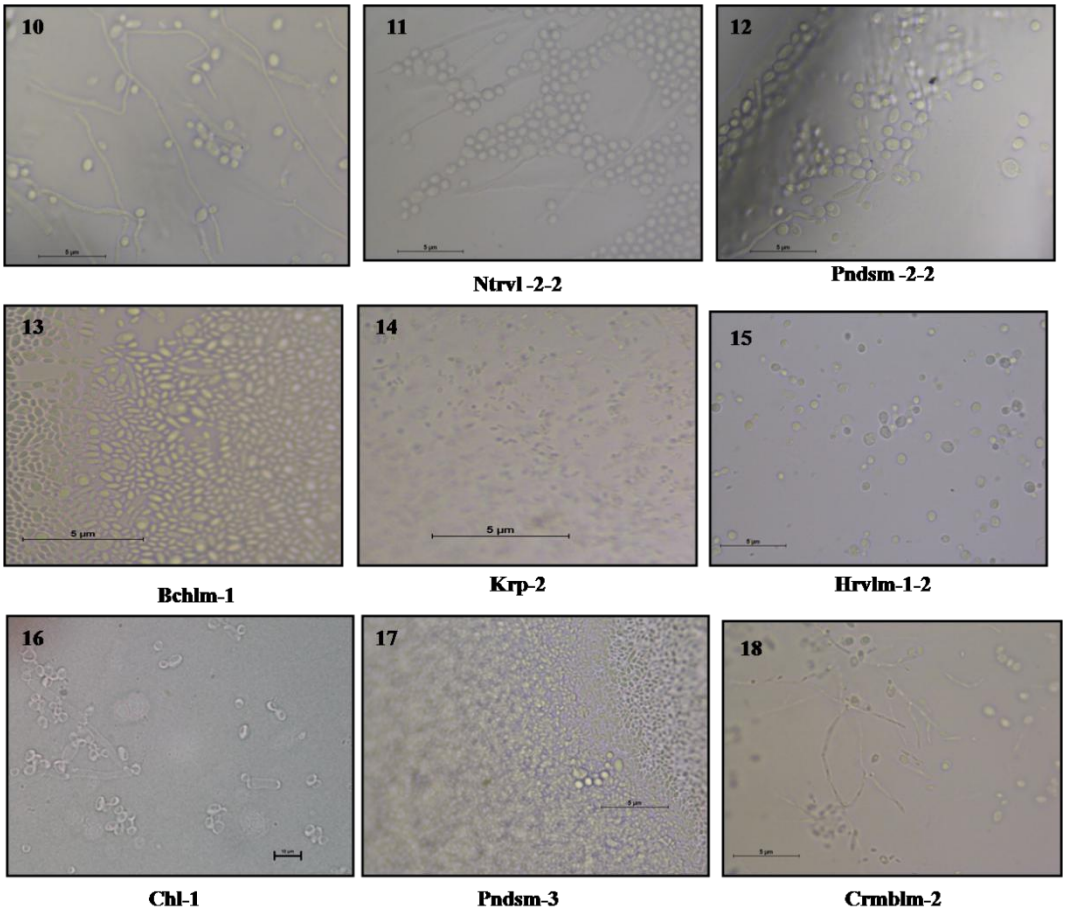


Figure.3.11: (10-18) Various form of pseudomycelium and non mycelia cells

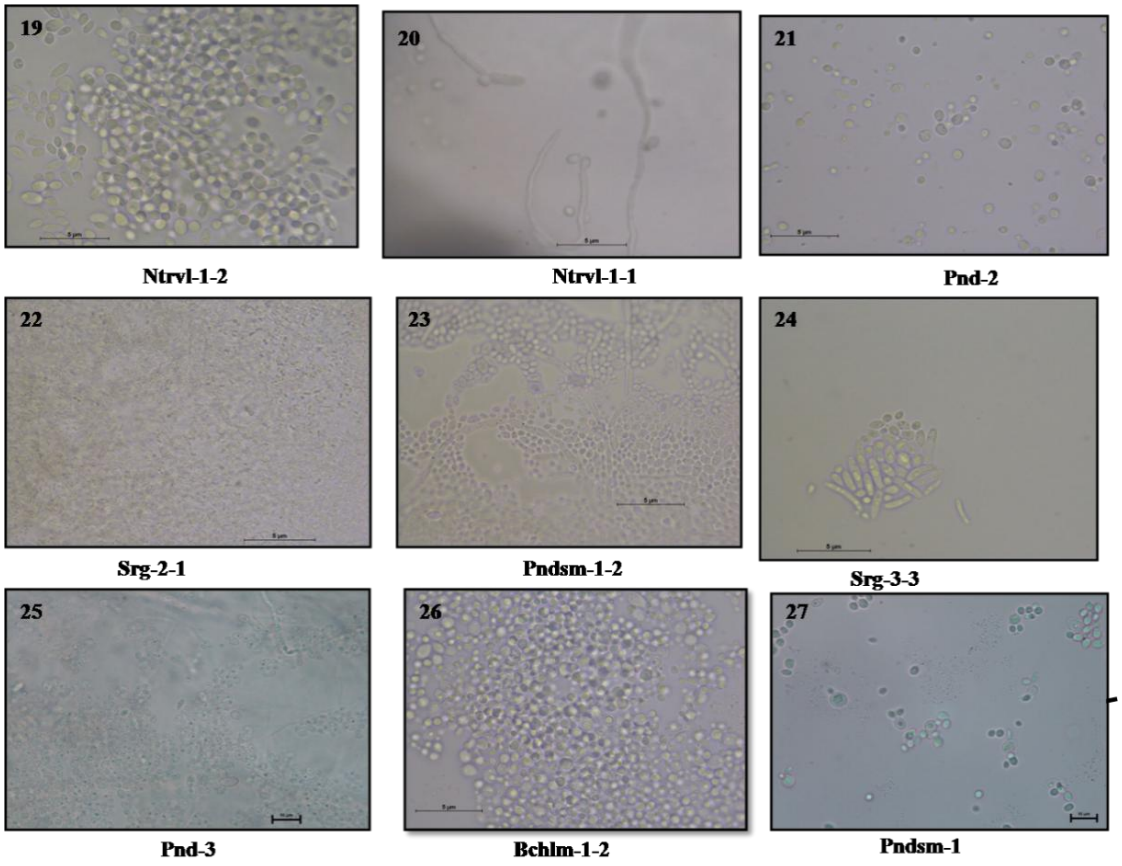


Figure.3.12 (19-27) Various form of pseudomycelium and non mycelia cells

Table.3.3 Yeast's colony characteristics in various mediums

Strain designation	cielum production	Nitrate agar	Sodium acetate agar	Sugar test			
				Glucose	Sucrose	Lactose	Maltose
Bchlm-1-2	–	+	+	+	+	–	+
Bchlm-1	–	–	–	–	–	–	–
BH_1	+	+	+		++	–	+
Bndl-1	+	–	+	+	+	+	–
Bndl-1-1-1	–	+	+	+	+	+	–
Bndl1-1-1	–	–	–	+	–	–	–
Bndl-2-2	–	–	–	+	–	–	–
Crmblm-2	+	+	–	–	NA	–	+
Chrl-1	+	+	+	–	+	+	+
Gh1-3	+	–	–	+	–	–	+

Pnd-3	Pnd-2	Ntrvl 2-3	Ntrvl-2-2	Ntrvl-1-2	Ntrvl-1-1-1	Ntrvl	Mym-1-2	Krpr-2	Hrvlm1-2	Gh1-5	Gh1-4
+	–	+	+	+	–	–	–	–	–	–	+
–	–	+	–	+	+	–	+	–	+	–	+
+	+	–	+	+	+	–	+	–	+	–	–
–	+	–	+	+	+	+	–	–	–	+	+
–	–	–	+	+	+	–	+	–	–	–	–
–	–	–	+	–	+	–	+	+	–	–	–
–	–	–	+	++	++	+	++	+	–	–	–

Tmrs-2-2-4	Tmrs-2-3	Tmrs-2-2	Srg-2-1	Srg-1	RW(2a)	Pndsm-3	Pndsm-2-2	Pndsm-1-2	Pndsm-1
+	–	–	+	+	+	–	+	+	–
+	–	–	–	+	+	–	–	+	–
Not grown	–	–	-	+	+	-	+	+	+
–	–	+	+	–	+	+	+	+	–
–	–	+	+	+	+	–	–	+	–
–		–	–	+	–	–	+	–	
–	–	+	–	+	+	–	+	++	–

Table.3.4 Colony characteristics on differential agar media ((The colour coding indicates colour observe in plates)

Strain Designation	Colour in D.A.	Colour in MEA	Strain Designation	Colour in D.A.	Colour in MEA
Bchlm-1-2	Blue	White	Ntrvl-2-2	Green	White
Bchlm-1	Not grown	Beige	Pndsm-1	Not grown	White
Bndl-2-2	Brown	White	Pndsm 1-2	Shiny brown	White
Bndl-1-1-1	Not grown	White	Pndsm-2-3	Blue	White
Bh-1	Purple	Cream	Pndsm-3	Brown	Cream
Bnd-1	Pink	White	Pnd-2	Coke	White
Bndl-3	Purple	White	Pndsm-2-2	Not grown	White
Bndl-2-1	Purple	White	Pnd-2-2	Dark Purple	Red
Chl-1	Not grown	Beige	Pnd-3	White	White
Crmblm-1-2	Not grown	White	Sc-1	Not grown	-
Gh-4	Peach Pink	White	Rw.	Coke	Beige
Gh2-1	Coke	White	Srg-3-3	Pink	White
Gh1-3	Not grown	Beige	Srg-1	White	Beige
Hrvlm-1-2	Pink	White	Srg-2-1	Not grown	White
Krpr-1	White	White	Bchlm-2	Green	White
Krpr-2	Not grown	White	Tmrs-2-1-2	Not grown	White
Mym-1-2	Green	Brown	Tmrs-2-2-4	White	White
Ntrvl-1-2	Green	White	Tmrs-2-3	Not grown	White
Ntrvl-1-1-1	Blue	White	-	-	-
Ntrvl-2-1	Not Grown	White	-	-	-

Table.3.5 Tentative identified cultures

S. No.	Strain designation	Genus
1	Bchlm-1-2	*Candida sp.* I
2	Bnd-1	*Candida sp.* II
3	Srg-3-3	*Candia sp.* III
4	Ntrvl 1-2	*Candida sp.* IV
5	Pnd-2	*Candida sp.* V
6	Ntrvl-1-1-1	*Candida sp.* VI
7	Pndsm-3	*Candida sp.* VII
8	Pndsm-2-3	*Candida sp.* VIII
9	Gh-4	*Candida sp.*IX
10	Bndl-3	*Candida sp.*X
11	Bndl-2-2	*Candida sp.* XI
12	Pnd-2-2	*Rhodotorula sp.*
13	Pnd-3	*Sacchromycopsis sp.*

3.2.5.1 Characterization of the most promising yeast

The dye adsorption capability of the most promising yeast (for biosedimentation) was high (fig.3.13 (b)) whole colony turned blue on the media and zone of clearance observed. The occurrence of salt deposition on the plate can be attributed to calcium oxlate (fig 3.13 (c, d)). With catalase, the most promising strain produced a large gas bubble (fig.3.13 (f)) provides insight of hydrogen peroxide reaction

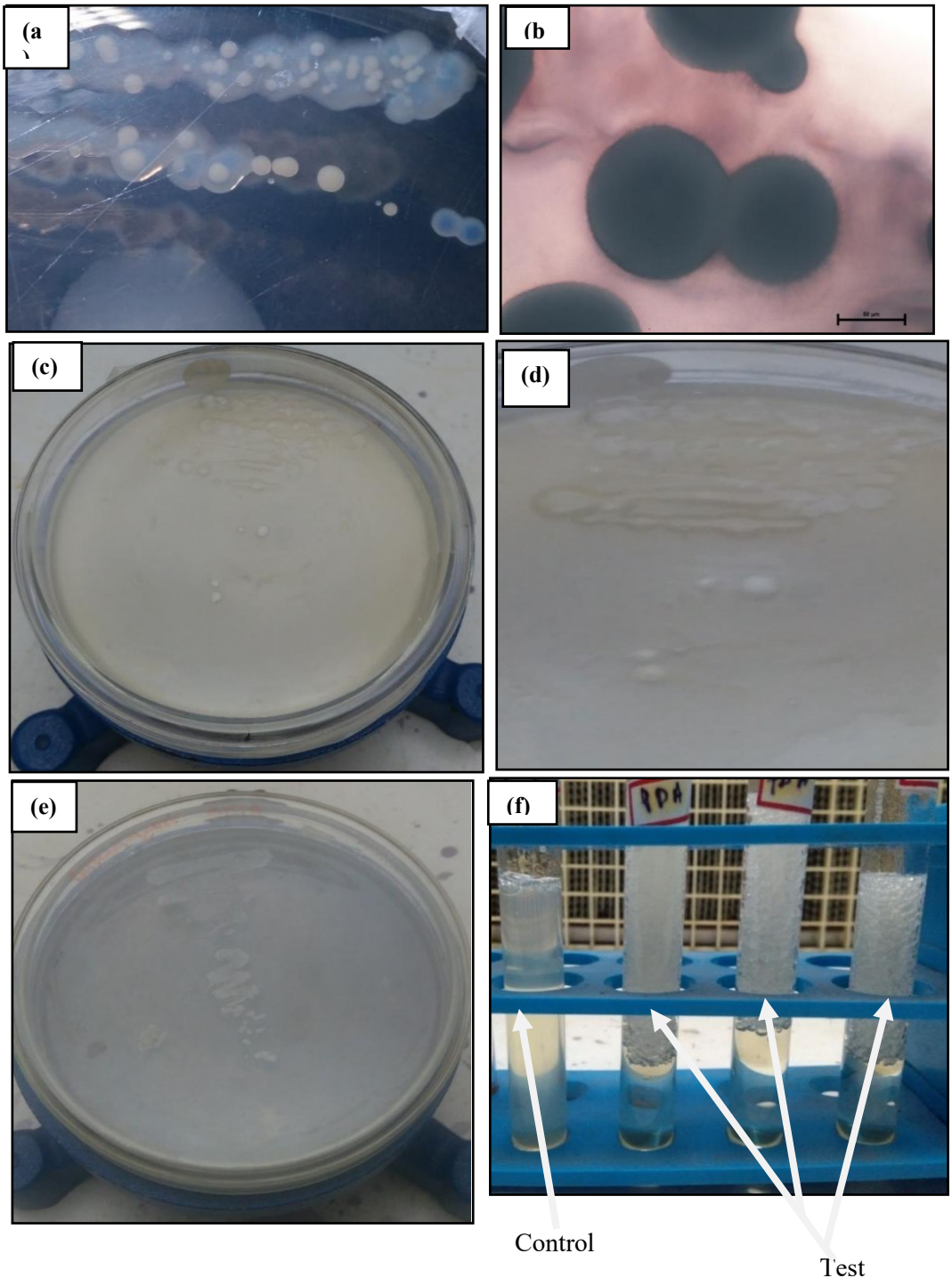


Figure.3.13: Yeast isolate Bchlm-1-2 (promising for biosedimentation) (a) showing strong dye absorption (b) colony turned blue (c) acid (b) crystals (d). (f) Strain showed huge gas bubble formation with H_2o

3.2.5.2 Phosphate solubilization efficiency

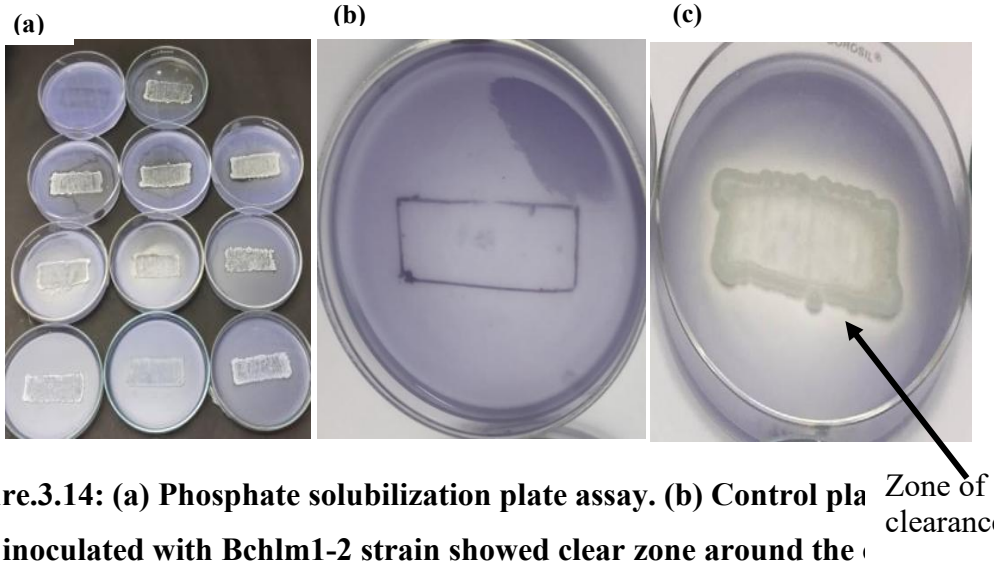


Figure.3.14: (a) Phosphate solubilization plate assay. (b) Control pla
inoculated with Bchlm1-2 strain showed clear zone around the

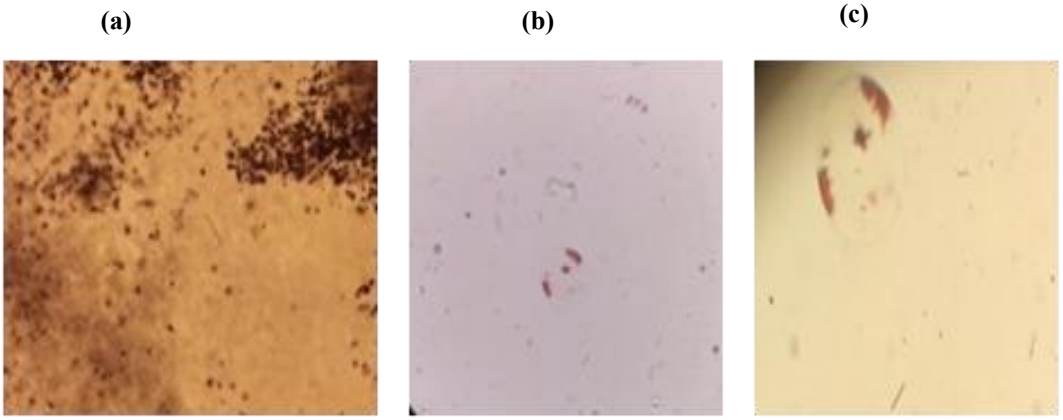


Figure.3.15: Inoculation plate for phosphate solubilization with crystal of calcium oxlate

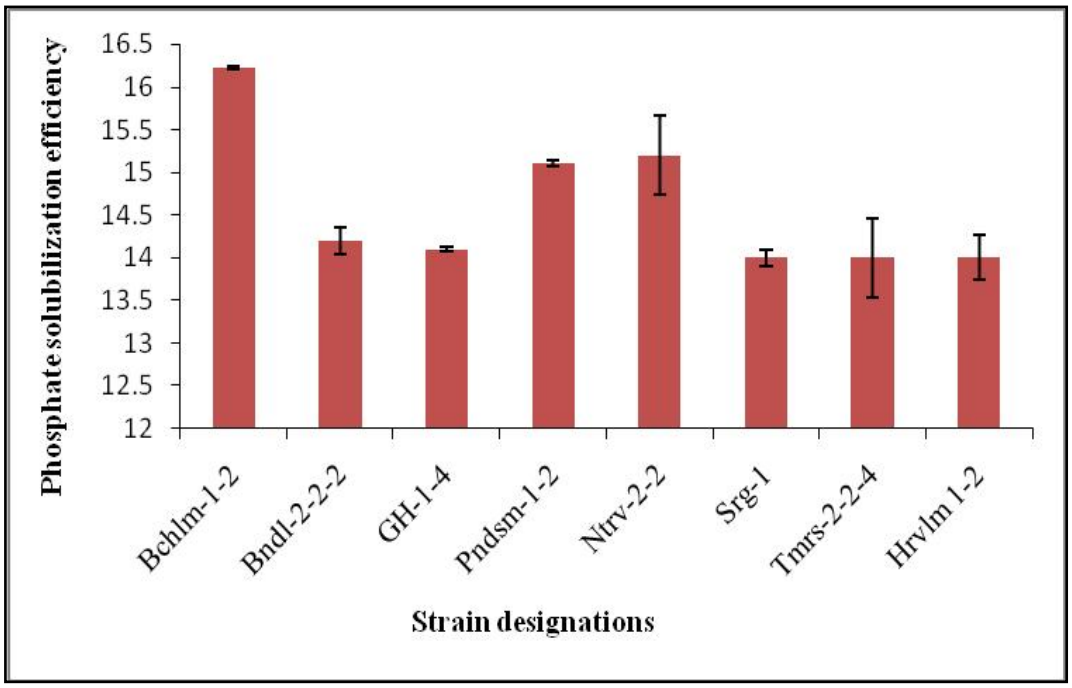


Figure.3.16: Phosphate solubilization efficiency

Five strains with high scores were discovered. Among all studied strains, the Bchlm1-2 strain received a perfect score. Furthermore, the 150 mm^2 area surrounding each implanted rectangular biofilm was more transparent than the rest of the petriplate, owing to the high oxalic acid concentration along the boundary. The chemogradient of oxalic acid decreases as one moves away from the biofilm border.

The Pnd-3 strain scored poorly in terms of phosphate solubilization. The quantification provides a more consistent metric for comparing and selecting multiple strains.

3.2.5.3 Surface charge test

According to the results of surface charge test it is found that test yeast has a significant capacity to bind cations on its surface. In comparison to commercial baker yeast, test yeast showed a strong affinity with alcian blue dye.

Table.3.6: Absorbance of test yeast, commercially available yeast, and dye

	Absorbance			Mean
Yeast+Alcianblue dye Supernatant	0.002	0.002	0.002	0.002
Sachromyces cereveciae+Alcian blue dye supernatant	0.004	0.004	0.004	0.004
Alcian blue dye	0.009	0.009	0.009	0.009

ABR= (AABsolution-Asupernatant) X 100 / AABsolution

ABR of test yeast = $\frac{(0.009-0.002)\ X100}{0.009}$

$=77.77$

ABR of Baker yeast = $\frac{(0.009-0.004)\ X100}{0.009}$

$= 55.55$

3.2.5.4 Hydrophobicity test

Baker yeast had a greater affinity with hexadecane, ethyl acetate, and chloroform. Moreover, the test strain's affinity for decane proved to be strong, it might be as culture was treated in buffer. Standard deviations of all replicates were negligible.

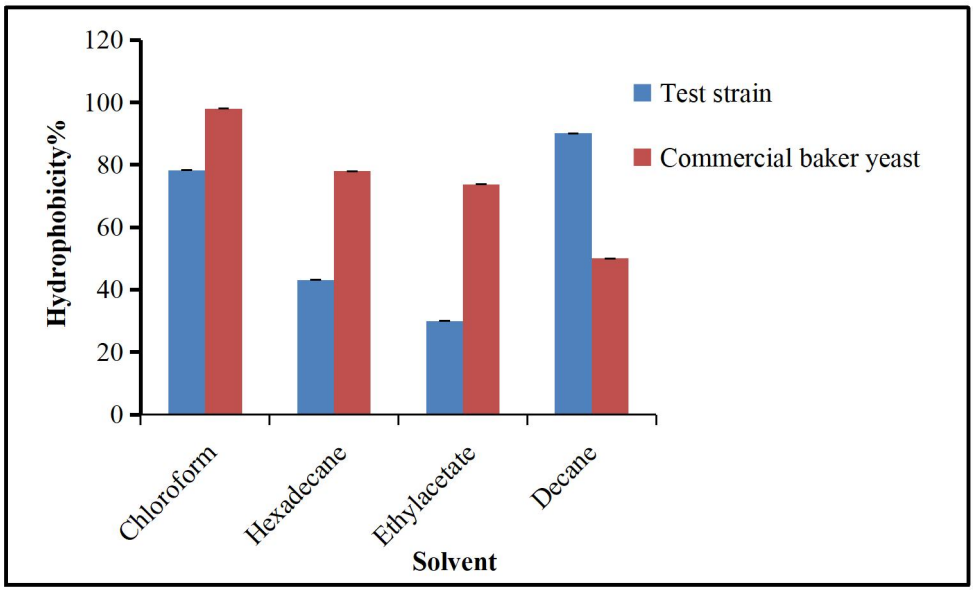


Figure.3.17: Hydrophobicity percentage of yeasts

3.2.5.5 Test for morphological stability of selected culture

The test culture turned black in the modified media, (prepared with mine tailing soil and agar), possibly due to metal adsorption, but it turned white again when restreaked on the nutrient-rich (MEA 2%) media. After seven days, colonies of both plate, MEA media and modified mine tailing soil medium turned slimy to protect themselves from infection and starvation. As shown in fig.3.18.

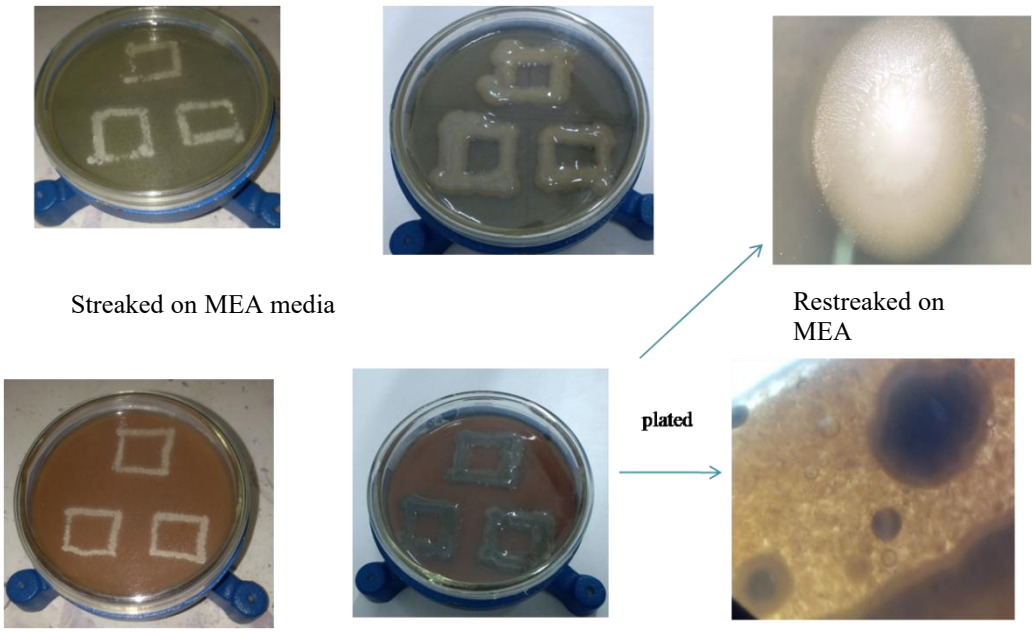


Figure.3.18: Bchlm1-2 strain grown on MEA and modified media

3.3. Biosedimentaion efficiency test

The bioefficiency scores of selected strains is presented here as discussed in chapter two (table 2.25). Highest score is obtained by strain Bclm-1-2.

Table 3.7 Biosedimentation efficiency test score

Strain designation	score
Bchlm-1-2	280
GH-1-1	105
Pnd-2	130
Pnd-2	130
Pnd-3	105
Pndsm-2-2	130
Tmrs-2-2-4	160

3.3.1 Fractality Index of selected strains

The results of Fractality Index of the colony margin (premiliary study) is presented in Fig.3.21, Further Fractality Index of best five strains, studied after slightly modification in method as showed in fig. 3.23 is presented. Strain designated as Bchlm-1-2 showed highest Fractality Index of colony margin (Fig 3.21;3.23)

3.3.2 Fractality Index of sediment

Strain Bchlm-1-2 showed highest Fractality Index of sediment (Fig. 3.22) formed by binding the suspended solid and yeast.

3.3.3 Preliminary study of Fractality Index of colony margin

A preliminary analysis of the fractality Index of the thirteen strains (as discussed in Fig.3. 21 and Fig 3.23) revealed that the inner margins have a large fractality Index compared to outer edges. Besides Fractal dimension of colony margins the F.I. of the sediment is showed highest by same culture, designated as Bchlm-1-2. (Fig 3.22 and 3.24).

Moreover, negligible deviation was observed in the Fractal dimension of the sediment formed by yeast and respective suspended solids. (Fig.3.22 and 3.24). Almost similar Fractality Index of sediment in the every replicates reveals, sediment structure were same, formed by specific culture and its respective clay.

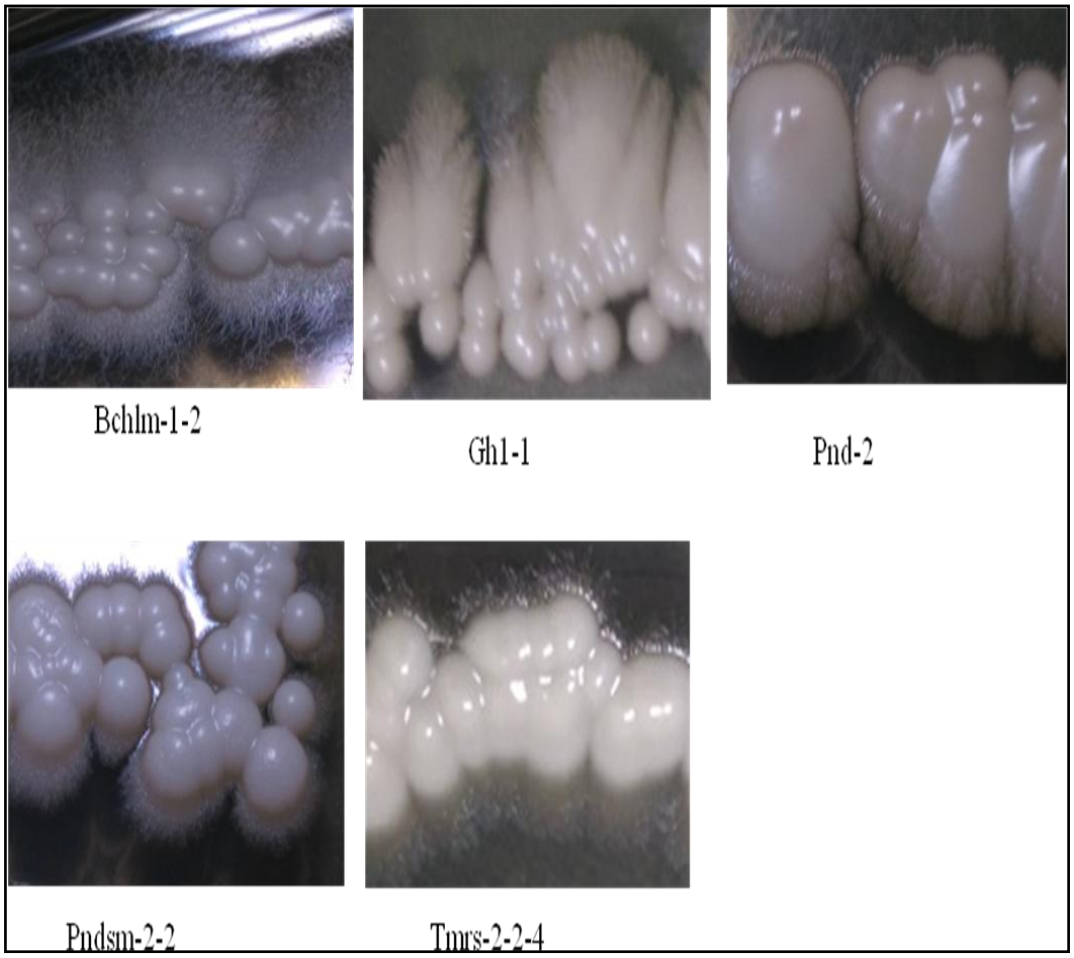


Figure. 3.19: The complex margins of yeast strains

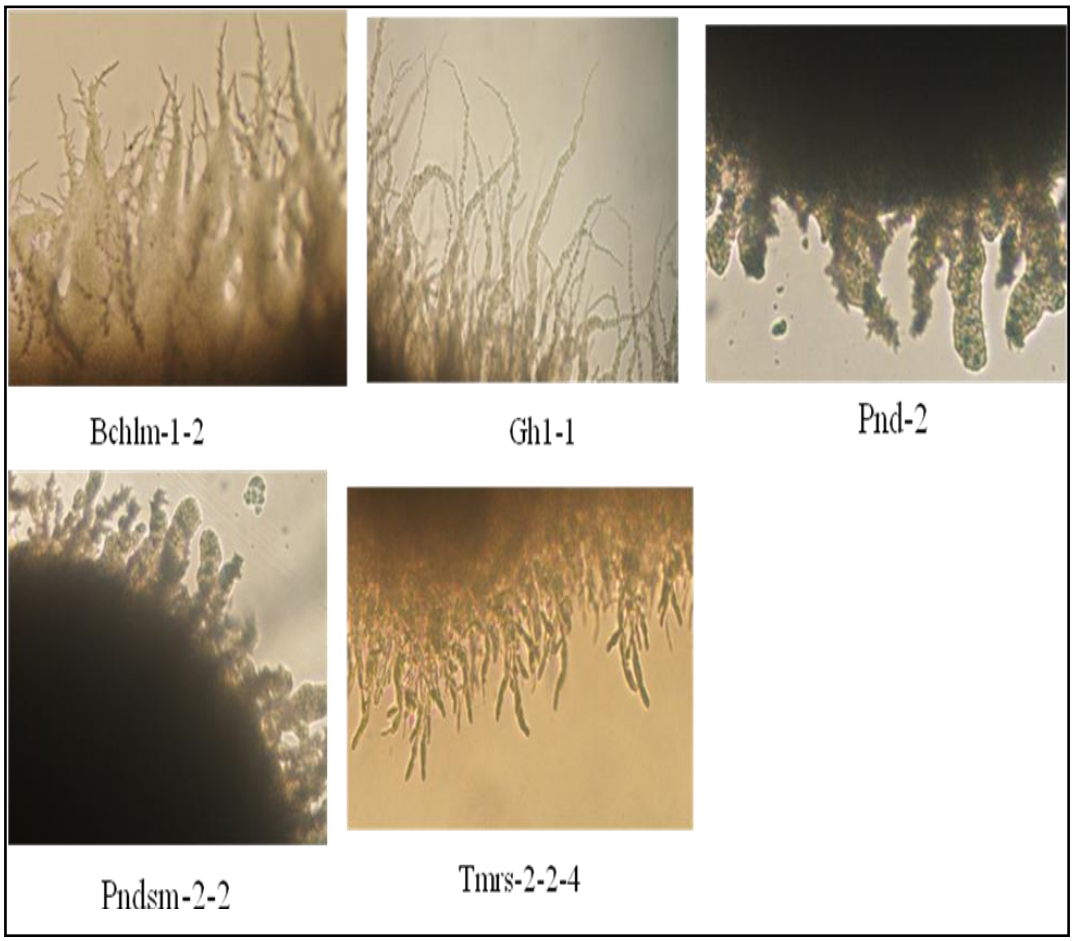


Figure. 3.20: The fractal margins of yeast strains under 100x magnification

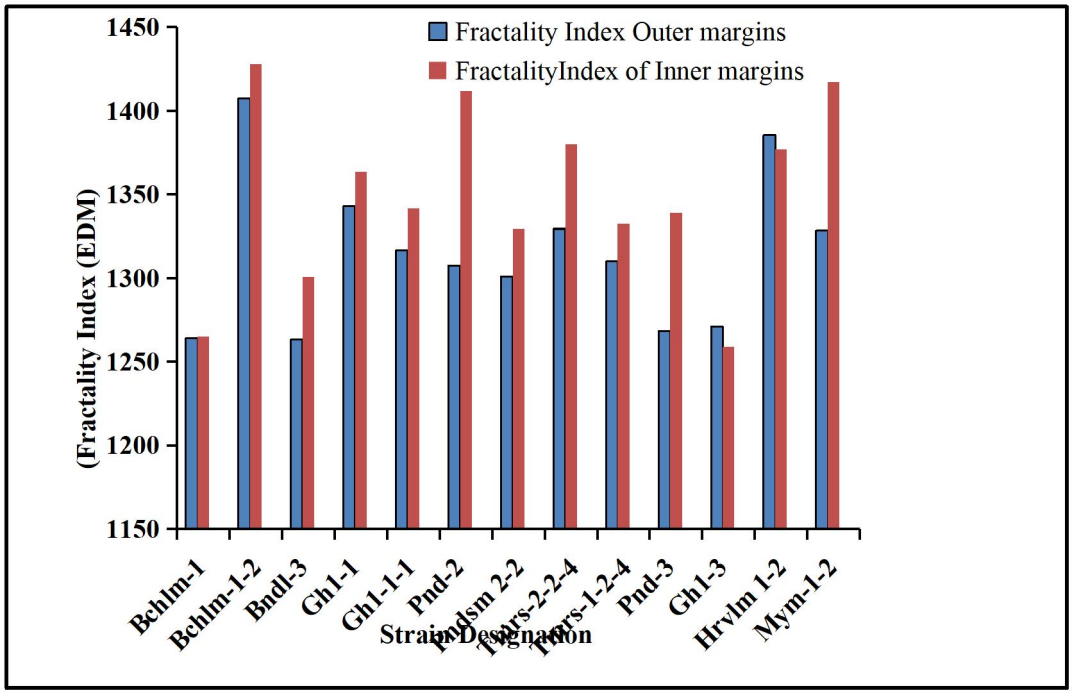


Figure.3.21: Fractality index of strains' inner and outer margins

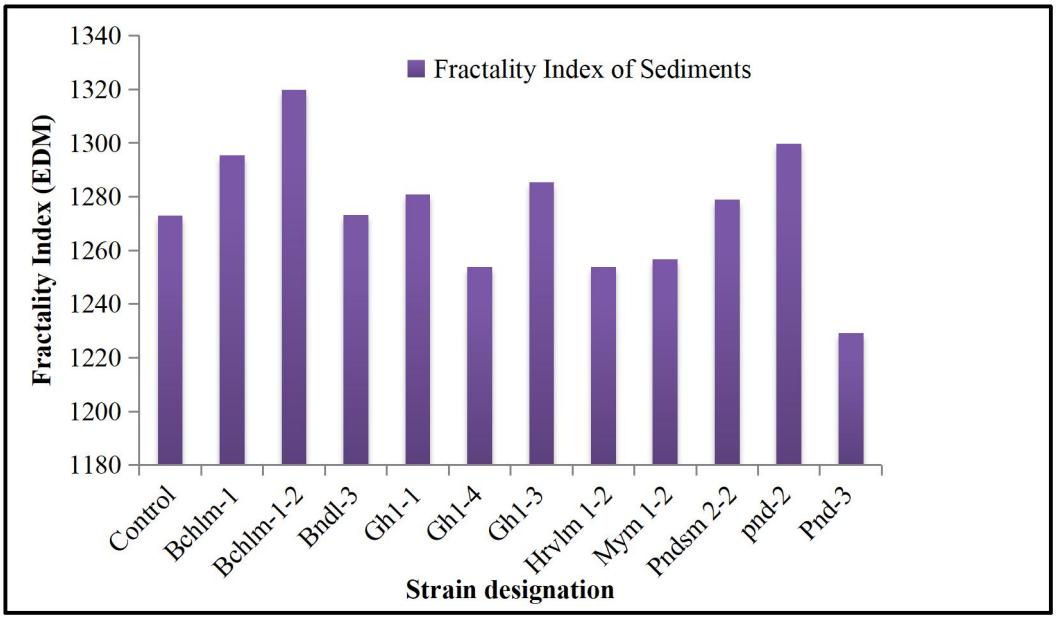


Figure.3.22: Fractality Index of sediments

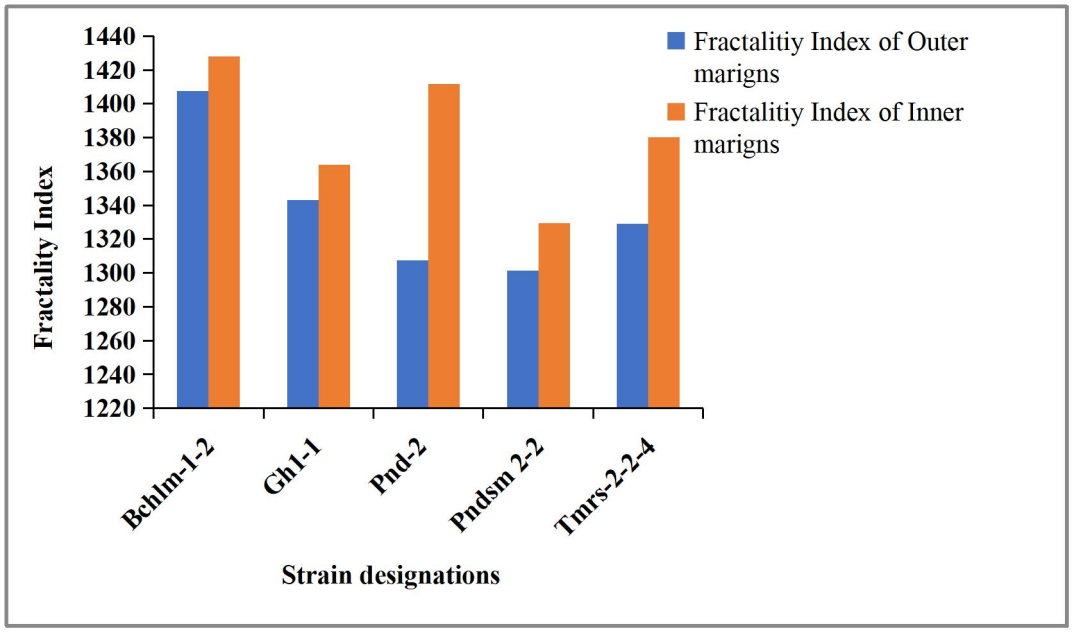


Figure3.23: Fractality Index of inner and outer margins

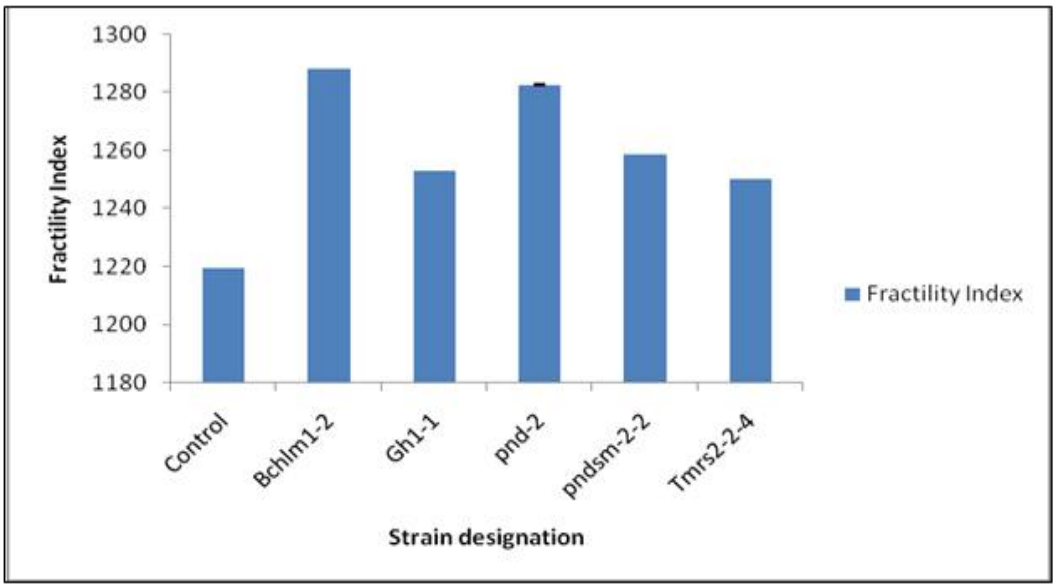


Figure.3.24: Fractiliity lndex of sediments

3.3.4 Complex colony margins

Colony margins in cultures were complicated. The colonies of representative cultures are shown in Fig.3.25. The inner borders of all strains were more complex than the outer margins. Each colony has a unique pattern of fractality structure. The micromorphology (under 100x magnification) of selected cultures were showed unique surface morphology, in detail, photo among all cultures with complex colony borders showed in (Fig.3.25).

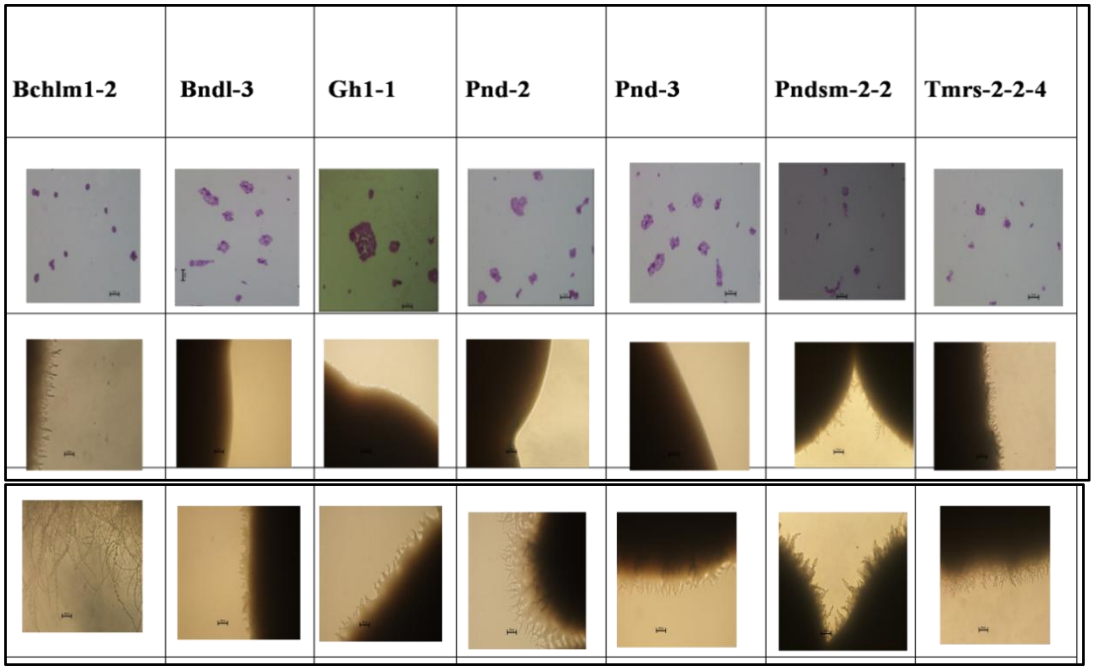


Figure 3.25: Outer inner margins of yeasts strains

3.3.4.1 Morphology of sediments

With clay colloids, all tested cultures formed solid sediment (Fig.3.26). Sediment showed compactness, compared to control sediment. Furthermore, among all the strains tested, Bchlm-1-2 exhibits compact sediment as it remained unchanged after disturbing the settled sediment (under40x magnification).

Control	
Bchlm1-2	
Bndl-3	
Gh1-1	
Pnd-2	
Pnd-3	
Pndsm-2-2	
Tmrs-2-2-4	

Figure.3.26: Micromorphology of sediments

The amplified picture of yeast strains taken on 6th day by 10x objective lens is shown in Fig.3.19 to show cause the fractal margins of the five strain designations. Micromorphology of colonies margins shows variation (Fig.3.20) in the colony margin among the cultures. The observations for different strains were as follows:

1. Strain designated as Bchlm-1-2 shows high growth rate of the complexity of colony margins and complexity on its edges within 24 hr of inoculation. Moreover, this strain shows more complex growth of margins with respect to time.
2. Strain designated as Gh1-1 shows less complexity on the surface of colony margin on the second day. However, its colony margin shows very complex structure after 3 days.
3. Strain designated as Pndsm-2-2 showed smooth edges at the beginning but after three days it exhibits complex growth of colony margins with respect to time.
4. Strain designated as Pnd-2 showed slow growth rate of its complexity of margins and after three days we observe irregular and complex colonies with increasing complexity with respect to time.
5. The cultures Tmrs-2-2-4 shows complex colony edges within two days.

Table.3.6 Fractality Index of colony margins at different time interval

Day	**Strains designation**	Peripheral		Middle		Centre	
		Outer	Inner	Outer	Inner	Outer	Inner
2	Bchlm-1-2	1194	1192	1194	1225	1224	1281
	Gh1-1	1185	1184	1186	1181	1185	1212
	Pnd-2	1191	1186	1207	1219	1184	1238
	Pndsm-2-2	1184	1191	1183	1190	1177	1186
	Tmrs-2-2-4	1174	1180	1178	1190	1054	1179
4	Bchlm-1-2	1287	1309	1302	1309	1300	1311
	Gh1-1	1191	1231	1227	1231	1262	1205
	Pnd-2	1213	1179	1226	1289	1231	1226
	Pndsm-2-2	1216	1235	1230	1249	1235	1311
	Tmrs-2-2-4	1182	1187	1180	1258	1207	1183
6	Bchlm-1-2	1410	1422	1357	1408	1425	1397
	Gh1-1	1304	1268	1288	1230	1280	1326
	Pnd-2	1204	1231	1242	1278	1221	1256
	Pndsm-2-2	1193	1215	1305	1216	1269	1353
	Tmrs-2-2-4	1247	1281	1269	1366	1331	1277

3.3.5 Colony margins

The colony border of the most promising culture had a complex shape. As shown in fig. 3.27, the inner layer of the colony margin's was more complicated than the outer layer under 1000X magnification.

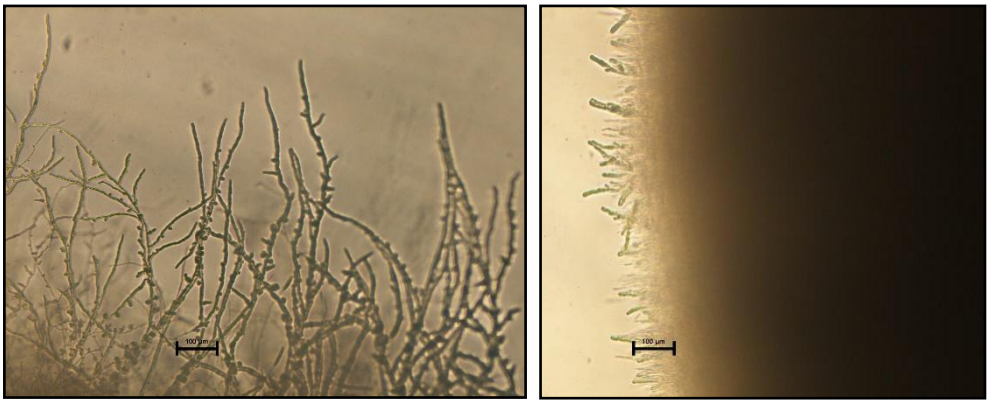


Figure. 3.27: (a) Inner (b) outer layer of promising yeast's colony margin

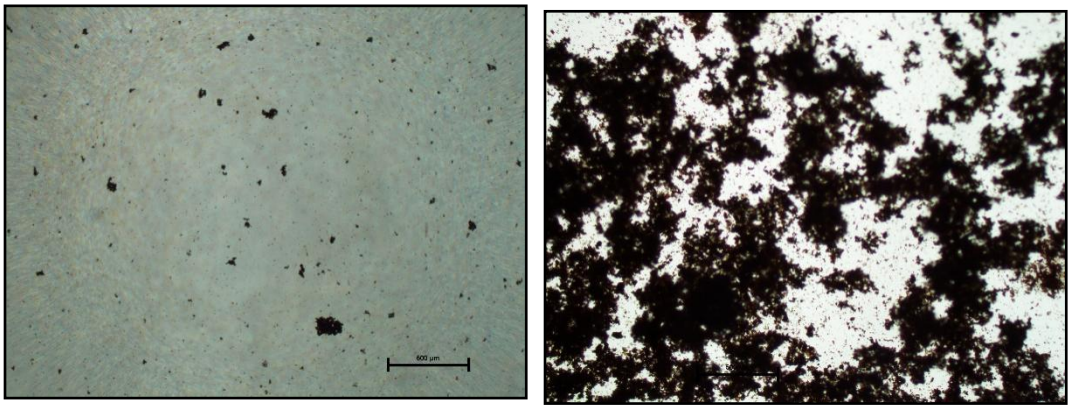


Figure 3.28: (c) Pinch of sediments of control tube (d) Pinch of sediments mixed with yeast

3.3.6. Particle size analysis of the test and control sediments

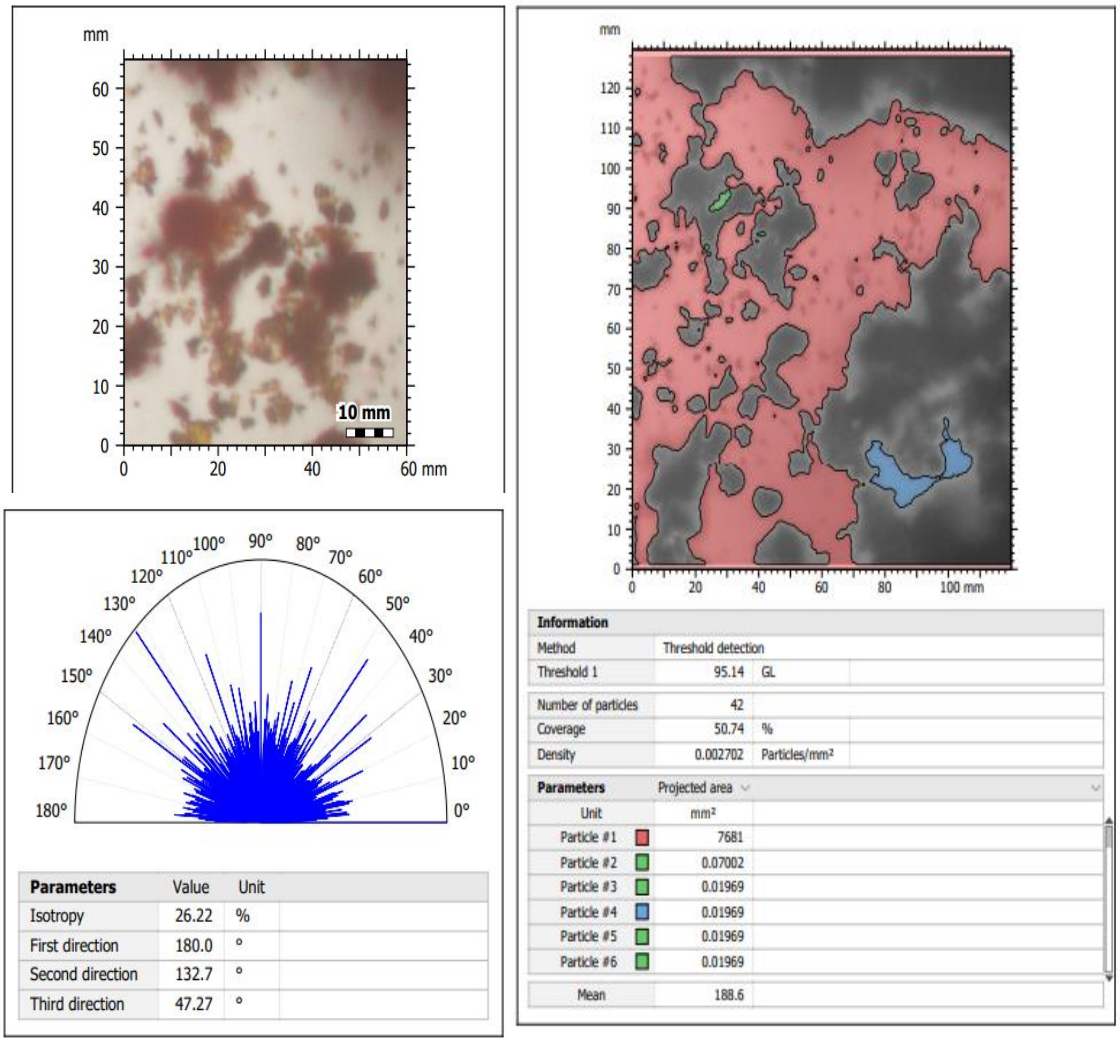


Figure. 3.29.: Particle size analysis of control sediments

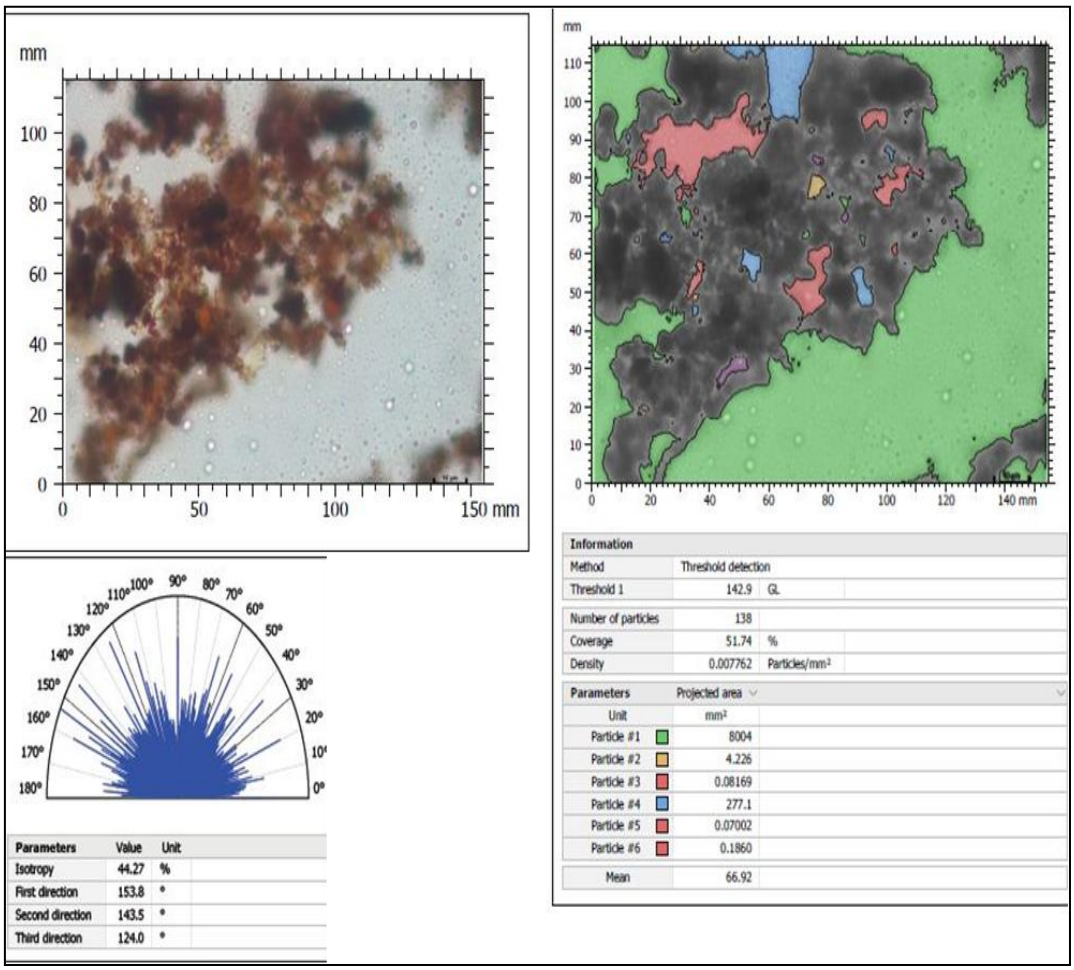


Figure. 3.30: Particle size analysis of test sediments

3.4. Control sediment, test sediment and yeast

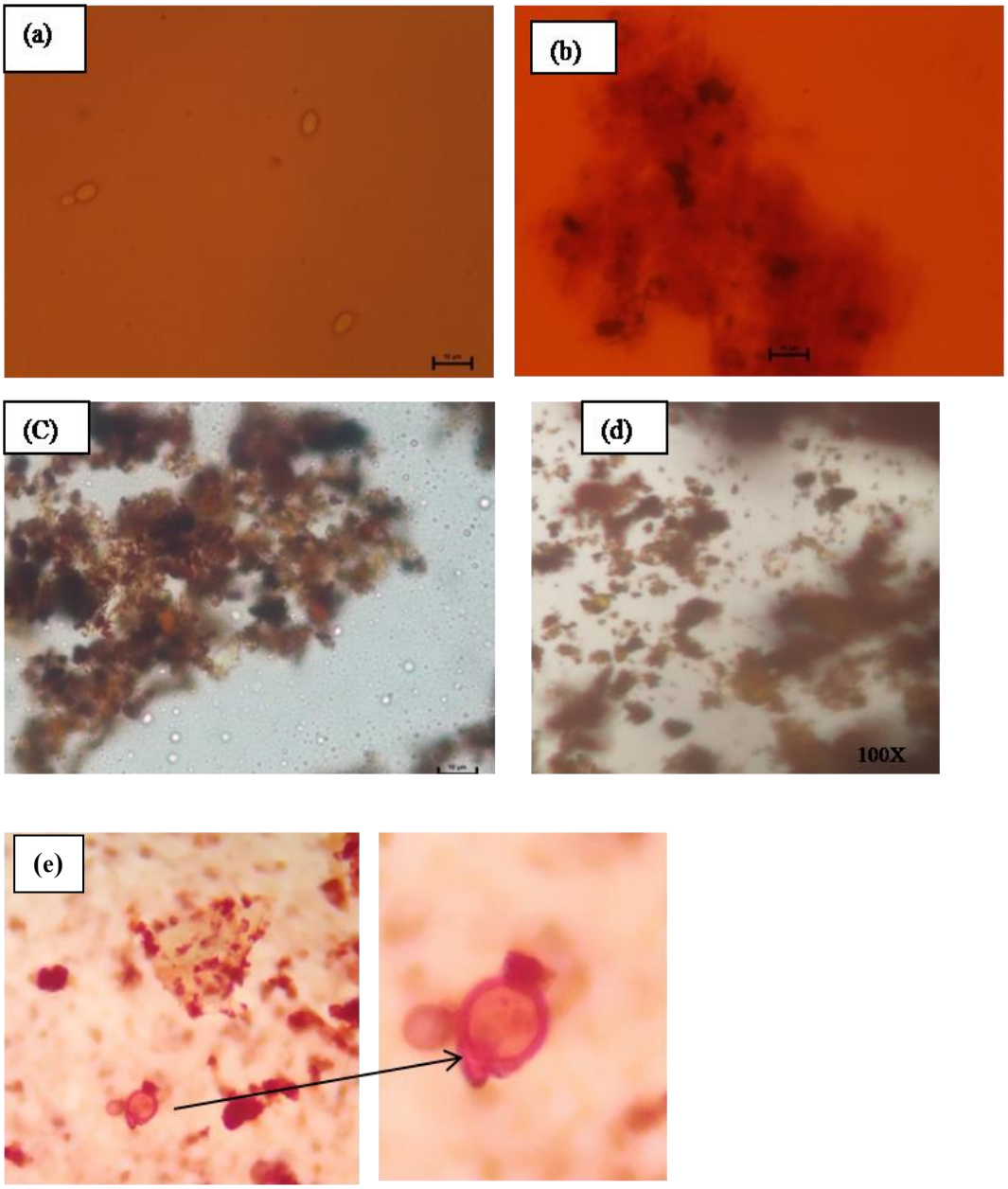


Figure.3:31 (a) Micromorphology of yeast cells (b) sediment mixed with yeast cells stained in Congo red (c).Test sediment (d) Control sediment mounted in DPX Sediment of Kaolin clay and yeast, under100X objective lens, stained in Congo red.

3.5.1 Water quality in mining region of Goa

Table.3.7 Water quality parameters of samples, mining areas, non monsoon season

Parameter (Average)	Arvalem water	Mayem lake	Morlem tank	Morlem spring	Bicholim tank	Pissrullum Pond
Turbidity (NTU)	**26** ±0.57	**9**±0.1	**10**±1	**15**±0	**22**±1.547	**22**±0.230
TDS	29±	31±1.15	76±1.527	77±1.15	40±0	40±0
EC µs	50±0	55±0	105±5.291	113±2.156	90±5.506	90±0.057
pH	7.2 ±0	6.8±1.73	7.4±1.09	7.2±0	7.4±0.05	6.87±0.0
Temp	28 ±0	29.7±0.1	27±0	27±0	28.9±1.6	26.9
D.O	7.25±0	7±0	7.9±0	7.2±0	7.2±0	7.1±0
Fe	**0.6542**	**0.8056**	**1.4812**	-	-	**0.560**
Mn	**0.5915**	**0.6460**	**0.6565**	**0.7262**	-	**0.898**
K	0.77	1.7	1.4	1.2	1.3	1.7
Na	7	7	7	7	7	7
Cl^-	51.8±0.2	33±0.058	51.6±0.79	35.92±0.4	33±0.208	44±0.1
NO_3^-	0.29±0.16	0.65±0.001	0.129±0.001	0.65±0.001	0.55±0.01	0.54±0.01
PO_4^{-3}	0.0176	0.0917	0.0787	0.0398	0.07863	0.04095
SO_4^{-2}	0.2±0	0.7±0.03	0.7±0.053	0.1±0.001	0.57±0.0	0.24±0.08
Alkalinity	62.33±1	68.3±1.5	88±3.6	96±2.65	86.33±2.	86.66±2.
Total Hardness	68±1.7	48.66±3.5	129.66±0.5	119.66±2.	111.66±2	112±1.73

*Temp=Temperature ,ºC All values are in mg/L except temperature, electrical conductivity,pH

Table. 3.8 Water quality parameters in mining areas in monsoon season

Parameter (Average)	Arvalem water fall	Mayem lake	Morlem tank	Morlem spring	Bicholim tank	Pissurlum Pond
Turbidity (NTU)	**18±0.577**	**8±0.1**	**12±0.577**	**23±2**	**20±0.1**	**18±0.577**
TDS ppm	28±1.12	30±1.154	76±0	77±0	40±0.577	40±0
EC µs	49±0.577	53±1.527	105±0.57	113±1	90±0.577	89±0.57
pH	7.2±0.057	6.7±0.05	7.4±0.057	7.2±0.05	7.4±0.1	6.87±0.5
Temp(°C)	27±0.57	29.7±0.1	27±0.058	27±0	28±0	26±0.57
DO	6.4±0.252	6.9±0.25	7.1±0	7.25±0.1	7.2±0.58	7±0.116
K	0.72±	0.7±0	0.74±	0.73±0	0.77±0	0.77±0
Na	7±0	7±0	8±0	8±0	6.9±0	7±0
Cl^-	54.9±0.23	30±0.088	50.31±0.6	40.7±0	45±0.17	44±0.08
NO_3^-	0.56±0.00	0.85±0.0	0.28±0.00	0.56±0.0	0.54±0.0	0.53±0.0
PO_4^{-3}	0.02±0	0.02±0	0.01±0	0.01±0	0.01±0.0	0.01±0
SO_4^{-2}	1.6±0.1	1.5±0.06	0.9±0	0.9±0	0.87±0.0	0.8±0.06
Alkalinity	62±0	60.33±1.	96±3.61	89±1	83.33±.1	86.66± 2
Total Hardness	59±1.185	48±2.645	130.66±0.08	122.3±0.52	111.66±02	112.09±2

*Temp=Temperature ,ºC All values are in mg/L except temperature, electrical conductivity,pH

3.5. Correlation coefficients among various water quality parameters

Table. 3.9 Arvalem waterfall (Non monsoon)

	Turbidity (NTU)	TDS ppm	EC µs	pH	Temp (°C)	DO	NO_3^-	PO_4 -	SO_4	Alkalinity	Total Hardness
Turbidity (NTU)	1	0	0	0	0	0.27	-0.5	-1	0	0.18	-1
TDS ppm		1	0.11	0	0	0	-0.32	0.46	0.9	0	0.9
EC µs			1	0	0	0	0.69	1	0.5	0	0.5
pH				1	1	0	-0.69	-1	-0.5	0	-0.5
Temp (°C)					1	0.69	1	0.5	0	-0.94	0.5
DO						1	0.69	-0.28	0	-0.89	-0.27
NO_3^-							1	0.5	0	-0.94	0.5
PO_4								1	0	1	1
SO_4^{-2}									1	0	0
Alkalinity										1	-0.18
Total Hardness											1

*Temp=Temperature All values are in mg/L except temperature, electrical conductivity, pH

Table.3.10 Arvalem water fall (Monsoon)

	Turbidity (NTU)	TDS	ECµs	pH	Temp	DO	NO_3^-	PO_4	SO_4^-	Alkalinity	Total Hardness
Turbidity (NTU)	1	-0.9	-0.5	0.5	-0.5	-0.46	0.11	0	0.44	0	-0.02
TDS		1	0.46	-0.46	0.46	0	0	0	0	0	0
EC µs			1	-1	1	0.43	-0.72	0	0.98	0	0.78
pH			1	1	-1	0	0	0	0	0	0
Temp					1	0	0	0	0	0	0
DO						1	-0.94	0	0.59	0	0.9
NO_3^-							1	0	-0.83	0	-0.9
PO_4^{-3}								1	0	0	0
SO_4^{-2}									1	0	0.8
Alkalinity										1	0
Total Hardness											1

*Temp=Temperature All values are in mg/L except temperature, electrical conductivity, pH

Table.3.11 Morlem pond (Non monsoon)

	Turbidity (NTU)	TDS ppm	EC µs	pH	Temp	DO	NO_3^-	PO_4^{-3}	SO_4^{-2}	Alkalinity	Total Hardness
Turbidity (NTU)	1	0	0	0	0	0	0	0	0	0	0
TDS	0	1	-0.12	0	0	-0.93	-0.5	0.9	0.37	0.32	-0.69
EC µs			1	0	0	0.273	0.92	0.024	-0.88	-0.98	0.8
pH				1	0	0	0	0	0	0	0
Temp (°C)					1	0	0	0	0	0	0
DO						1	0.13	-0.95	0.7	0.7	0.06
NO_3^-							1	0	-0.42	-0.6	-0.98
PO_4^{-3}								1	-0.4	0.24	0.240
SO_4^{-2}									1	0.35	0.59
Alkalinity										1	0.96
Total Hardness											1

*Temp=Temperature ºC, All values are in mg/L except temperature, electrical conductivity, pH

Table.3.12 Morlem pond (Monsoon)

	Turbidity (NTU)	TDS	EC µs	pH	Temp (°C)	DO	NO_3^-	PO_4^{-3}	SO_4^{-2}	Alkalinity	Total Hardness
Turbidity (NTU)	1	0	0	0.86	0	0.86	1	0	0	1	-0.3
TDS			1	-	-	-	-	-	-	-	-
EC µs				-	-	-	-		-	--	-
Temp (°C)					1	0.5	0.86	0	0	0.87	0.18
DO						1	0.86	0	0	0.87	0.86
NO_3^-							1	0	0	1	-0.32
PO_4^{-3}								0	0	0	0
SO_4^{-2}									0	0	0
Alkalinity										1	-0.32
Total Hardness											1

*Temp=Temperature ºC All values are in mg/L except temperature, electrical conductivity, pH

Table 3.13 Morlem tank (Non monsoon)

	Turbidity (NTU)	TDS	EC µs	pH	Temp	DO	NO_3^-	PO_4^{-3}	SO_4^{-2}	Alkalinity	Total Hardness
Turbidity (NTU)	1	0	0.5	0	0	0	0.18	-0.5	0	0	-0.24
TDS		1	0	0	0	0	0	-0.5	0	0	0
EC µs			1	0	0	-0.95	0.5	0	0	0	0.97
pH				1	0	-0.95	0.5	0	0	0	0
Tem (°C)					1	0	0	0	0	0	0
DO						1	0.76	0	0	-0.9	0
NO_3^-							1	0	0	0	0.96
PO_4^{-3}								1	0	0	0
SO_4^{-2}										0	1
Alkalinity										1	0
Total Hardness											1

*Temp=Temperature ºC All values are in mg/L except temperature, electrical conductivity,

Table 3.14 Morlem tank (monsoon)

	Turbidity (NTU)	TDS ppm	EC µs	pH	Temp (ºC)	DO	NO_3^-	PO_4^{-3}	SO_4^{-2}	Alkalinity	Total Harness
Turbidity (NTU)	1	-0.98	-0.76	0	0	0.66	0	0.9	-0.87	-0.27	0
TDS		1	0.62	-3.80	0	-0.79	0.19	0.94	-0.67	0.45	0.18
EC µs			1	0	0	-2.19	-0.66	0.33	-0.99	-0.41	-0.65
pH			0	1	0	-9.5	1.96	-7.85	-2.03	0	-2.
Temp (ºC)					1	0	0	0	0	0	0
DO						1	-0.76	-0.95	-0.91	-0.91	-0.76
NO_3^-							1	0.5	0.59	0.91	1
PO_4^{-3}								1	-0.40	0.34	0.5
SO_4^{-2}									1	0.35	0.59
Alkalinity										1	0.96
Total Hardness											1

*Temp=Temperature ºC All values are in mg/L except temperature, electrical conductivity,

Table.3.15 Mayem lake (Non Monsoon)

	Turbidity (NTU)	TDS	EC	pH	Temp	DO	NO_3^-	PO_4^{-3}	SO_4^{-2}	Alkalinity	Total Hardness
Turbidity (NTU)	1	-0.8	0	-2	-0.8	0.8	-3	0	1	0.2	-0.6
TDS		1	0.1	0.5	1	-0.8	-4	0	-1	-0.2	0.6
EC µs			1	0.9	0.18	-0.8	-4	0	-1	-0.2	0.6
pH				1	0.5	0.9	-0.5	0	0.8	-0.3	-0.9
Temp					1	0	0	0	0.8	-0.3	0
DO						1	0.	0	0.8	-0.4	0
NO_3^-							1	0	4.	0.9	0.7
PO_4^{-3}								0	0	0	0
SO_4^{-2}									1	0.1	-0.6
Alkalinity										1	0.6
Total Hardness											1

*Temp=Temperature ºC, All values are in mg/L except temperature, electrical conductivity, pH

Table.3.16: Mayem lake (Monsoon)

	Turbidity (NTU)	TDS ppm	EC µs	pH	Temp (°C)	DO	NO_3^-	PO_4^{-3}	SO_4^{-2}	Alkalinity	Total Hardness
Turbidity (NTU)	1	-1	-1	0.8	0	-0.5	-0.5	-0.8	0.5	-0.9	0.2
TDS		1	1	-0.8	0	0	0	0	0	0	0
EC µs			1	-0.8	0	-0.5	0	-0.8	0.5	-0.9	0.2
pH				1	0	0.5	0.5	0.8	-0.5	0.9	-0.3
Temp (°C)					1	-0.5	-0.5	-0.8	0.5	-0.9	0.23
DO						1	1	0.8	0.5	0.3	-0.9
NO_3^-							1	0.8	0.5	0.3	-0.9
PO_4^{-3}								1	0	-0.6	-0.6
SO_4^{-2}									1	-0.6	-0.6
Alkalinity										1	-0.03
Total Hardness											1

*Temp=Temperature All values are in mg/L except temperature, electrical conductivity,pH

Table.3.17: Bicholim tank (Non Monsoon)

	Turbidity (NTU)	TDS	EC µs	pH	Temp (°C)	DO	NO_3^-	PO_4^{-3}	SO_4^{-2}	Alkalinity	Total Hardness
Turbidity (NTU)	1										
TDS	0	1									
EC µs	0.51	0	1								
pH	0.5	0	0.11	1							
Temp (°C)	0.55	0	0.99	0.9	1						
DO	0.18	0	0.94	0.9	0.92	1					
NO_3^-	0.5	0	-0.49	-0.5	-0.4	-0.75	1				
PO_4^{-3}	0.67	0	0.97	0.97	0.98	0.84	-0.29	1			
SO_4^{-2}	0.51	0	0.7	-0.49	-0.44	-0.75	0.99	-0.28	1		
Alkalinity	0.97	0	0	0.69	0.73	0.42	0.28	0.8	0.29	1	
Total Hardness	-0.27	0	0.7	0.7	0.6	0.9	-0.97	0.5	-0.9	-0.03	1

*Temp=Temperature All values are in mg/L except temperature, electrical conductivity, pH

Table.3.18: Bicholim tank (Monsoon)

	Turbidity (NTU)	TDS ppm	EC µs	pH	Temp (°C)	DO	NO_3^-	PO_4^{-3}	SO_4^{-2}	Alkalinity	Total Hardness
Turbidity (NTU)	1	-1	-1	0.8	0	-0.5	-0.5	-0.8	0.5	0.9	0.28
TDS		1	1	-0.8	0	0	0	0	0	0	0
EC µs			1	-0.8	0	0	0	-0.8	0.5	0.97	0.2
pH				1	0	0	0.5	0.8	-0.5	0.97	-0.27
Temp (°C)					1	-0.5	-0.5	-0.8	0.5	-0.9	0.27
DO						1	1	0.8	0.5	0.27	-0.9
NO_3^-							1	0.8	0.5	0.2	-0.9
PO_4^{-3}								1	0	0.6	-0.6
SO_4^{-2}									1	0.6	-0.6
Alkalinity										1	-0.03
Total Hardness											1

Table.3.18 Pissurlem tank (Non Monsoon)

	Turbidity (NTU)	TDS ppm	EC µs	pH	Temp (°C)	DO	NO_3^-	PO_4^{-3}	SO_4^{-2}	Alkalinity	Total Hardness
Turbidity (NTU)	1	0	-0.9	0.7	0.2	0.4	-0.9	0.4	-0.3	0.1	-0.3
TDS		0	0	0	0	0	0	0	0	0	0
EC µs			1	-0.6	0	-0.2	1	0	0.4	-0.2	0.5
pH				1	0.7	0.9	-0.6	-0.22	0.3	-0.5	0.3
Temp					1	0.9	1.0	0.80	0.8	-0.9	0.8
DO						1	-0.2	-0.6	0.7	-0.8	0.6
NO_3^-							-0.2	-0.6	0.7	-0.8	0.6
PO_4^{-3}								1	-0.9	0.93	-0.9
SO_4^{-2}									1	-0.9	0.9
Alkalinity										1	-0.9
Total Hardness											1

*Temp=Temperature ,ºC All values are in mg/L except temperature, electrical conductivity,pH

Table.3.19 Pissurlem tank (Monsoon)

	Turbidity (NTU)	TDS ppm	EC µs	pH	Temp (°C)	DO	NO_3^-	PO_4^{-3}	SO_4^{-2}	Alkalinity	Total Hardness
Turbidity (NTU)	1	0	1	-1	0	0.5	-1	0	-1	-0.2	0.27
TDS		0	0	0	0	0	0	0	0	0	0
EC µs			1	-1	1	0.5	-1	0	-1	-0.2	0.27
pH				1	-1	-0.5	1	0	1	0.2	-0.9
Temp (°C)					1	0.5	-1	0	-1	-0.2	-0.2
DO						1	-0.5	0	-1	0.6	0.6
NO_3^-							1	0	1	0.2	0.27
PO_4^{-3}								0	0	0	0
SO_4^{-2}									1	0.2	1
Alkalinity										1	-.9
Total Hardness											1

*Temp=Temperature ,ºC All values are in mg/L except temperature, electrical conductivity,pH

3.6.1. Standardization of parameters for efficient biosedimentation of SMTTW

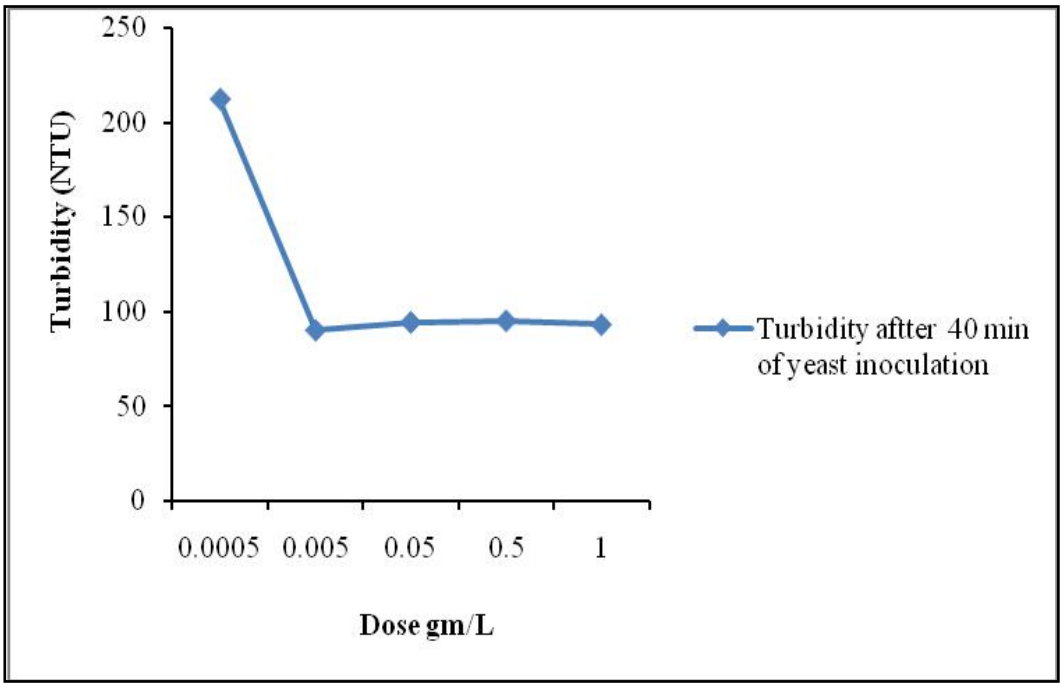


Figure 3.32: Turbidity of mine tailing water at yeast's different doses

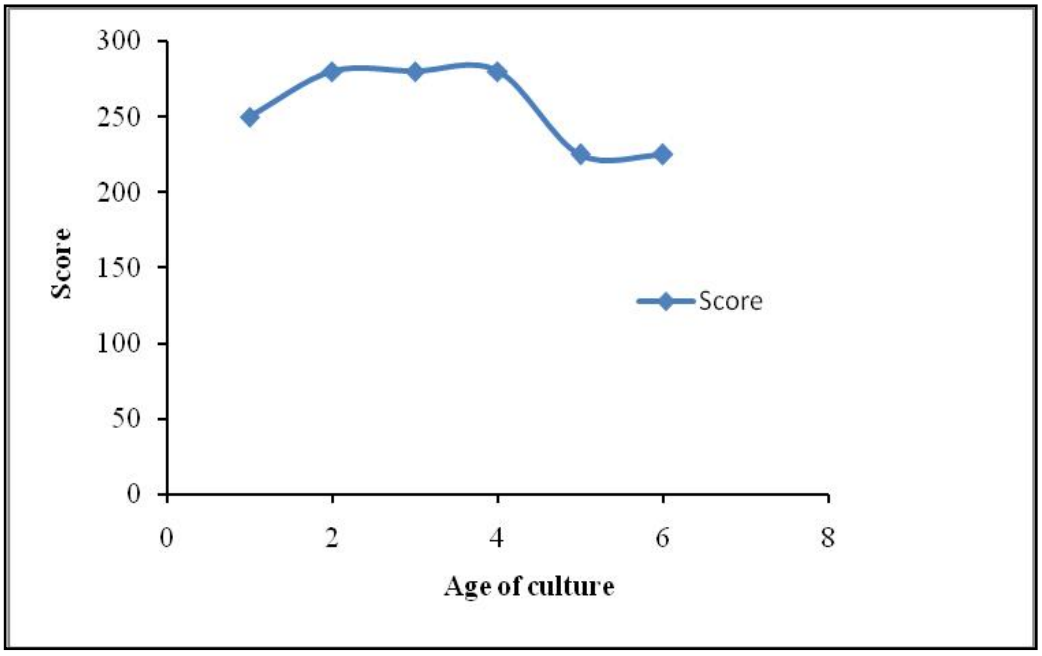


Figure.3.33: Biosedimentation efficiency score at different age of selected strain

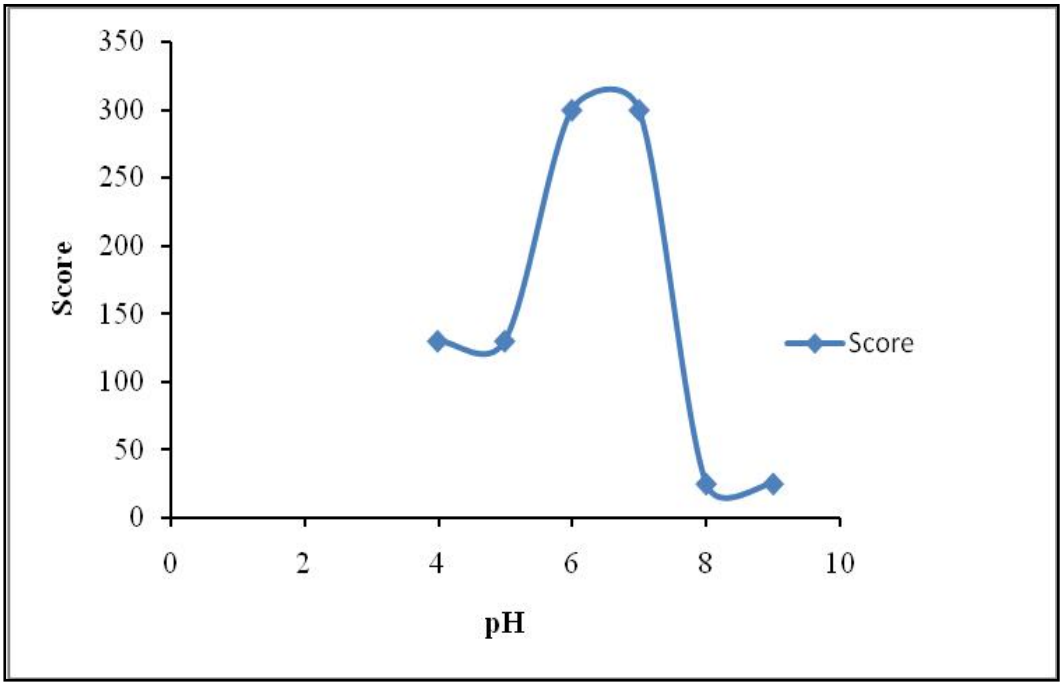


Figure.3.34: Efficiency score of biosedimentation at different pH of selected strain

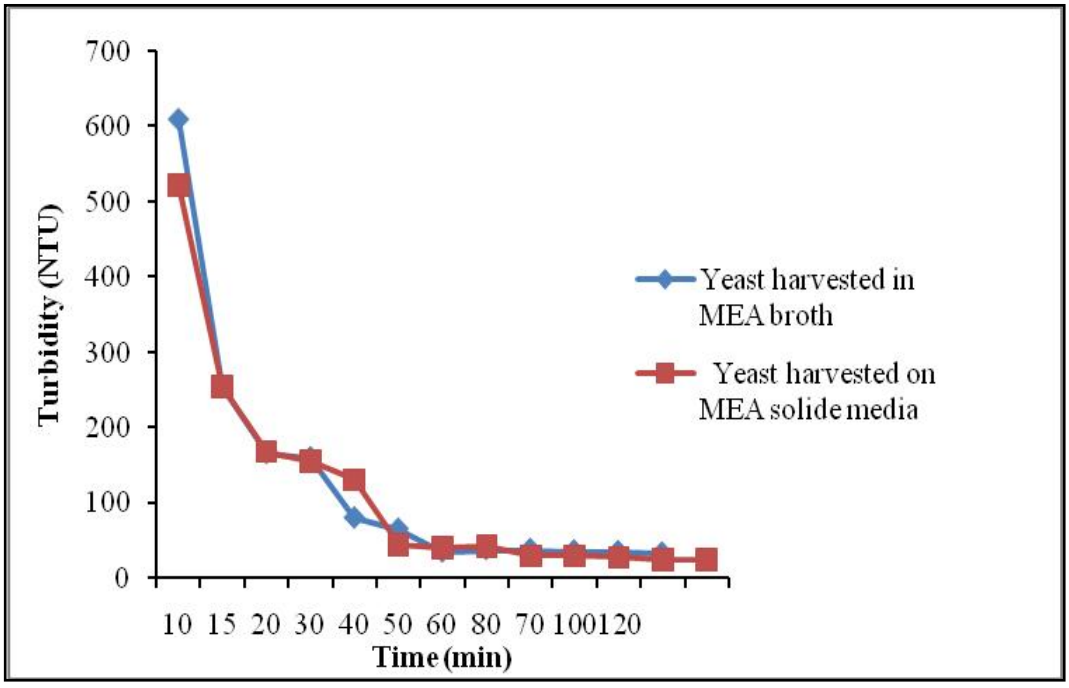


Figure.3.35: Kinetics of turbidity reduction

Biosedimentation efficiency score was highest for 4th and 5th day old culture, and biosedimentation score was highest at pH six and seven. (Fig.3.33). Moreover, appropriate dose for efficient biosedimentation is 0.005gm/L (Dry weight).

3.6.2. Biosedimentation efficiency score (BES) for floc assay after different heat treatment of strain

Table.3.20 Growth of yeast plated on MEA(a) and BES after heat treatment(b)

(a)

Treatment time (Sec)	Plate observation after 48 hr of inoculations
0	Mate growth
5	Mate growth
10	Circular white colony 200 in number
20	Colony 100 in number
30	10 colony in number
40	4 colony in number
50	No growth
60	No growth

(b)

Treatment time (Sec)	Biosedimentation efficiency score description			
	A	**B**	**C**	**Total**
0	80	100	100	280
5	50	80	80	210
10	25	80	80	185
15	25	80	80	185
30	80	100	100	180
40	50	80	100	130
50	25	80	100	180
60	25	25	100	150
Control	100	25	0	125

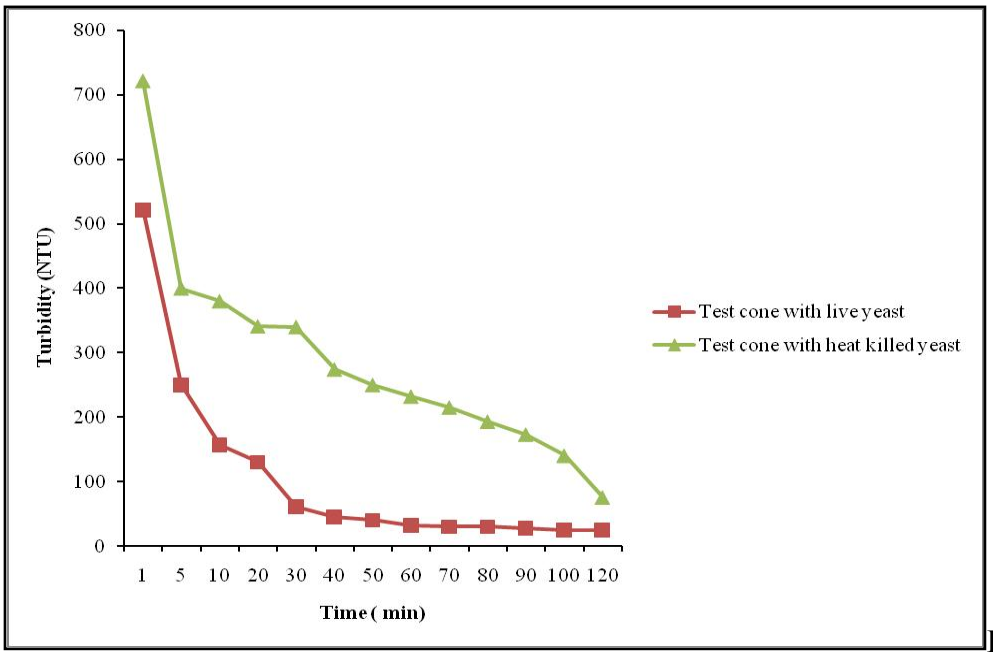


Figure.3. 36: Biosedimentation kinetics with live yeast and heat killed yeast

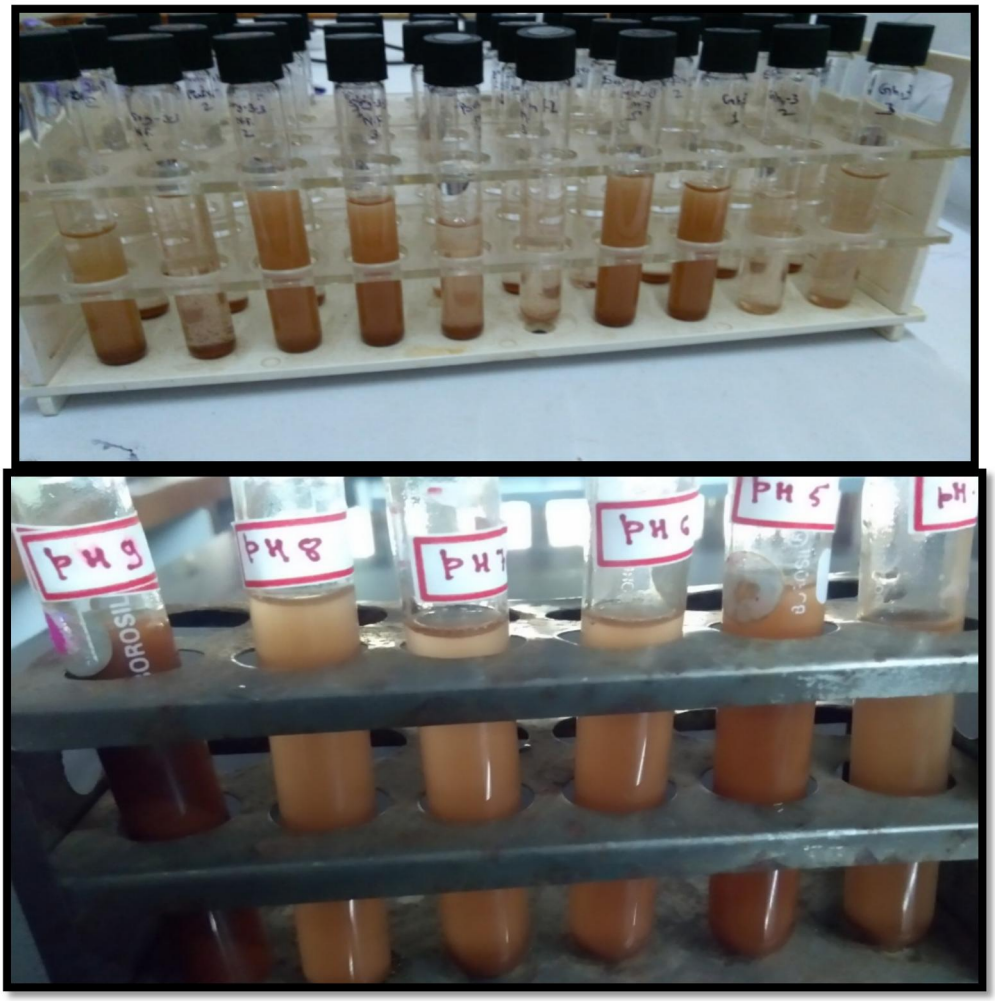
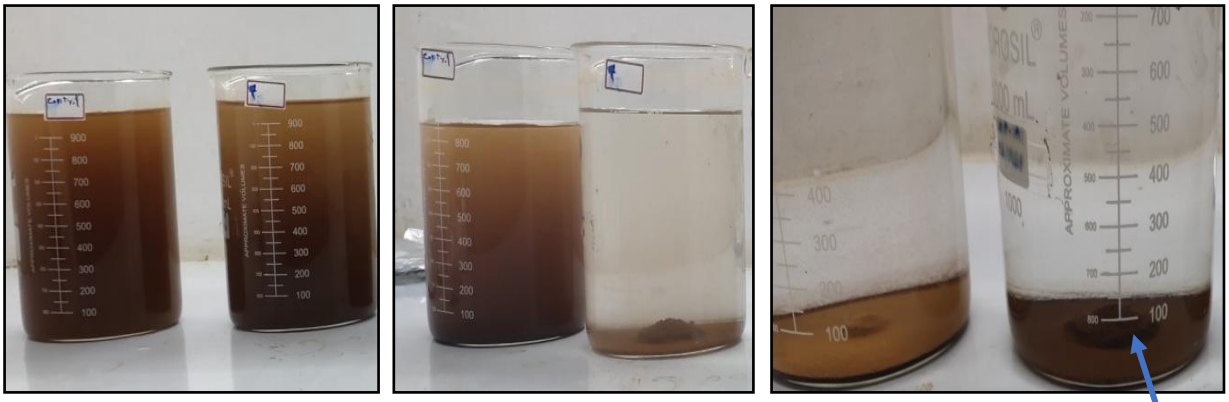


Figure. 3.37: Biosedimentation test assay



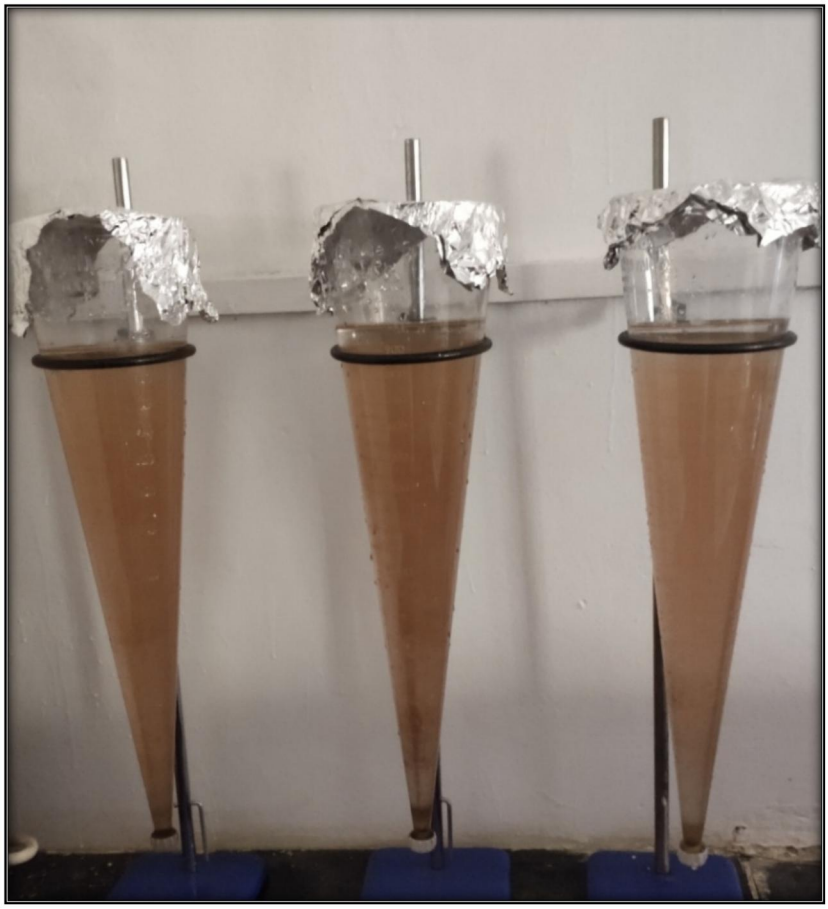


Figure. 3.38: Setup for biosedimentation test at 1 L scale

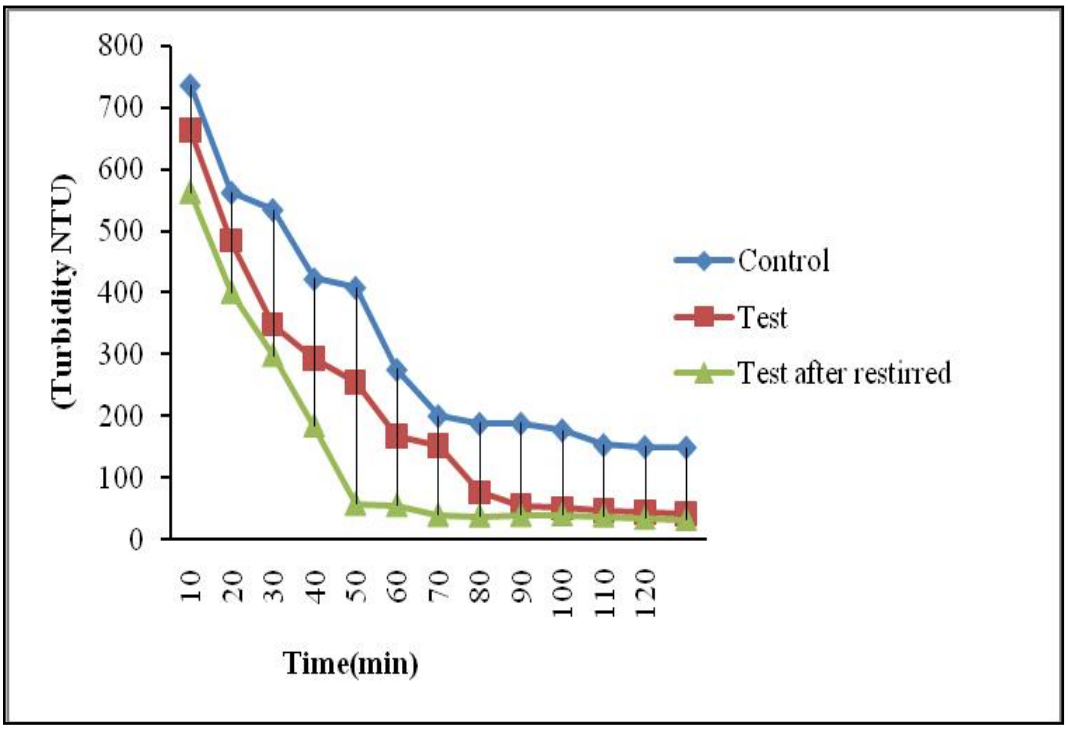


Figure 3.39: Kinetics of turbidity reduction in 1L Imhoff cone

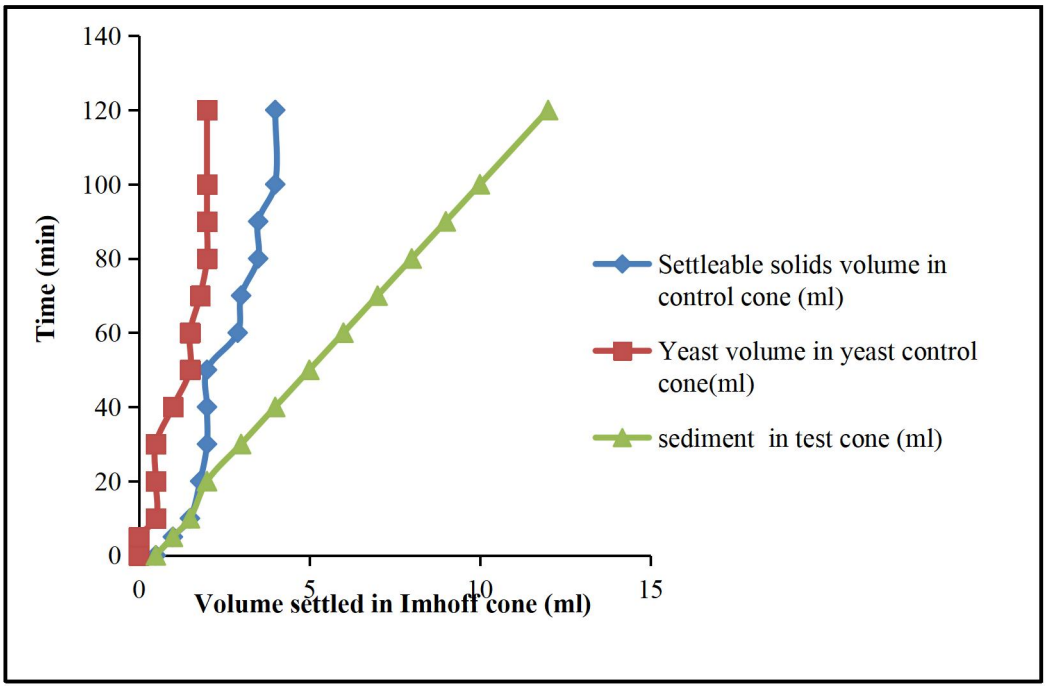


Figure.3.40: Sediment volume (ml) in Imhoff cone

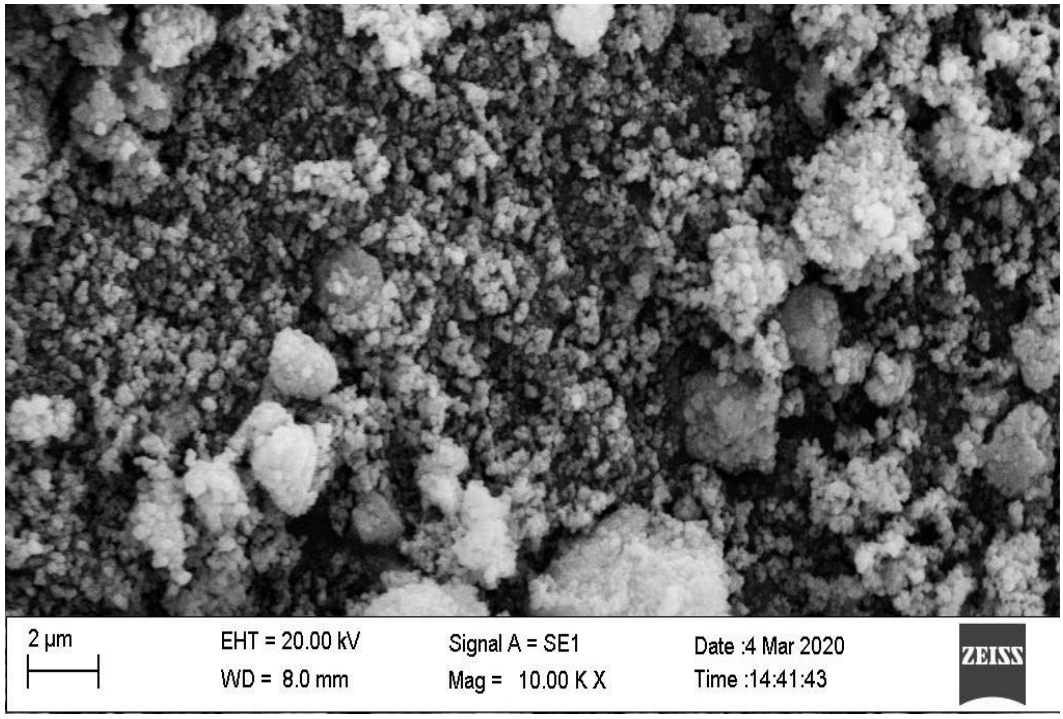
The reduction in turbidity of mine tailing water was higher in the test cone than in the control cone (Fig.3.36). Alive yeast reduced turbidity better than heat-killed (Fig.3.35). Further yeast grown on MEA plates and in MEA broth under shaking conditions demonstrated the similar trend of turbidity decrease (Fig.3.35). Moreover, soil sediment was higher in test cone with compared to control cone (Fig.3.40), 10 to 15 ml sediment was observed after 40-60 min of yeast addition. Sediment deposition was not compact. Sedimentation pattern revealed porous or filled with water or air. While in control cone sediment volume was less than 5ml within 120 min. Moreover sediment were tightly packed in control cone bottom.

Hence structural as well as quantity changes were observed among control and test cone sediment.

3.6.3. SEM analysis of the sediments

The sediment of SMTTW showed rough morphology under SEM. After yeast was added, it became more flat. SEM Images of yeast revealed a spherical cell shape (fig.3.41(b)). Yeast cells are budding in the nature. The size of each cell varies. In figure(fig.3.42(c)), It can be seen yeast cells are not visible due to particle depositions, (with microscopic clay particles). SEM morphology of test yeast, test sediment, and control sediment revealed that clay particles were rough, and round in shape but sediment texture changed after interacting with yeast. Sediment is looking like flat sheets. Average size of control sediment and test sediment was found 0.232 and 3.567 µm respectively using SEMJ software. Hence SEM images providing prove of interaction of clay colloids and yeast cells.

(a)



(b)

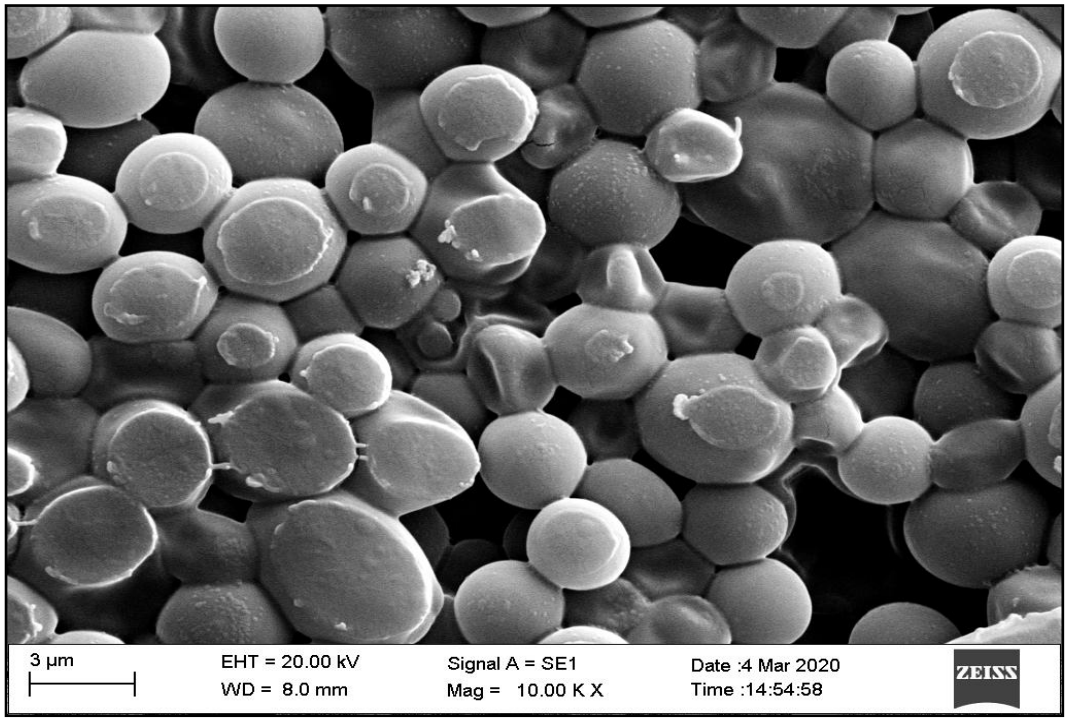


Figure. 3.41: (a) SEM images of control sediment showing absence of microbial biofilm (b) Yeast cell

(c)

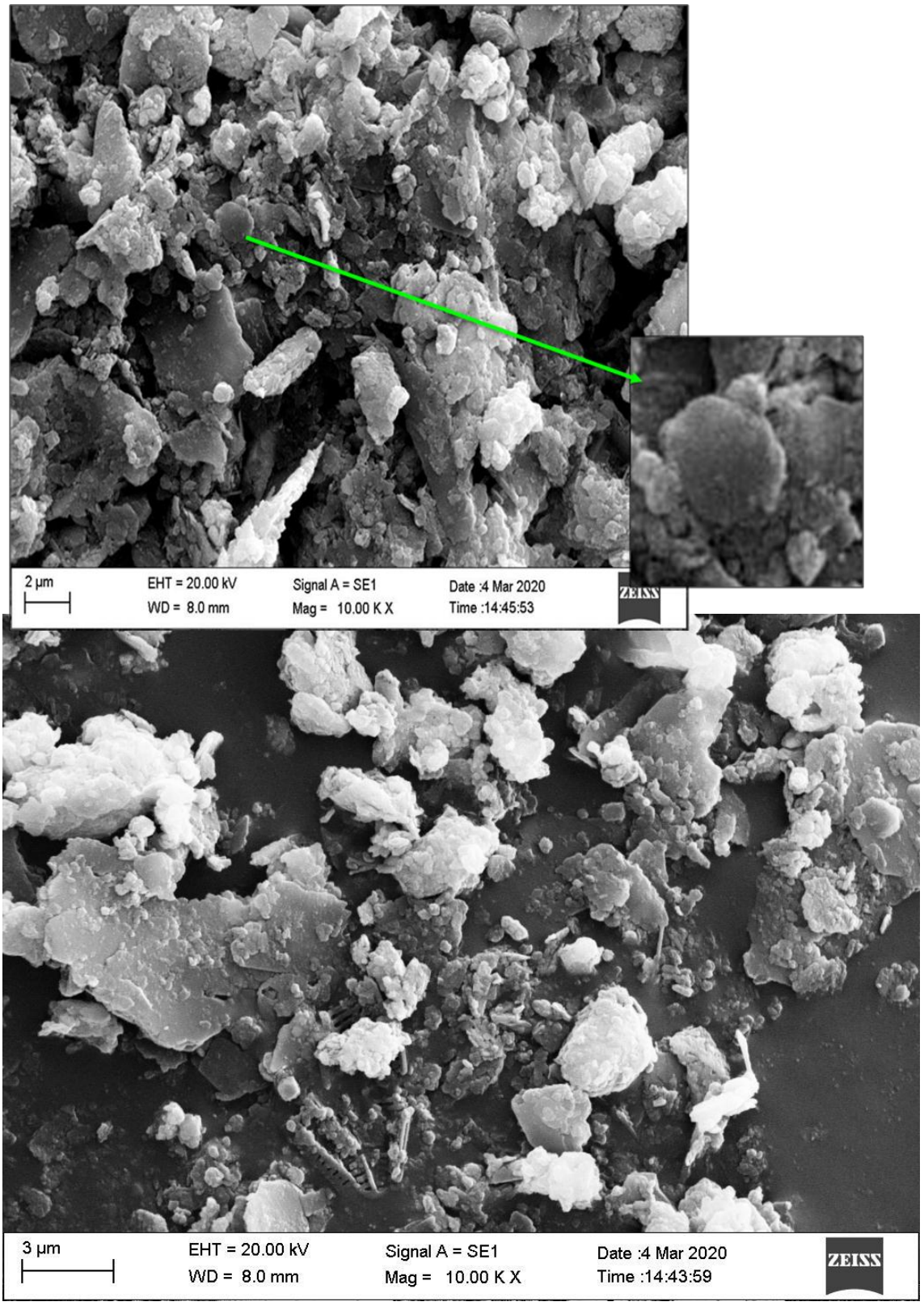


Figure. 3.42: (c) SEM images of test sediments

3.6.4. EDX study of sediments

The presence of Fe, Mn, Al, Pd, Au, and Si in mine tailing soil sediment (Control sample) was revealed by EDS (Fig.3.43 (a)). The presence of carbon element was detected by EDS in the mixture of yeast and mine tailing soil sediment (Test sediment) (Fig.3.43 (b)), in addition to all other elements observed in the control sediment. EDS spectra revealed, not much chemical composition changed in the soil sediment after yeast addition

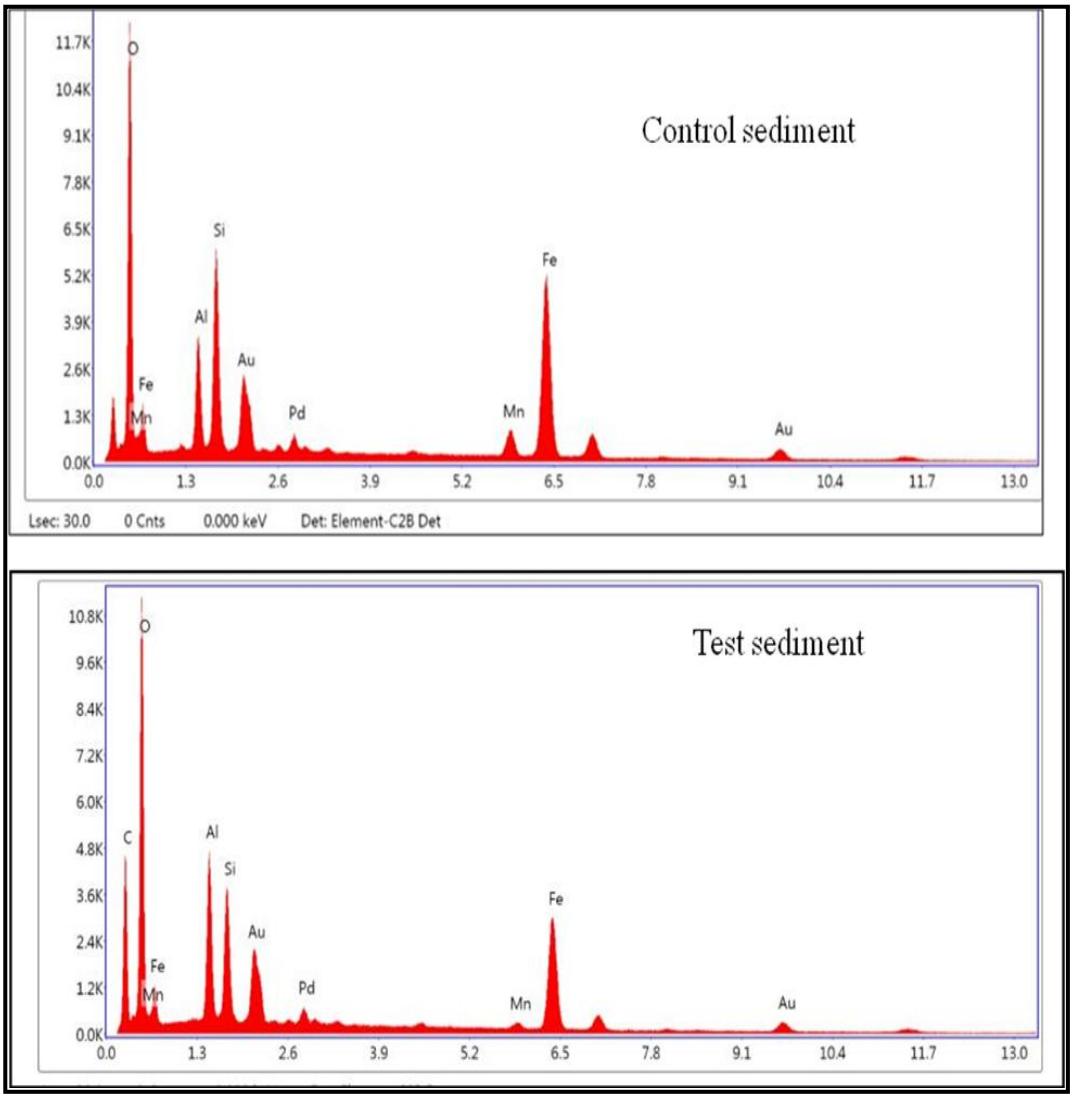


Figure. 3.43: EDS spectra of control sediment and test sediment

3.6.5. The FTIR spectra of control and test sediments

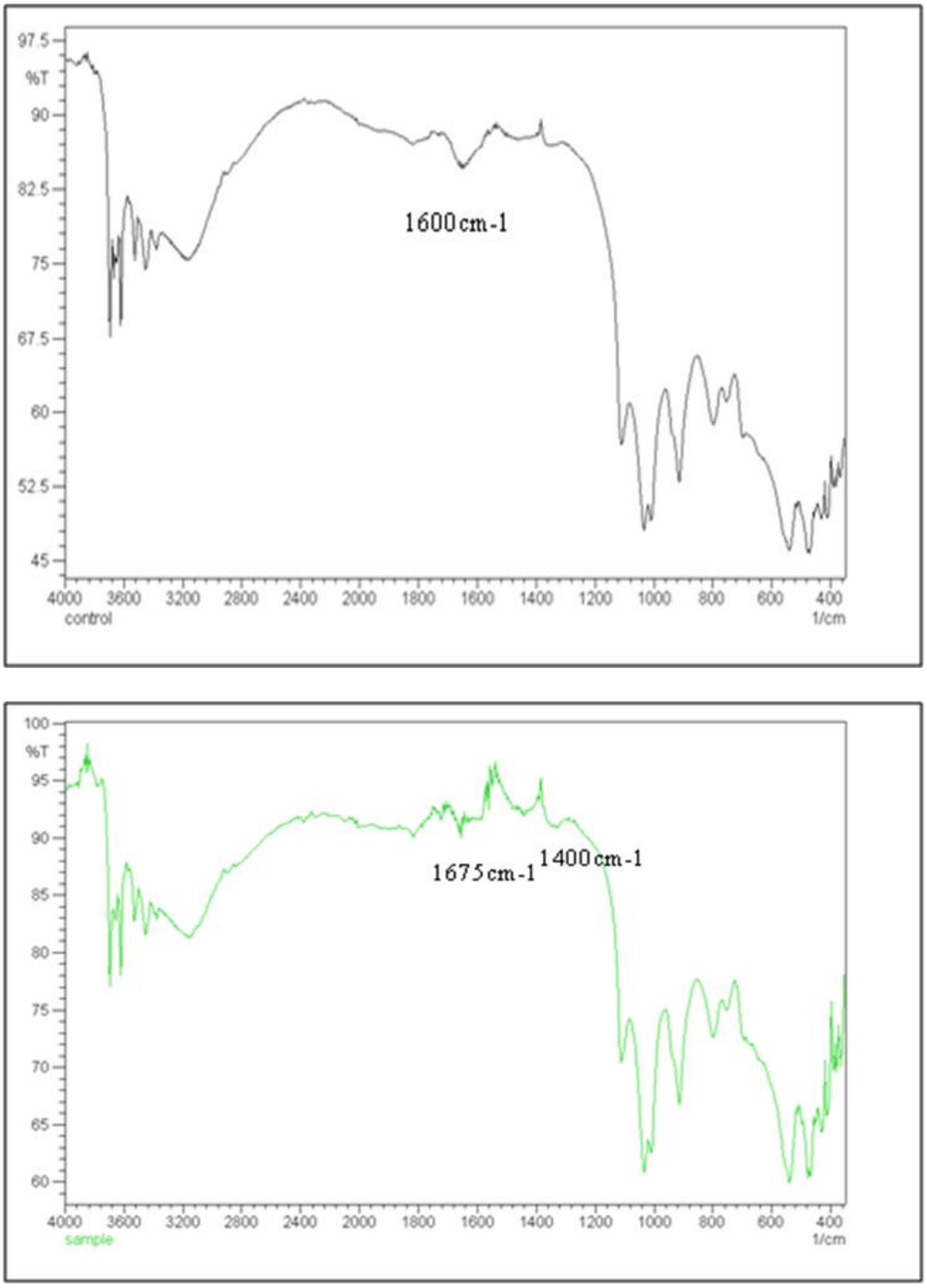


Figure.3.44: Spectra of control and test sediments

3.6.5.1 FTIR Spectra of sediments.

The IR spectra of soil sediment, soil sediment with yeast, were collected between the wave numbers of 4000 and 400 per cm and are shown in fig.3.44. sediment, and sediment with yeast spectra were studied.

As seen in fig. A peak indicating P=O extending at 1,217 per cm occurs in the IR spectra of soil sediment. Peaks were identified in the soil and yeast mixed sediment IR graph ranging from 1,100 per cm to 500 per cm. In IR of yeast cells, a peak from 1,750 per cm appeared to be an aliphatic ketone.

The presence of amide is shown by the peak range 2800-3000 cm-1, which shows N-H stretching. The existence of an O-H bond in the peak range 2500-2000 suggests the presence of a carboxylic or hydroxyl group. Due to the presence of carbonyl chemical, the spectra exhibit a peak in the 1600-1750 range. $1200cm^{-1}$ is the highest point. At $1200cm^{-1}$, the C-O group reaches its pinnacle. The presence of aromatic amines and nitro compounds is indicated by the presence of 525.12 cm1 peaks that show distinctive combinations of N=O and C–N stretching. Similar to the vibrating absorption of asymmetric and symmetric stretching in alkanes, alkenes, and alkynes, the peaks ranging between 1442.35 and 1056.50 cm1 were features of CC, C=C–H, and H–C–H, respectively. In general, the existence of organic molecules, compounds, and functional groups recorded for the sample demonstrates ion exchange capabilities that would confirm the attachment of clay colloids cations. Surface functional group dissociation aids dispersion by generating a negative or positive charge and/or supplying hydrophilic spots on a hydrophobic surface (Choudhary and Neogi 2017; Dupuy et al. 1997; Simate et al. 2012; Boehm 1994).

3.7. Biosedimentaion study (20- 100 L)

3.7.1. Biosedimentation test in the 20 L bell jar

Biosedimentation efficiency from 1 L to 100 and results are follows

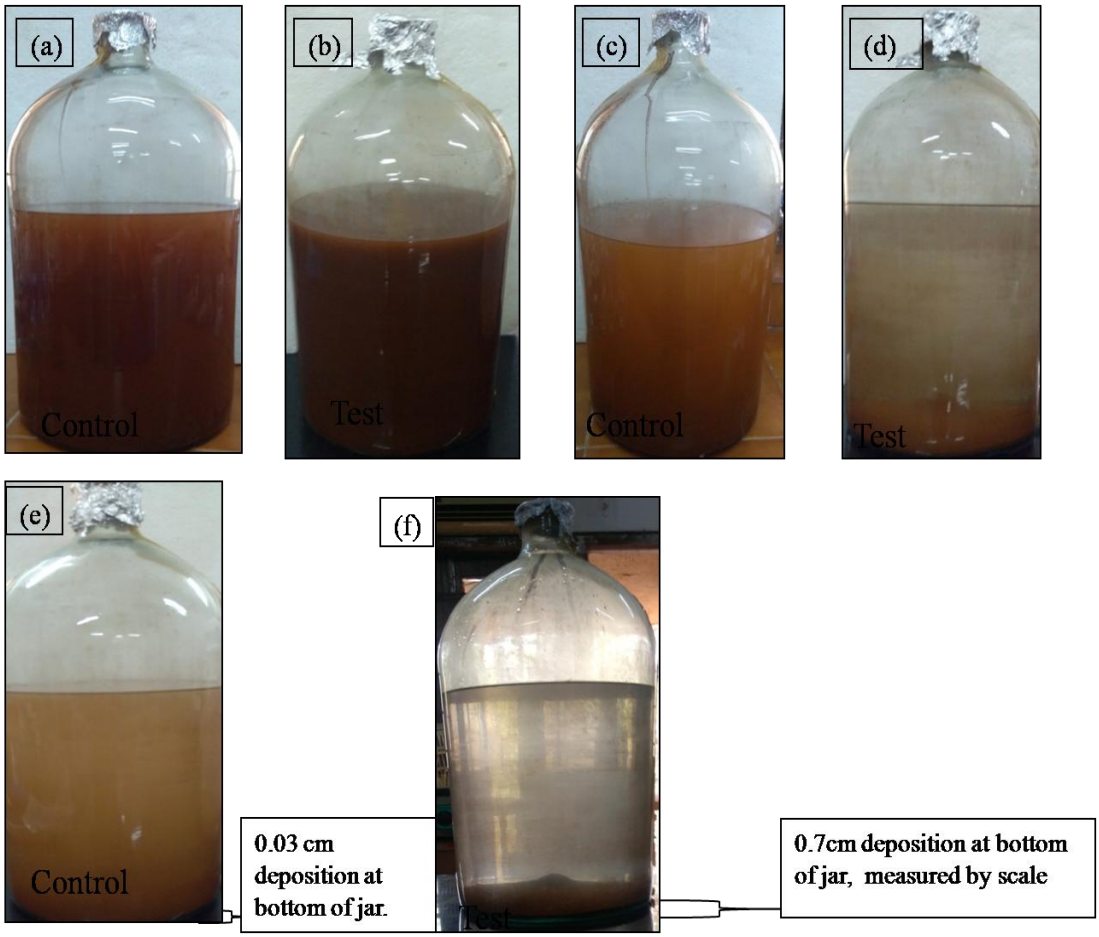
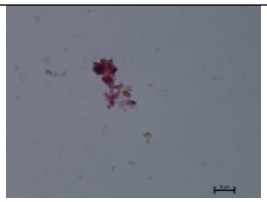
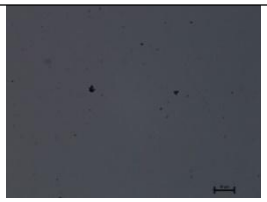
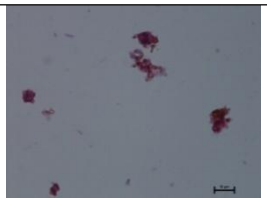
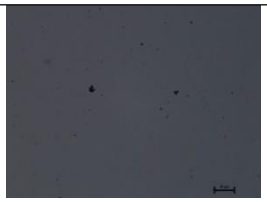
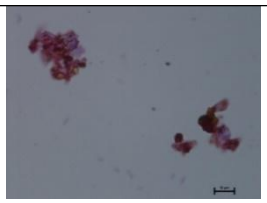
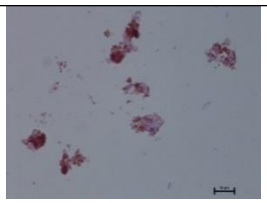
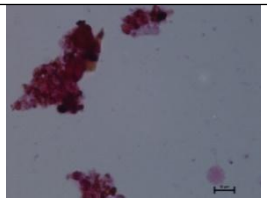
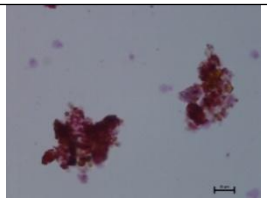
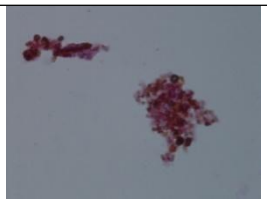
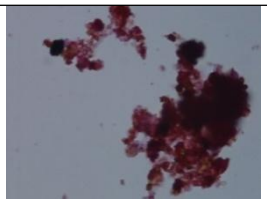
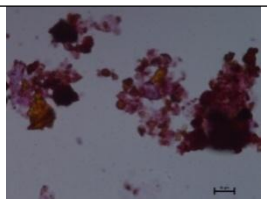
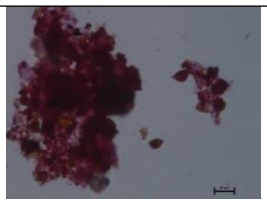


Figure.3.45: Turbidity in Bell jar. Bell jar was kept on vibration free platform. (a) Control (b) Test at zero time. (c) Control (d) Test bell jar, after 10 minute of yeast addition in test jar) (e) Control (f) Test after 120 minute of yeast addition.

Table.3.21 Sediments morphology (at 100x magnification)

Volumetric depth ml	Control	Test
14000		
12000		
10000		
8000		
6000		
4000		

3.7.2.2. The biosedimentation kinetics of (SMTTW) in 20 L Bell jar

The biosedimentation kinetics of mine tailing water in 20 L at various depths revealed a decreasing tendency of turbidity at all depths. Turbidity in the test jar was lower than in the control jar after 60 min of yeast addition.

3.7.3. The biosedimentation kinetics of (SMTTW) in 100 L tank

In 100 L at various depths revealed a decreasing tendency of turbidity at all depths. Turbidity in the test jar was lower than in the control jar after 60 min. Moreover, sediment were compact as settled down rapidly after vigorous stirring, Fig3.47.

Moreover, sediment compactness was high, as after restirring the water column turbidity reduced rapidly.

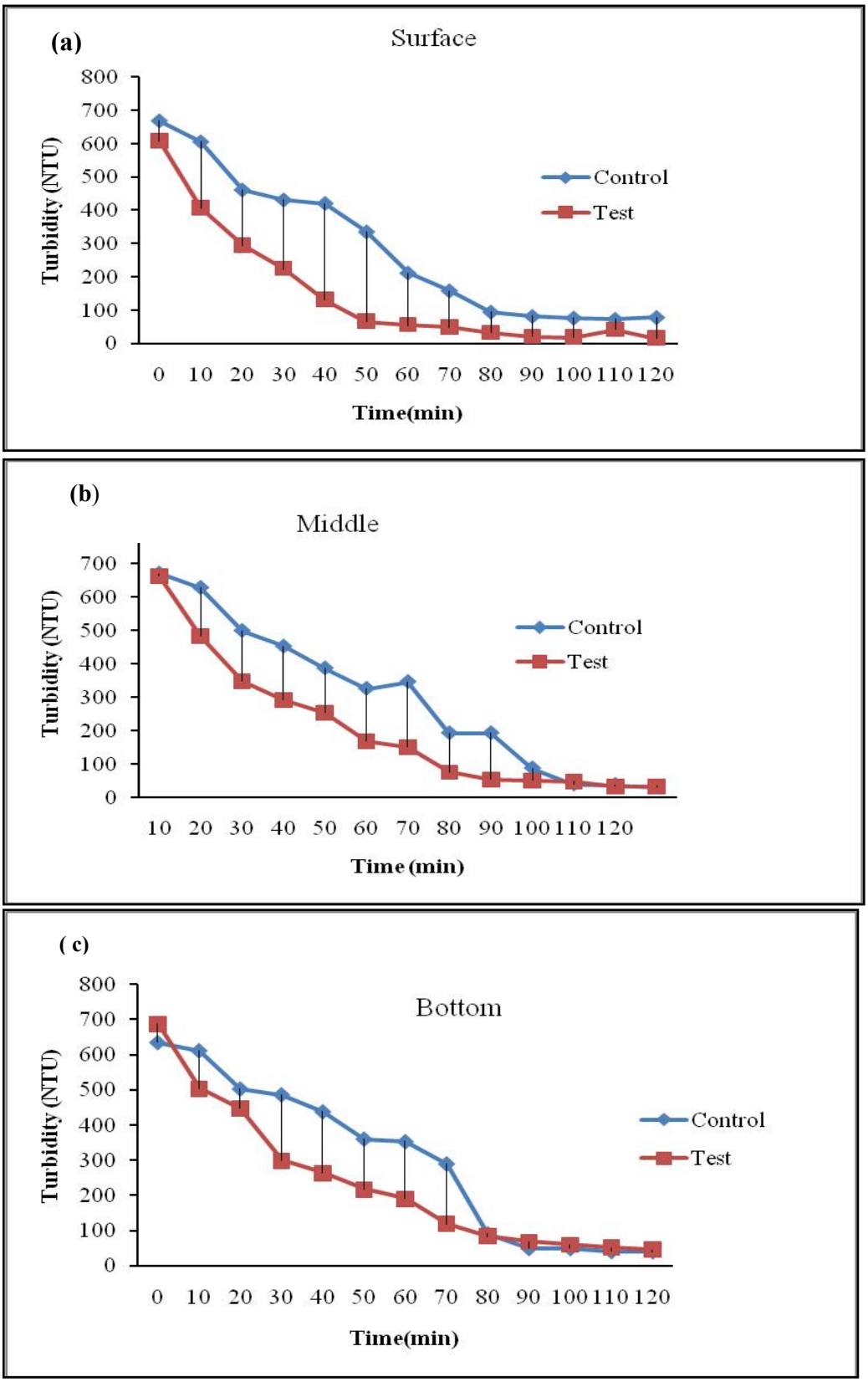


Figure.3.46 Biosedimentation kinetics at different depths of bell jar (a) surface (b) middle (c) bottom of the Bell jar

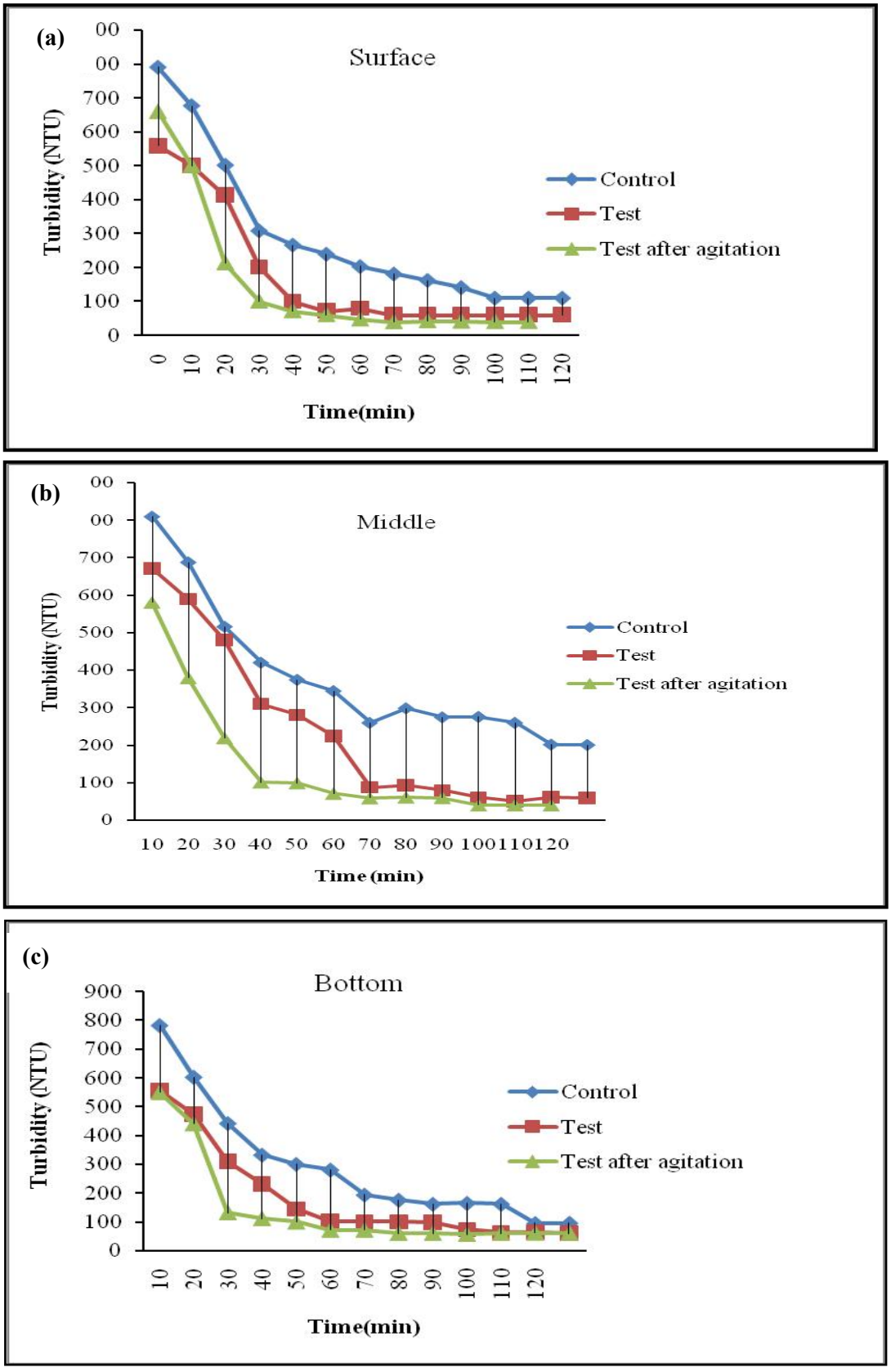


Figure.3.47: Biosedimentation kinetics at different depths of 100 L tank(a) surface(b) middle(c) bottom of the tank

3.8.1. Biosedimentation using selected cultures scale 500-1000 L

Figure. 3.48: Visible turbidity in control tank, (a) after 0 min of stirring (b) after 1 hrs of stirring In the 1000L of tank.

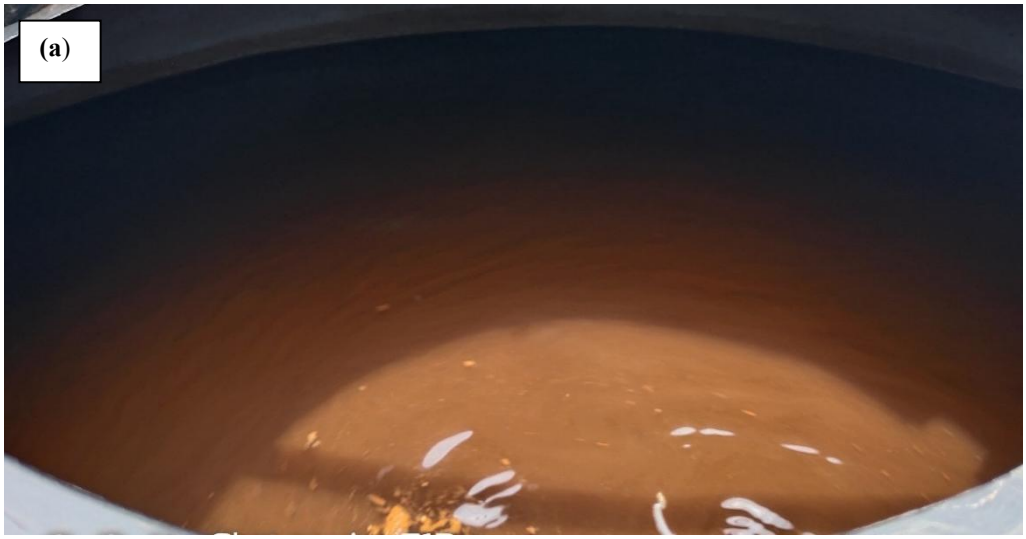


Figure.3.49: Visible turbidity in reactor tank, (a) after 0 min of stirring (b) after 1 hrs of stirring in the 1000L of tank

3.8.2. Out flow water collected from control tank, test tank and their respective sand bed

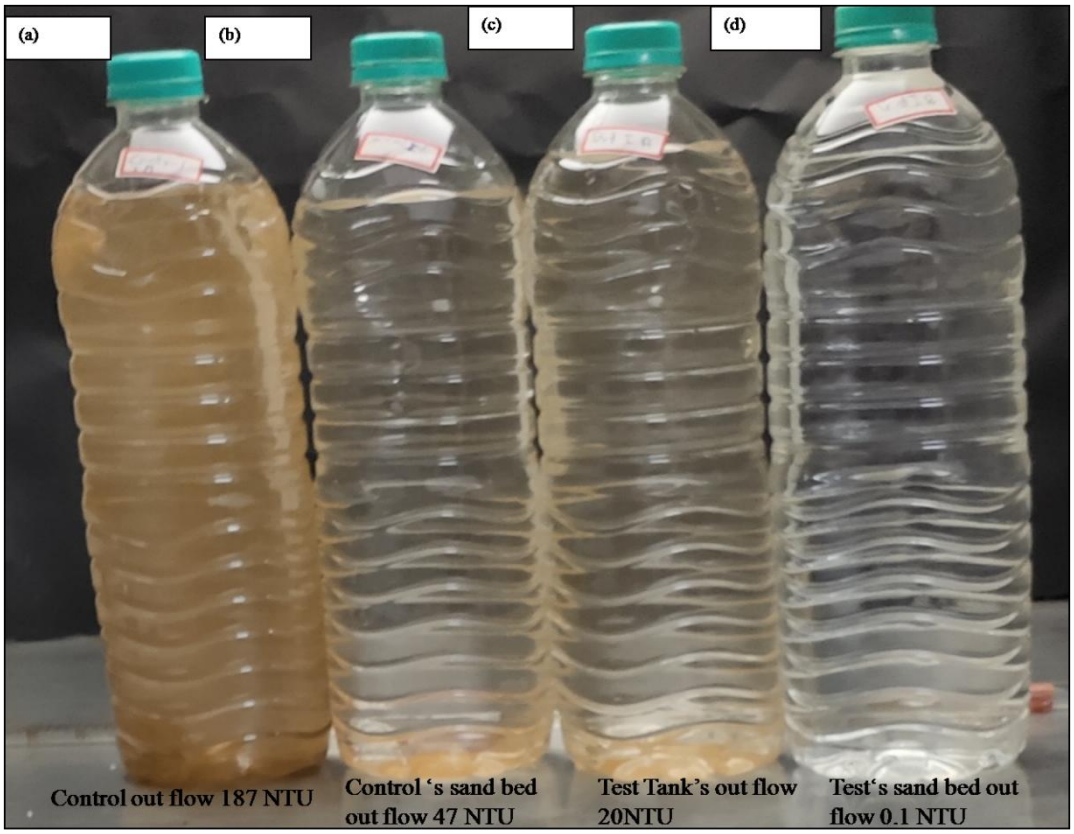
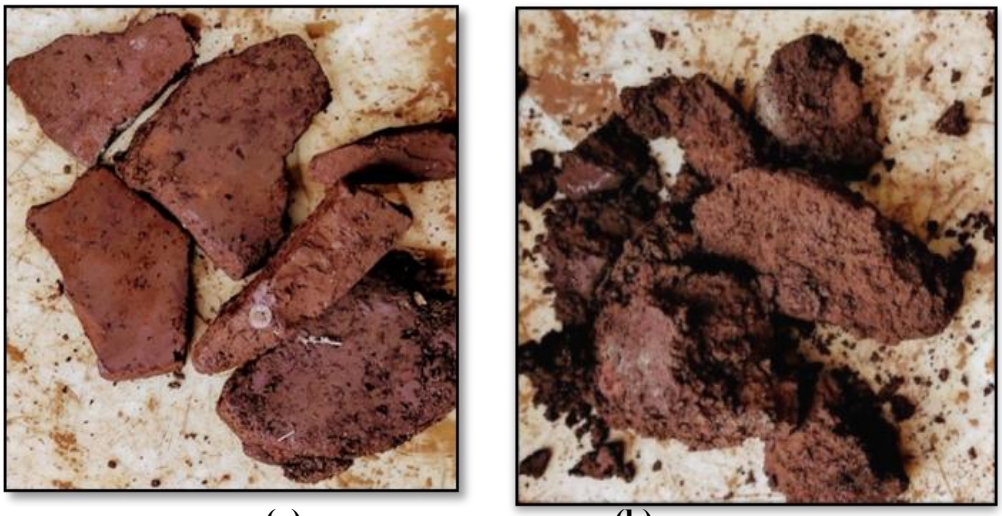


Figure3.50: Turbid water, after 1 hr of stirring, collected from middle point of the tank height (a) Control tank outlet sample, (b) Control sand bed outlet sample (c) Reactor tank outlet sample (d) sand bed outlet sample connectedto reactor tank.



(a) **(b)**

Figure.3.51: (a) Settled Sediment from control tank showed clay like smooth texture. (b) Settled Sediment of reactor tank showed a coarsely aggregated outlook pattern of the texture.

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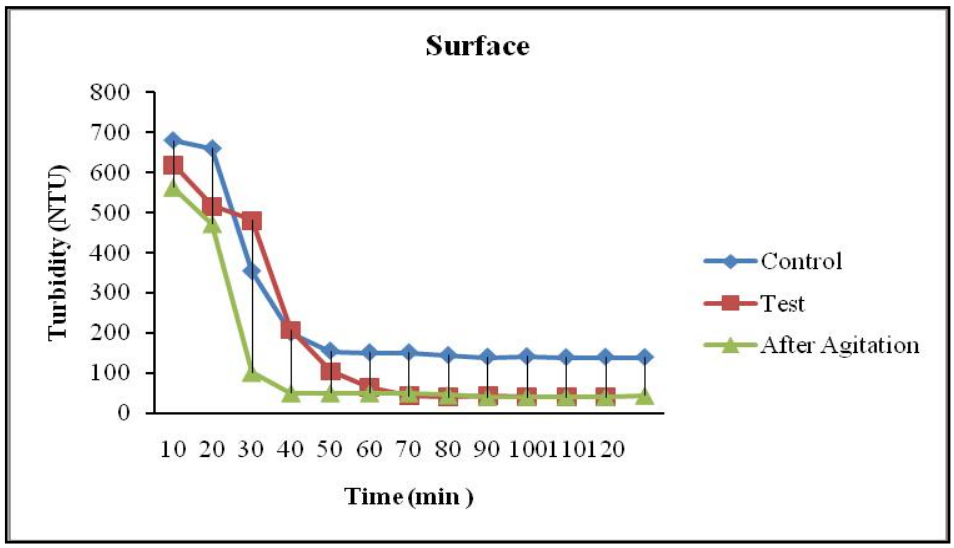
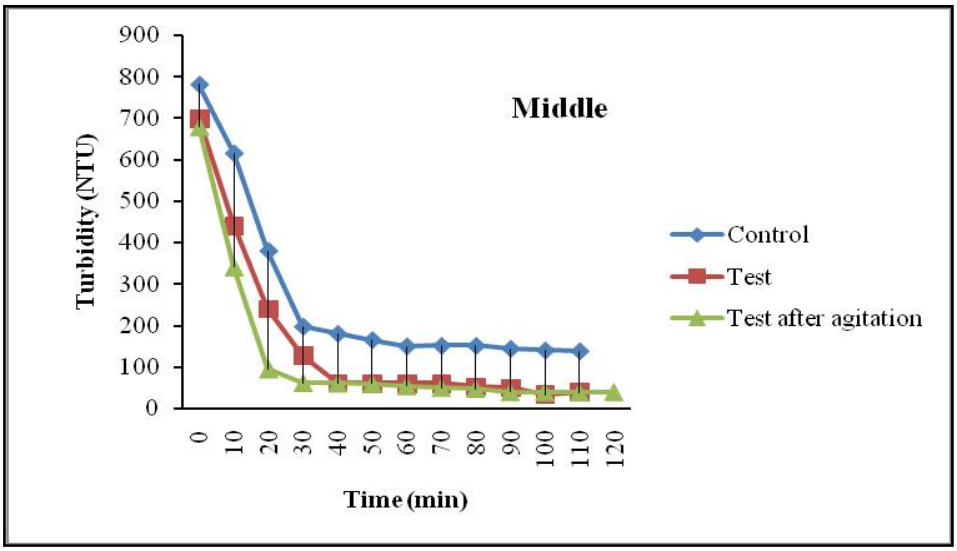
3.8.3. The biosedimentation kinetics of mine tailing water in 500L-1000L tank

The results demonstrated that sedimentation occurring rapidly in the test reactor, or reactor in which yeast cells were inoculated. Moreover, sediment were compact as after restirring sediment did not broke and settled down rapidly. Results showed yeast mediated bioflocculation is scale independent.

(a)



(b)



(c)

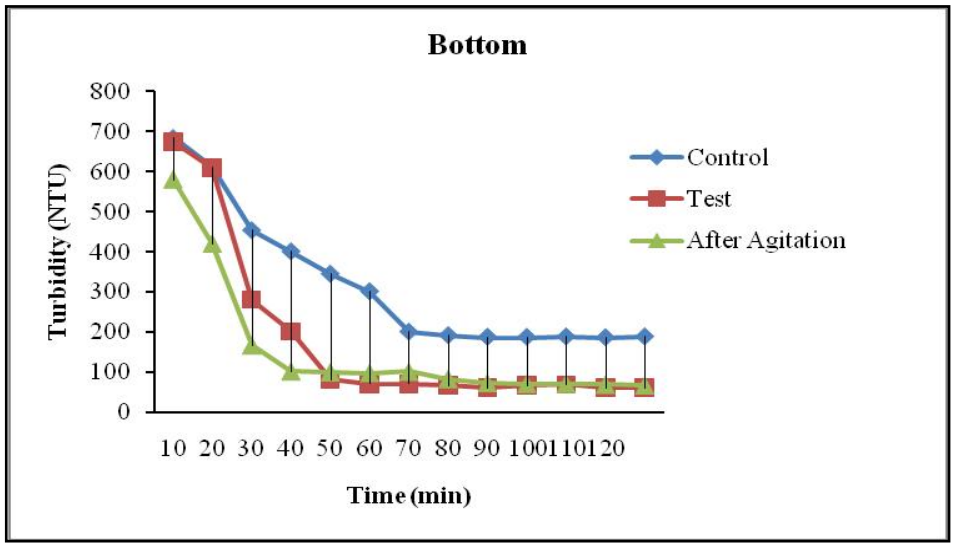
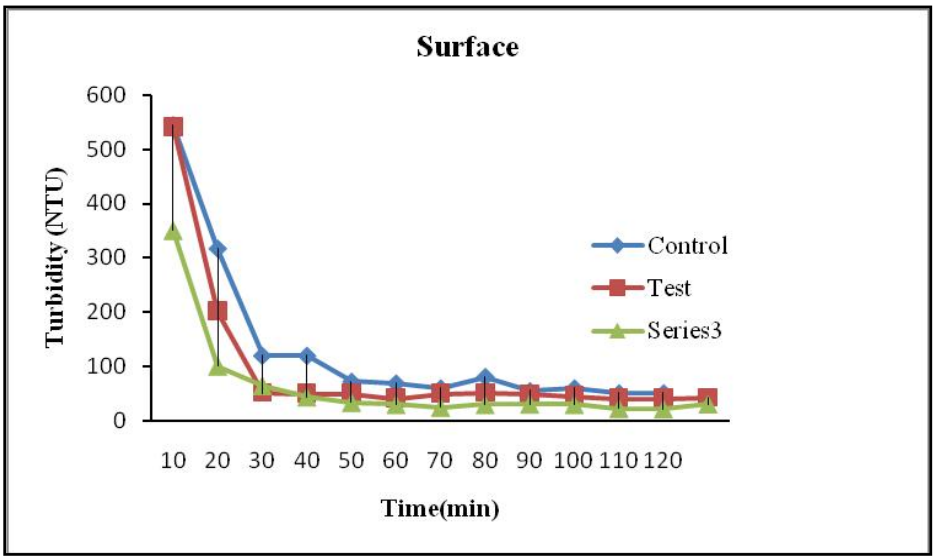
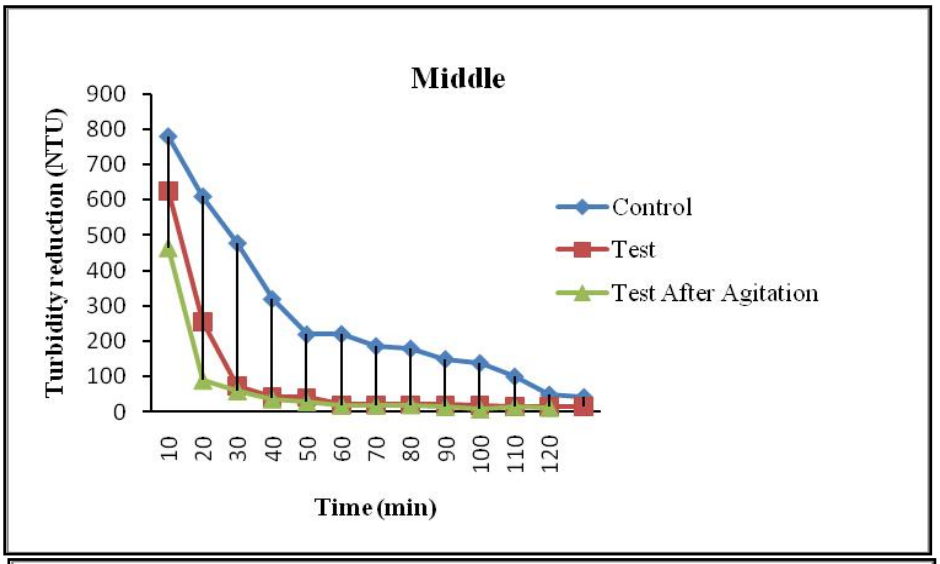


Figure.3.52: Biosedimentation kinetics in 500 Ltank(a)surface (b) Middle (Bottom)

(a)



(b)



(c)

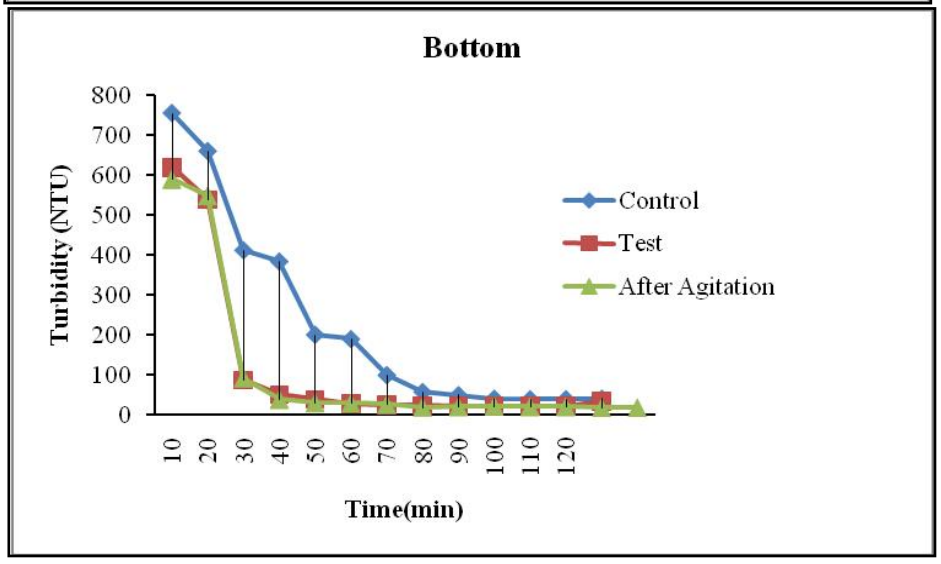


Figure.3.53: Biosedimentation kinetics in 1000Ltank (a)surface (b)Middle (Bottom)

3.8.4. Adsorption efficiency of biosedimentaion test

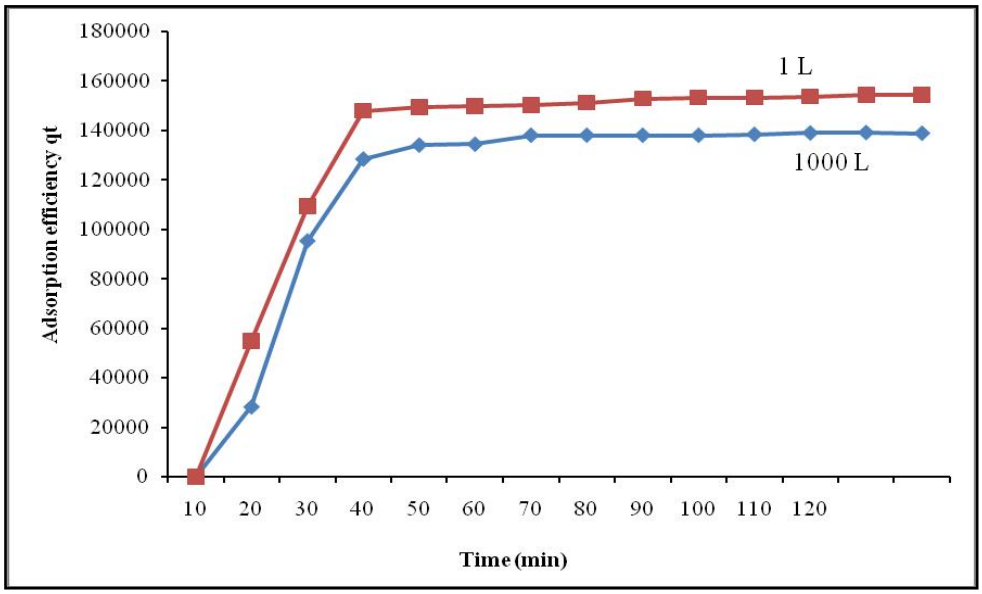


Figure.3.54: Plot of adsorption efficiency of biosedimentation test using yeast in1L and 1000 L mine tailing water

3.8.5. Microbial study of effluent of tanks and its respective sand bed

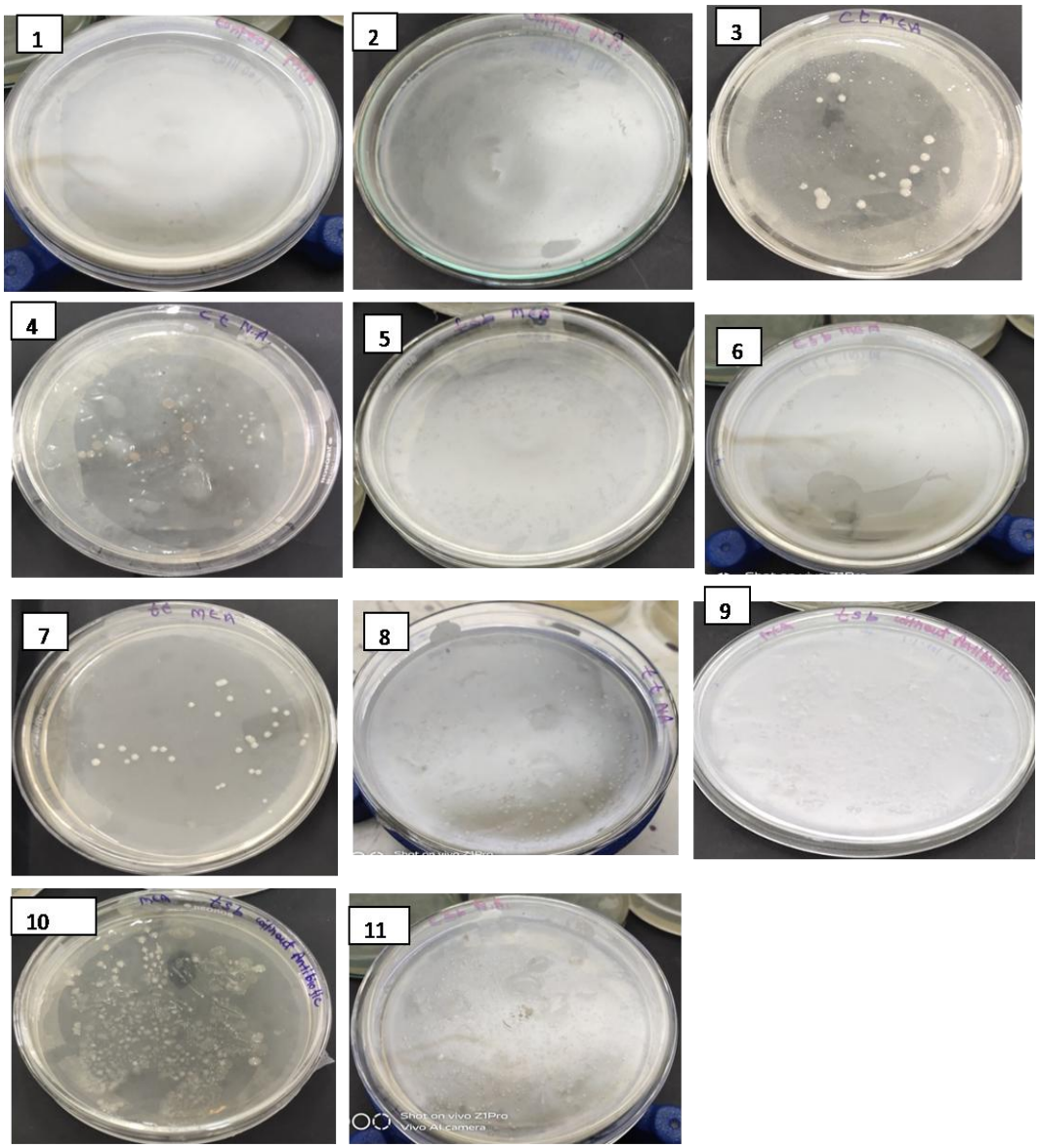


Figure.3. 55: Colonies from outflow of tanks

Table.3.22 Colony characteristics

S. No.	Sample designation	Colony characterization
1	Control MEA	No growth
2	Control NA	No growth
3	Control tank effluent Plated on mea (ct MEA)	Small white colony
4	Control tank effluent Plated on nutrient agar (ct NA)	Small colony
5	Test tank sand bed effluent Plated on MEA (ttsb MEA)	No growth
6	Control tank sand bed effluent Plated on MEA (ctsb MEA)	No growth
7	Control tank sand bed effluent Plated on nutrient agar (ctsb NA)	Small colony
8	Test tank effluent Plated on MEA (tt MEA)	Small white colony
9	Test tank effluent Plated on nutrient agar (tt NA)	Small colony
10	Test tank sand bed effluent Plated on MEA without antibiotics (ttsb MEA)	Small colony
11	Test tank sand bed effluent Plated on nutrient agar (ttsb NA)	Small colony

3.8.6. As a criterion for flocculant performance, the critical coagulant rate constant

The suggested criterion's validity is demonstrated by the results of a biosedimentation test in 1000L of turbid water in terms of residual turbidity of the flocculation process in order to meet the (WHO) turbidity standard for drinking water.

c: equals (5 NTU), which refers to the World Health Organization's (WHO) water turbidity standard (WHO 1996, 2006, 2008). The concentration of synthetic turbid water levels including: low (40 NTU), middle (70 NTU), and high (100 NTU) is the concentration of synthetic turbid water levels (780 NTU) **t:** equals the end of the chosen settling time (60 min) Substituting (c, c0, t) in Eq. (3) to obtain (kc) kC= 1/c-1/co/t for second order reaction (Elimelech *et al* (1995)

Lower level ($NTU^{-1}\ min^{-1}$**)**

Kc= 1/0.1-1/40 /60 **(**$NTU^{-1}\ min^{-1}$**)**

Kc=0.1629 **(**$NTU^{-1}\ min^{-1}$**)**

Middle level

Kc= 1/0.1-1/70 /60 $NTU^{-1}\ min^{-1}$

Kc=0.1664 $NTU^{-1}\ min^{-1}$

High level

Kc= 1/0.1-1/780 /60 $NTU^{-1}\ min^{-1}$

Kc=0.1666 $NTU^{-1}\ min^{-1}$

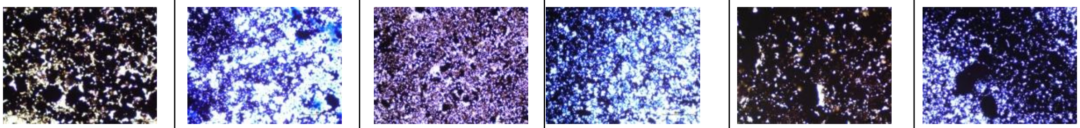
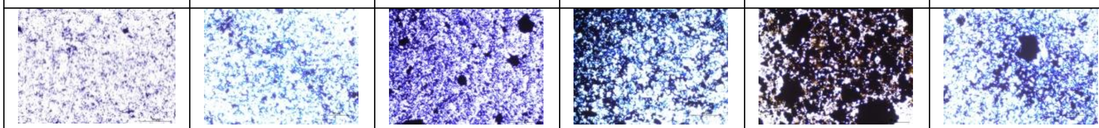
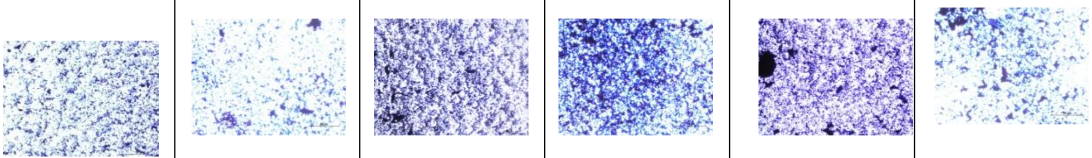
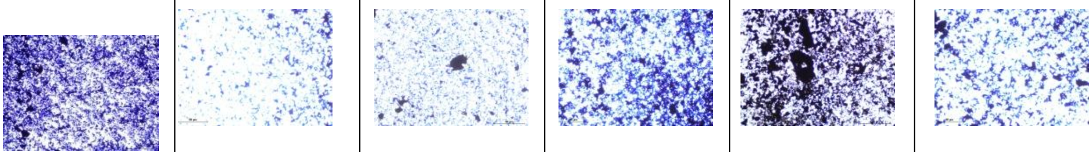
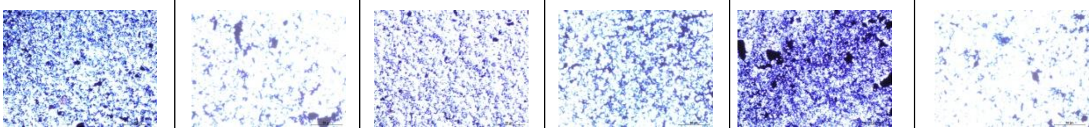
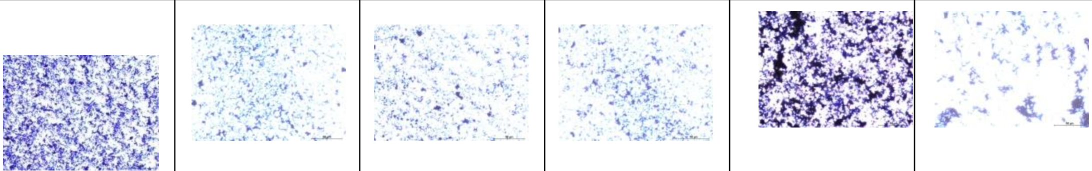
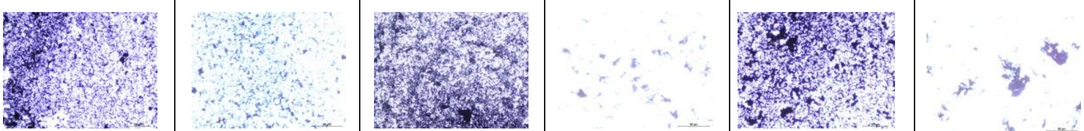
Rate constant for control tank and test tank at time 60 min

Crc=(1/c_t-1/c_o)/t =(1/187-1/780)/60=0.000067 $NTU^{-1}\ min^{-1}$

Trc=(1/c_t-1/c_o)/t=(1/40-1/780)/60=0.000396 $NTU^{-1}\ min^{-1}$

As a result, after employing yeast to remove turbidity in mine tailing water, the rate constant increased by more than 6 times. As a result, yeast may have a substantial role in reducing turbidity.

Table.3.23 Morphology of sediment at different depth of the 1000L tank (at 100x magnification)

Depth					
Surface (Control)	Surface Test	Mid (Control)	Mid Test	Bottom (Control)	Bottom Test
					
					
					
					
					
					
					

3.8.2.7. Sediment micromorphology

Micromorphology of the sediment revealed the concentration of suspended particles reducing with respect of time and it is reducing faster in the test reactor (Table3.27).

Table.3.24 Water Parameters for 1000L biosedimentation test

Parameter	Control tank	Control tank (sand bed)	Test tank	Test tank (sand bed)	Permissible Limit (WHO)
pH	6.4 ±0.05	6.40±.00441	6.40±.004	6.4±0.0044	6.5-8.5
Temp (°C)	29+1.06	29.1±.11	29±.88	29±0.99	-
TDS (ppt)	74.93±0	66.189±0	68.30±0	69.91±0	500
Conductivity µs	136	127.6±	129	127.3	500
D.O.	6	6	6	6	No standard
Po_4^{-3}(mg/L)	0.75±0.306	0.75±0.614	0.25±0.16	-	0.1
So_4^{-2}(mg/L)	70.5±0.81	59.4285±.29	52±.25	-	400
No_3^{-1}(mg/L)	1.17±.0027	1.901±.003	0.6559±0.004	1.13±.0046	10
Alkalinity (mg/L)	4±0.44	4±0.333	3±0.5	2±0.5	200
Cl	6.025 0±.25	5.6 ±0.17	5.672±0.118	5.67 0±.27	≤200
Hardness (mg/L)	34±0.29	28±0.12	24±0.101	24±0.17	500
Na (mg/L)	6.484±0.5175	7.8480±.53	6	6.848	250
K (mg/L)	0.6432±.0.31	0.6432±.480	0.6432±.0.3	0.6432±.0.41	-
Fe (mg/L)	0.1928±0.01	0.0178±0.003	0.1427±0.008	0.0025±0.001	0.5
Mn (mg/L)	0.21145±0.01	0.00227±0.0002	0.12798±0.005	0.00224±0.00032	0.3

3.9.1. Observation of beads after and before immobilization and its turbidity removal efficiency

Beads from both the test and control beakers were thoroughly washed and examined under a stereomicroscope. Sediment were found in the test beads' surface interiors, but not in the control beads. In the test beaker, the drop in turbidity was not considerable. Furthermore, control beads turned yellowish in colour, whereas test beads stayed white and did not alter colour. Though sediment were visible in the test beads, they were not present in the control beads.

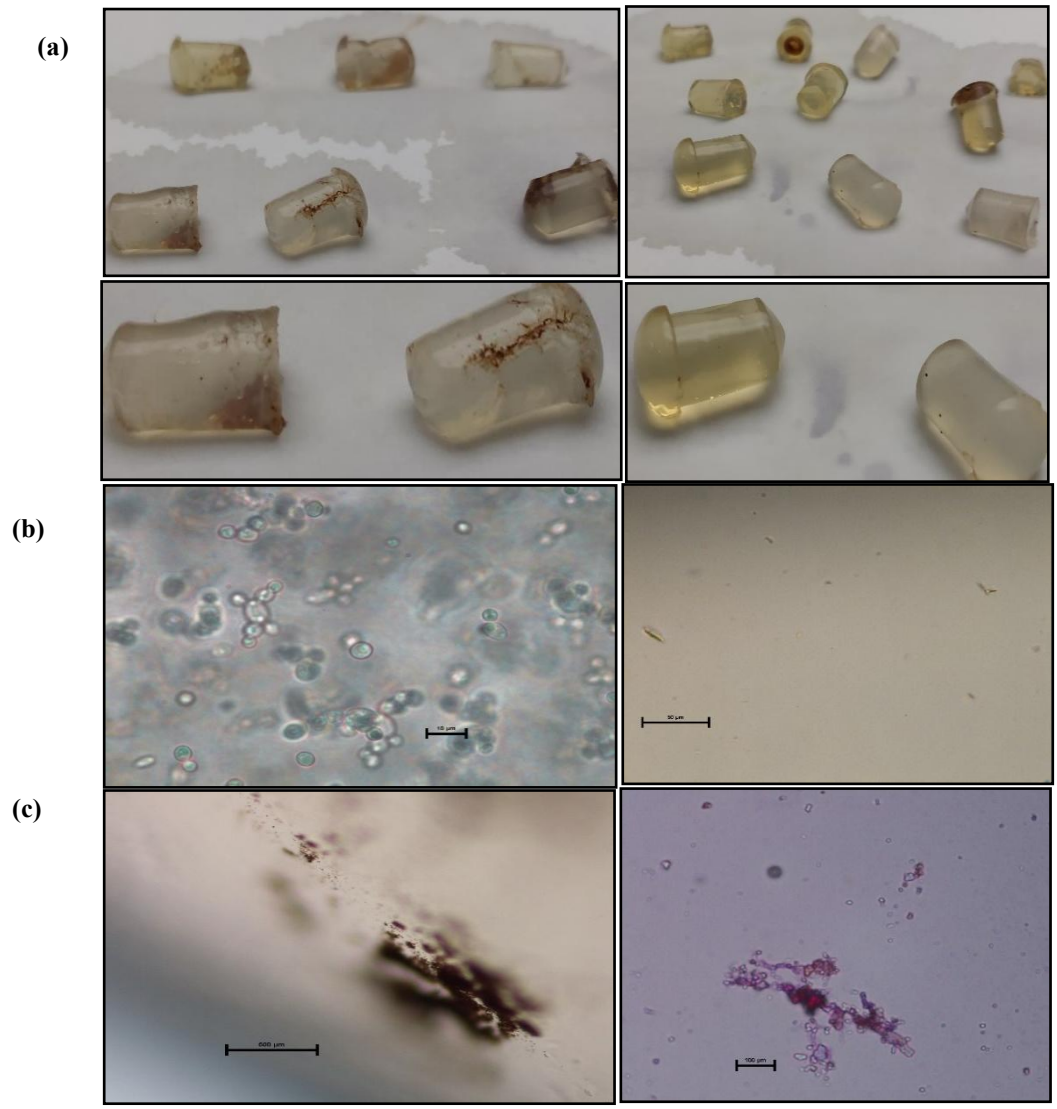


Figure.3.56: (a) Beads with yeast (left) and without yeast (right), longitudinal section of beads with yeast revealed presence of yeast in the layer of agar (left). (b) Longitudinal section of beads without yeast (right). (c)Beads surfaces (left), longitudinal section with yeast, (right)

3.10.1. Biosedimentation efficiency score for floc assay for yeast cultures combination study

The combination of the test strain and its own strain yielded the highest flocculation efficiency score, followed by Ntrvl-2-2.

Table.3.25 Biosedimentation efficiency score of combinations of cultures

Strain designation	score	Strain designation	score	Strain designation	score
Bchlm-2	100	Gh1-4	130	Srg-3-3	180
Pndsm1-2	180	Gh1-6	-	Krp-3	130
Srg-2-2	75	Krpr-2	-	S.C.-2	160
Bndl-1-1-1	50	Bndl-1-1-1	50	Srg-1	105
Pndsm-3	75	Bnd-2-2	50	Tmrs-2-2-3	105
Gh1-5	105	Crblm -1	-	Bndl-1	105
Ch1-1	105	Srg-3-3	-	Pnd-2	50
Krpr-1	75	Bnd-3	50	Bnd-2-2-1	130
Crblm-2	160	Tmrs-2-2-4	200	Ntrvl-2-2	50
Pndsm-1	130	Bchlm-1-2	280	Ntrvl-2	200
Pndsm2-2	130	Mym1-2	160	Hrvlm	130
Pndsm WA	130	Bchlm-1	160	Bh1	160
Gh1-1	130	Pnd-3	130	Control	25

Figure. 3.57: Biosedimentation assay for combinations study of yeasts cultures

3.11. Biosedimentaion study of natural mine tailing water

The turbidity of mine tailing water remained high for a long time (Control cone), however yeast treatment reduced turbidity to less than 200 NTU after 60 minutes.(Fig3.58). In the control cone, the turbidity of natural mine tailing water stayed at 300 NTU till 60 minutes. Mine tailing turbidity remained same in the control cone for natural turbid water.

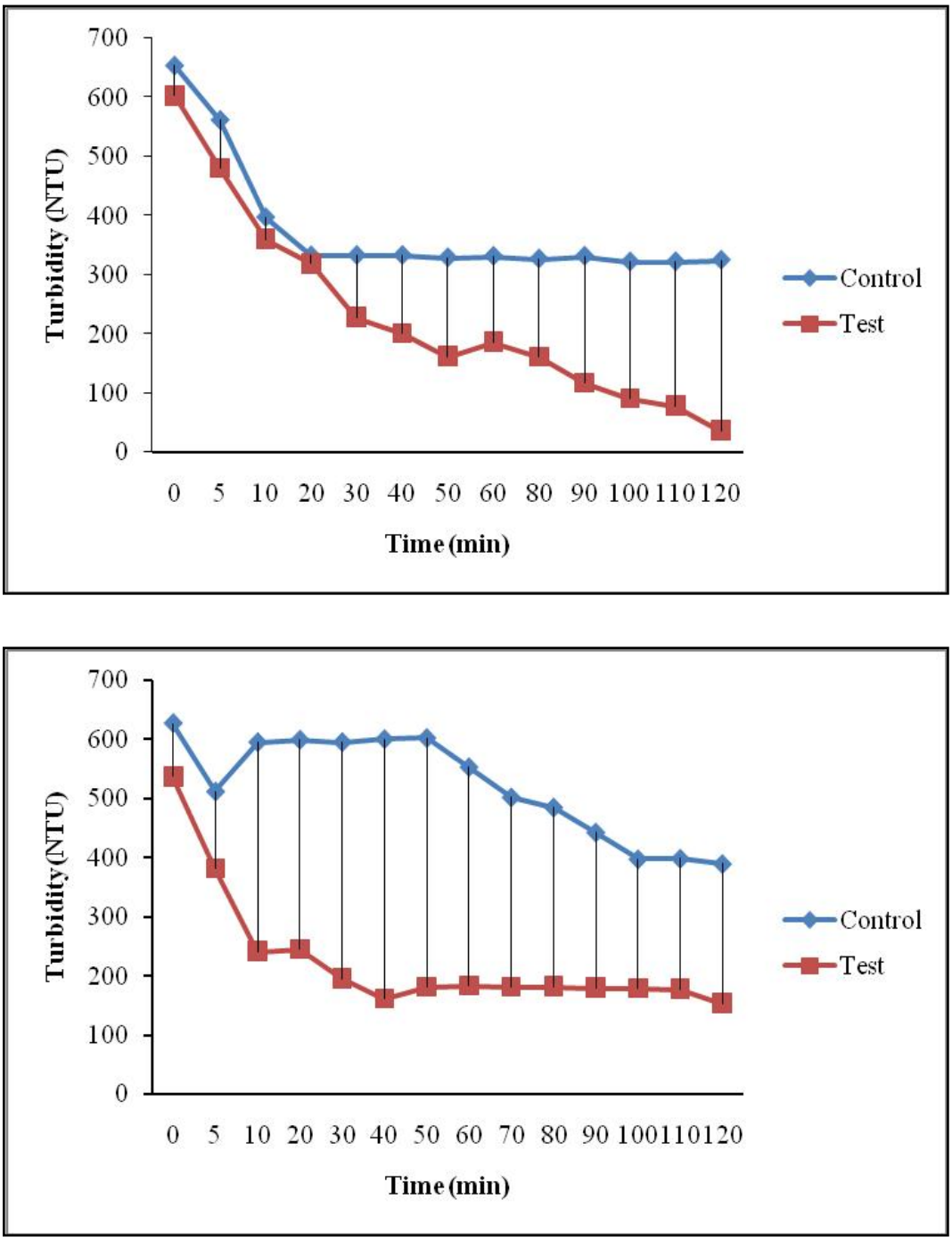


Figure.3.58: Biosedimentation kinetics of natural mine tile water in Imhoff cone (a) at 900 ml of Imhoff cones depth (b) at 600 ml of Imhoff cones depth (All turbidity reduction values are the average of 9 turbidity reduction values

3.12. Molecular Identification of promising culture

Candida Orthopsilosis was shown to be the most effective strain for biosedimentation after molecular identification. The cultures had a strong resemblance to *Candida Orthopsilosis* isolated from Ireland.

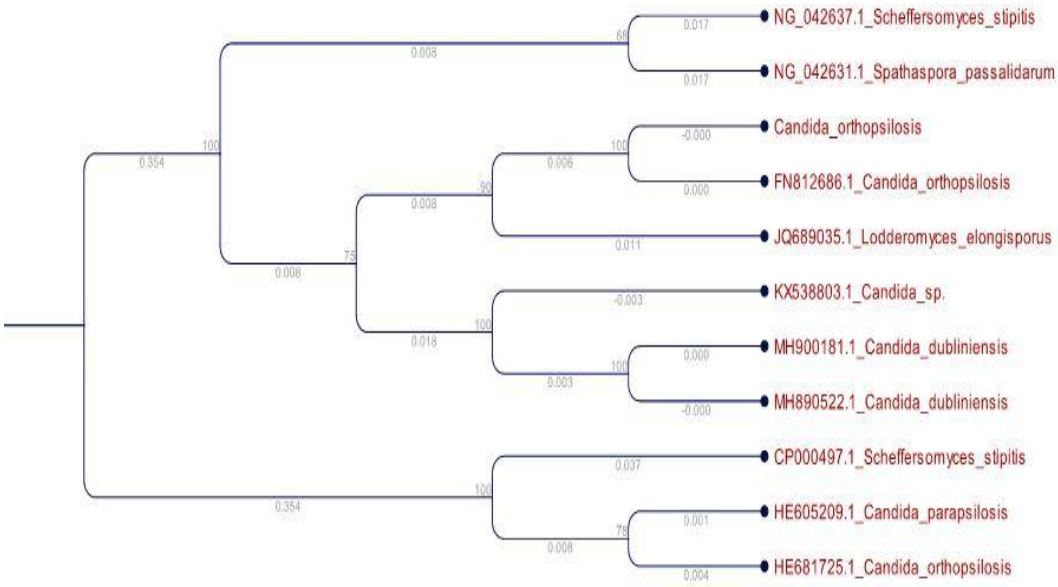


Figure.3.59: Phylogenetic analysis of *Candida orthopsilosis.* Isolate showed 0.006 dissimilarity with type species *Candida orthopsilosis* (FN812686.1)

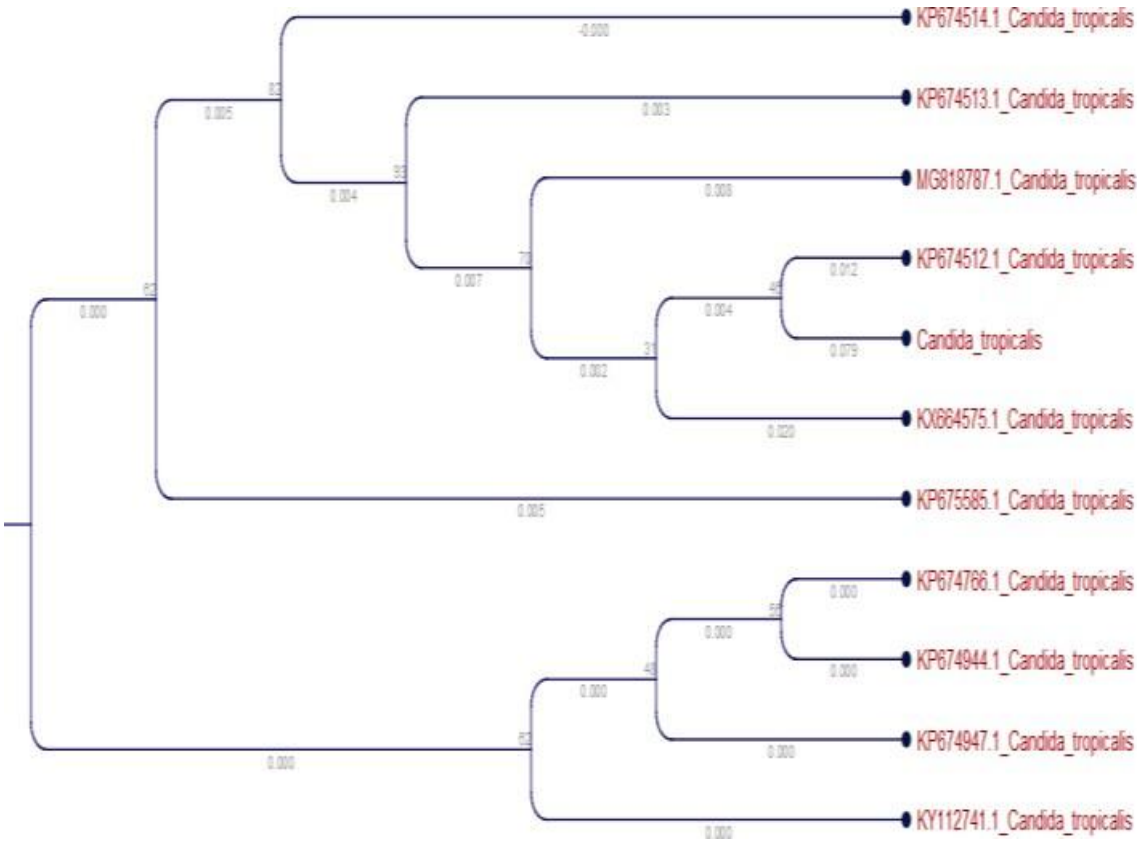


Figure.3.60: Phylogenetic analysis of *Candida tropicalis.* Isolate showed 0.012 dissimilarity with type species *Candida tropicalis* (KP674512.1) obtained from gastric mucosa, China.

CHAPTER IV
DISCUSSION

4.1. Survey of freshwater habitats from mining and non-mining areas of Goa

Goa showed a huge number of freshwater, bodies such as seasonal pond, during monsoon season. Most of the reservoir source was rainwater (e.g., cemented temple pond or tank and ground water (e.g., springs).

Since mining activities were not going on from the past twelve years, so the value of water parameters of these water bodies under study remained below the permissible level. Temperature varied from 24.5 to 30 °C and pH showed a variation from 6.3 to 7.8 variations in the studied seasonal water bodies.

Moreover, because of anthropogenic activity, slightly alkaline pH was observed at Netravali's freshwater bodies. The Electrical conductivity of water of these sampling sites under study varied from 40 to 140 µs and remained almost in same range in monsoon and non monsoon season of every sites indicates the constant condition of the water body.

TDS were found to be within range of 15-130 ppm. Furthermore, Shirigao pond showed low TDS as no human activity was observed. Safa masjid pond showed highest TDS 140 ppm as the fauna population were high.

Renu et al., (2014) reported various physico-chemical parameters of the freshwater bodies of Goa. The pH in all locations was neutral to slightly acidic, varying within a small range of 6 to 7. And max pH limits of 7.9 was found during monsoon season.
The results validated Rump et al., (1988) assertion that any scenario when the water is neither excessively acidic nor very alkaline, it may be believed that pH is regulated by carbon dioxide, bicarbonate-carbonate system, which was first proposed by pioneer limnologist Hutchinson (1976).

4.2. Isolation of natural aquatic yeast cultures

This is first-time time that aquatic yeast were isolated successfully from mining areas of Goa by Kamat et al., (2010; 2013) using the membrane filter technique.
So after getting leads from the previous work freshwater yeast were isolated succefully. Among all the isolation plates, petriplates without antibiotics (MEA 2 %) showed growth of both yeast and bacteria. So it can concluded that antibiotics was effective for

the isolation of pure yeast cultures. This antibiotics faropanem was used first time by our group. Pure yeast culture growth was observed in the antibiotic-treated petri plates, and a similar pattern of growth was observed in all the triplicate plates. Further, the purified yeast's fourty cultures were maintained on MEA (2%) showed huge diversity of yeast.

Further, research can be done on freshwater yeast variety in Goa's water bodies, which revealed a great diversity of aquatic yeast and can be used for human welfare. All talukas showed huge yeast diversity. Bihcolim, and Ponda followed by Netravali waterfall, University seasonal pond, and then Sirigao showed yeast diversity.

Many different natural and artificial habitats, such as soil, freshwater and marine environments have yielded yeasts (Fell et al., 2001; Samarasinghe et al., 2021). Freshwater yeasts have not been much studied. However, no yeast was reported except some fungal species e.g., *Penicillium, Aspergillus, Rhizopus* in the lakes (Bandh et al., 2019). *Candida sp*. Have been isolated from resort salt water and the Shind river, Sonamar, and Kashmir (Bandh et al 2011; Wani et al., 2014). Vidya 2021 reported yeasts from the mangrove soil sediments of Kerala. In Kerala *Aspergillus* was reported as freshwater fungi isolated from Vembandu lake (Tomas et al., 2017).

Arora et al., 2021 isolated yeasts from coastal wetlands of Andaman Islands, India. *C. auris.* yeast isolated from mangroves soil sediments in Gujrat (Patel., 2020).

Most of the freshwater yeasts are underexplored in the world (Grossart et al., 2019). Previously pigmented yeasts was isolated from the river of mining area of Goa (Garg et al., 2011). So further studies need to be conducted to focus on isolation of freshwater yeasts from different freshwater bodies.

The highest number of aquatic yeasts are isolated and reported by Argentina Carlos et al., (2010)

4.2.1. Characterization of yeasts cultures

Yeast designated as Bchlm-1-2 had a high phosphate solubilization property, was robust to various drastic environmental conditions such as fluctuation in pH changes and high temperatures and grew on mine tailing media as black colonies. Colony colour changed from black to white on MEA media.

Besides from their biosedimentation activity, all strains can fruther investigated for their biotechnological applications.

Strains, such as Crmblm, Ntrvl-1-2, Ntrvl-2-2, and Srg-2-1, showed the presence of pseudo mycelium when cultivated on specified media, as demonstrated by various chemical tests.

Bchlm-1-2 tested positive for catalases. Bchlm-1-2 produced a positive test result with sucrose. All sugar tests were negative for Pnd-3, Pndsm-1, Hrvlm-1-2, Tmrs-2-3, and Tmrs-2-2-4, Ntrvl-2-3. With all sugars, Bh1 and Pndsm-2-2 were weak. Bndl-1-1-1, Pndsm-1-2, and srg-2-1 all tested positive for sucrose but negative for maltose. Ntrvl, Krpr-2, Pndsm-2-2 were positive in the presence of maltose sugar but negative in the presence of sucrose; moreover, Pnd-2 generated gas bubbles. Bndl-1-1, Gh1-5, showed positive with glucose and negative with all sugar.

Among all the studied cultures, seven freshwater cultures gave a positive test in phosphate solubilization and three cultures gave a negative results. Bchlm 1-2 was the best strain, displaying a very clear zone of phosphate solubilization around the colony on the media. The clean zone reached its maximum within 48 hours of strain inoculation and maintained a constant size until the seventh day of the inoculation.

Some soil fungal inoculants reported optimum phosphate solubilization effectiveness even after the 15^{th} day of inoculation, while others did not (Elias F.,2016). Furthermore, microscopic analysis revealed that crystal deposition was present on test plates but not in control plates. Calcium oxlate, a reaction product, was predicted as the crystalline structure. Crystal concentrations were highest near the edges of colonies and least near the inner edges of petriplates.

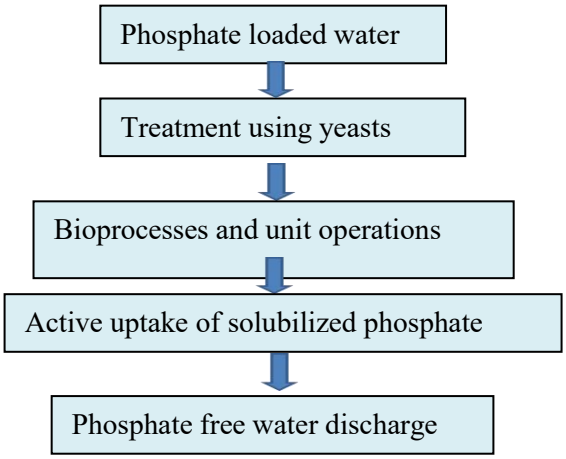


Figure.4.1: Schematic diagram for biotreatment of phosphate loaded water

Among all yeasts strains, 12 strain were tentatively identified up to genus level using morphological and biochemical features. Almost 28% cultures belongs to *Candida sp.*, 2.5% belongs to *Rhodotorula sp.*

4.3. Screening of strain capable of biosedimentaion of colloidal turbidity.

4.3.1. Promising strain for biosedimentation of clay colloids was screened manually, using the biosedimentation efficiency score.

4.3.2. Cultures were classified and quantified for their biosedimentation property based on margin characteristic. Dimorphic behaviour was visible in micromorphology of stained unidentified yeast cultures. The fractal analysis of oligotrophic freshwater yeasts and its relationship to their bioflocculation capacity is little understood. For the first time, fractal analysis of colony edges of freshwater yeast isolated from Goa's several freshwater reservoirs has been performed.

Previously yeast colonies were characterized by its whole surface (Prado., et al 2014). Growth of yeast colony with respect to colony height studied earlier (Ravindranath., et al 1998). Papagianni et al., (2006) quantified the fractal nature of mycelial aggregation in *Aspergillus niger*. Morphological study of yeasts on the basis of the margins of colonies with image analysis was not much done so far.

4.3.2.1. The image analysis-based technology was used to quickly screen many yeast strains for application in wastewater treatment polluted by Goa's mining industry.
The fractal dimension of colony margins has been used to grade the yeast strains in terms of efficient biosedimentaion.
Those yeast with a complex edge were examined for biosedimentaion of colloidal turbidity in mine tailing water and were identified using CmiesJFrad.

Tables 3.21 and 3.23 in result section demonstrates that Bchlm-1-2 had the highest fractal dimension of its colony margins and created compact sediments with clay colloids, while Srg-3-3 did not have complex margins but generated compact sediments with clay colloids and showed the highest reduction in colloidal turbidity in NTU among the cultures with simple colony margins.

The Fractal analysis of yeast colony margins could be a beneficial tool for characterizing freshwater yeast strains and establishing a clear positive or negative link with their bioclarification capability using the fractal dimensions.

Because the same procedure and results were repeated with five best strains, it is possible to conclude that the sediments formed by different strains in simulated turbid mine water were first characterized by a mathematical tool to analyse their fractal dimension to correlate with its biosedimentaion capacity, and it was discovered that those strains with complex margins were forming sediments with a high fractal dimension.

Apart from mathematical analysis, the most notable strains were visually examined for floc compactness. All cultures with complex margins developed compact sediments eg. Bchlm-1-2, formed compact sediments. The fractal dimension of colony margins and their compact floc formation feature were compared for the first-time using fractal analysis of isolate colony margins.

In Fig. 3.19, a snapshot of colony borders of freshwater yeast cultures is shown, and their fractality indices are compared in terms of growth functions and placement
New technique enables a simple study approach. Yeasts develop in predictable patterns, and even after streaking, they do not distribute evenly throughout the plates. It was

simple to watch, photograph, and generate a random data set from a predetermined nested pattern of established colonies on solid media. Furthermore, the square pattern has allowed repeated colonies to expand in a linear fashion.

4.3.2.1. Colony margin growth was monitored and defined using temporal and spatial changes. Because the colonies were inoculated on the same day and in the same conditions, growth and morphological behaviour for each colony could be easily compared.

Three nested colonies grew in the same pattern, increasing in complexity in their margins over time, but their fractality indices differed depending on their position.
The underlying cause could be colony heterogeneity or a genetic switch to live in nutrient-depleted media. With increasing margin complexity, we also see an increase in the fractality index. Because these traits may be ruled by chemo-taxis interaction or gene expressions, more research into colony margins and their interactions is needed to determine the reason of variance in growth patterns.

Ruusuvuori et al., (2014) used an image-based technique to conduct the qualitative analysis of yeast colony margins. Two-dimensional top-down binary pictures were used to examine the morphology of *Saccharomyces cerevisiae* colonies (Gontar., 2018).
Present study claims usefulness of image based technique for the screening of most promising strain designated as Bicholim1-2 shown to be the most promising strain for biosedimentation of clay colloids.

4.4.1. Study of potential test yeast morphology due to change in medium:
The test strain was tested for a change features when grown in nutrient rich medium (Commercially available MEA), which is usually rich in carbon source for microbial growth and simulated mine water to verify its wild behaviour.

Wherase test strain was cultivated on two separate medium, significant morphological changes were observed. The colour of yeast colonies changed after strain transfer to the changed media could be due to phenotypic changes in response to nutritional stress conditions. After 7 days of inoculation strain which were grown on MEA media remained white shiny smooth and gelatinous surface with complex margins while strain

which were grown on modified media turned black in colour with shiny smooth and gelatinous surface with complex margins. Strain grown on modified medium were spread plated on fresh MEA and the modified medium and observed that strain retained black colour on the modified medium while it regains its white color on MEA medium (Fig.3.18). Bicholim1-2 demonstrated remarkable resistance in the modified medium which was simulated to the mine environment.

4.4.2. Characterization of soil sediment and yeast using SEM and FTIR

The isolate was found to have a large surface area and surface charge. Catalases are reduced. Phosphate solubilization efficiency was high. Strain can also reduce Fe and Mn in the mine tailing water.

The results of the FTIR analysis used, to examine the functional groups present in the soil sediment and yeast mixed soil sediment. The functional Groups of the yeast mixed sediment, and control sediment did not show much variation. Little displacement was observed in the mixed sample.

Both sample had comparable spectra. The OH functional group and the O-H stretching of the polymeric molecules were attributed to the prominent peak seen at 3400-3294 cm^{-1}. Additionally, the peak between 2929 and 2928 cm^{-1} was identified as C-H groups. Around 1660–1657 cm^{-1} was the typical stretching peak of the carboxylic COO-double bonds of deprotonated carboxylate functional groups. Most of the peaks of studied samples showed peak at 2900 cm^{-1}.

4.4.3. The extent of the dye's adsorption (Aclian blue) indicates the strength of the negative charge on the cell surface. The presence of hydrophilic groups at the surface was primarily indicated by yeast's relative hydrophobicity. According to Laurent et al. (2009), hydrophilic molecules are often polar or charged while hydrophobic molecules are non-polar. A better availability of polar/charged groups like carboxyls at the yeast cell surface can be correlated with a lower relative hydrophobicity and a larger negative surface charge. The greatest concentration of negative and/or polar sites on the yeast surface probably corresponds to the greatest concentration of particle fixation sites. This results is matching with work of Kordialik (2008) concluded Copper and Lead removal

from yeast cells is less effective when their relative hydrophobicity is higher and their surface charge is lower.

Many researcher have reported that a hydrophobicity percentage of between 30 and 40% could ability to interact with epithelial cells and at least perform transient adhesion (Abdulla et al. 2014; Sidira et al. 2015; Ilavenil et al. 2016). In this study, the selected strain showed less hydrophobicity compared to baker yeast so it can be predicted the test strain is less virulent.

4.5. Assessment of the quality of drinking water in mining areas of Goa

Physicochemical analysis of different water samples were confirmed to be within acceptable limits. Furthermore, results of all parameters were found to be nearly identical in monsoon and non-monsoon seasons. Because mining did not take place on a large scale, turbidity was not as high as it was previously reported. Even though turbidity was found to be above the permissible levels in all water samples sampled from various locations.

The temperature of all the freshwater bodies under study closely matched with the general trend of ambient temperature and stayed consistently. In general, similar range of temperature was also reported by Sawaikar (2016),Vikrant (2001), Renu (2014).

There were no seasonal differences in the values of water parameters of the samples. Renu et al., (2014) reported nitrate concentration in Carambolim lake, Pilar lake was 0.38 and 1.31m respectively. And Phosphate concentrations reported 0.70 and 0.570 m respectively. Present study observed concentrations of Phosphate and nitrate ranged from 0.01 to 0.02 m and 0.28 to 0.85 respectively in monsoon sesason. The observations were similar to those by Sawaikar et al., (2016) and Vikrant et al., (2001). Water samples from Arvalem in monsoon showed strong correlation of turbidity with Nitrate, Phosphate, Sulphate. While Morlem pond showed strong correlation between turbidity and nitrate. Moreover, Morlem tank showed strong co- relation coefficient between turbidity and Phosphate. Bicholim tank showed modrate co-relation coefficient of turbidity with Nitrate, Phosphate, Sulphate non monsoon.

So, it is concluded that the low concentration of Nitrate and Phosphate indicate in the freshwater bodies were oligotrophic in nature.

4.6.1. Lab scale bioflocculation using natural yeast cultures at upto scale 1-100 liters.

The rate of biosedimentation of suspended particles in the test jar was higher than the control jar. The suspended particles settled to the bottom of the bell jar, and there was some wall deposition on the wall of the test bell jar. The water composition was essentially identical in slides taken from different depths of the bell jar and tank after 2hr of yeast inoculation and agitation. At a depth of 2 L(11cm), near bottom of the bell jar yeast cell hyphae were discovered with bacterial cells. Single coccid and yeast cells were found at the top of the jar at a depth of 14L (volumetric depth) from the bell jar's bottom.

Furthermore, water samples collected at various depths in the tank and plated on MEA media revealed the same microbial makeup and colony size. Within the same time period, triplicates of withdrawn water samples exhibited identical turbidity reduction values.

The control tank, in which yeasts suspensions were not added, the turbidity was found to decrease slowly, and sedimentation also occurred slowly, whereas the turbidity in the test tank was found to decrease swiftly.

The treated water was decanted and then passed through a sand bed filter in order to get 100 percent turbidity-free water in a short period of time.

4.6.2. After 30 minutes of yeast addition to turbid water, the decanted of water can be run through a sand bed filter to obtain clean water with low turbidity, according to the results.

4.6.3. To enable clay colloids and microbial surface interaction, a mechanical stirrer is necessary.

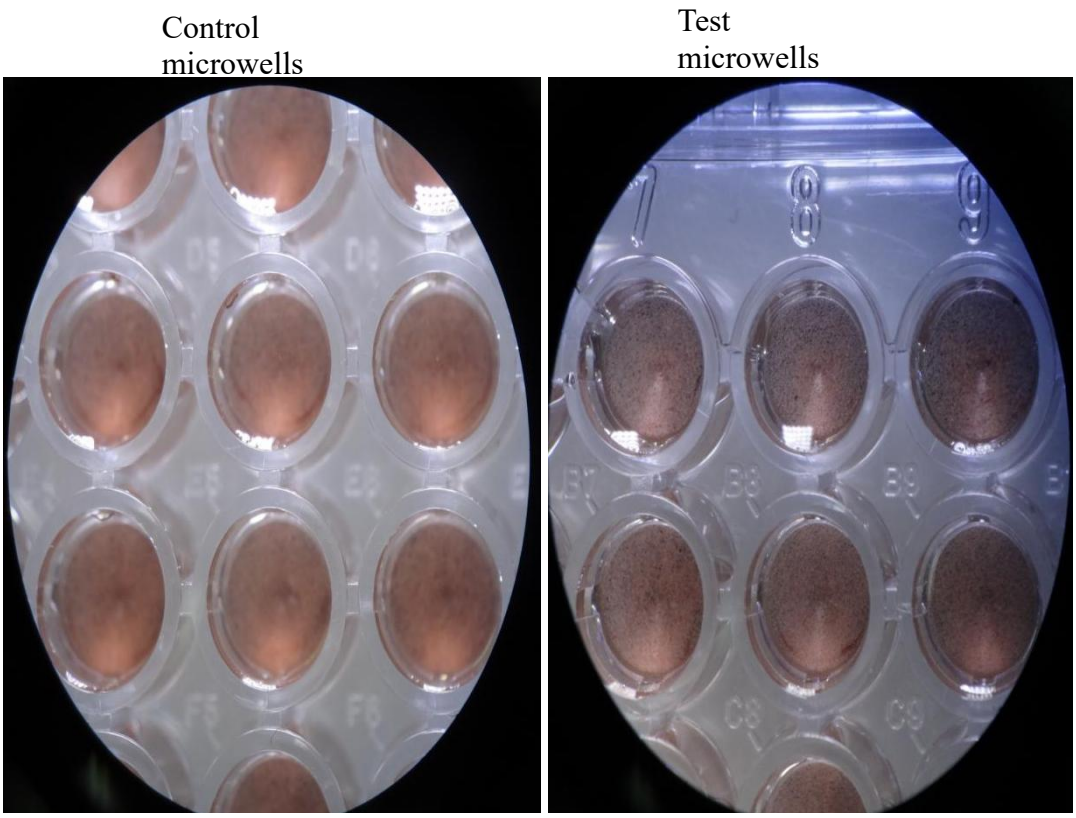


Figure.4.2: SMTTW in the micro well. (a) Control well showing turbid mine water, (b) Test well showing granular sediments, settled in the bottom due to binding of clay colloids with yeast.

The benefit of yeast treatment is that it cuts down on the time, takes to settle the suspended particles and can be highly successful in removing the small particles that are difficult to settle out.
Furthermore, significant in turbidity reduction was seen in natural mine tailing water due to the addition of yeast.

While the turbidity remained high in natural mine tailing water compared with simulated mine turbid water in control reactor. The reason could be because natural mine tailing water contained clay colloids with proper water saturated.

Moreover, the sediment deposition was observed 1.5 and 4.5 ml respectively in control and test cone respectively during biosedimentation test of the natural mine tailing water.

So, it can be confirmed, and concluded that yeast mediated biosedimentaion occurs in mine tailing turbid water as the sediment deposition in test cone is 3 times more than control cone in which the particles settled naturally.

The compactness of sediments formed were high, due to binding of yeast and clay particles. It can be observed from the biosedimentation kinetics graph that the sediments were compact since the turbidity quickly decreased when the test water column was restirred. And no breakage of biosediments were observed. Sediments settled fast after stirring the water. Moreover, Yu et al. 2011 studied the breakage of sediments formed by alum salt and concluded that sediments can break and regrow when the water column disturbed.

Leila Mosleh et al., (2014) reported a reduction in turbidity of water from 500 NTU to 85 NTU when using alum salt (0.005gm/L). Moreover, Aslani et al., 2012 reported a reduction of turbidity of water from 500 NTU to 10 NTU using alum salt (0.045gm/L), for a reduction in turbidity in an efficient time the dose of coagulant should be increased. While the present study can cause a reducution in turbidity of water using little amount of biomass (0.005 gm/L) from 780-800 NTU to 20-40 NTU within 1 hour, without breaking and remixing of the biosediments.

The findings could provide an insight into how yeast culture interacts with wastewater systems to eliminate colloidal turbidity. Yeasts can also be used as a coagulant because it is a good supply of fungal by products such amylase, chitin, and lactic acid (Oliveira et al., 2015). In comparison to bacteria, yeast can be also examined for its tolerance to inhibitory compounds.

4.7. Standardization of bioflocculation process for field level application scale 500 litres-1000 litres.

For the first time in the world yeasts isolated from freshwater habitats were used to study the biosedimentation of the clay colloids of the SMTTW. Mining activity contributes to water pollution, resulting in severely turbid water with SPM ranging from 4 to 22 gm per liter (AEQM, 1997) due to runoff of mine tailings soil during the southwest monsoon season, in Goa as we discussed.

Because freshwater yeasts have potent cell wall polysaccharides capable of attaching to inorganic colloids, the main goal of our research was to determine the practicality and bioefficacy of using them as active, live microbial cellular coagulants. After successfully screening, the most promising yeast strains (for biosedimentation of clay colloids), was investigated under various conditions.

For an efficient biosedimentation of clay colloids, all parameters were tuned, including SMTTW pH, system temperature, biomass of bioflocculant, age of yeast cultures, and coefficient of mine tailing water and yeast biomass. The stoichiometric connection between a flocculating yeast strain and a turbidogenic suspended material was investigated.

pH values were remained between 6-7 and temperature observed between 24-29 degree centigrade (ambient) for efficient sedimentation (throughout the experiment). Using an 0.005 gm/L (dry weight) of yeast suspension showed compact floc formation with clay and an efficient turbidity reduction.

Turbidity removal is maximum at an optimized dose of yeast suspension and when the dose is increased turbidity tends to reappear due increase number of yeast cells. 4^{th} and 5^{th} day old yeast proved more efficient as it coincided with the log phase of the selected strain. Bioflocculation kinetics were found to be greater after almost 40-60 min in the test reactors.

Hence pH and age of culture required to achieve a high reduction in turbidity ,as found 6-7 and 4^{th}-5^{th} day old respectively, using the scoring scheme (Table.2.3). When the pH was lower than 6-7, the water column was less clear, and when the pH was greater, the water became opaque (Fig. 3.34). Furthermore, wall deposition was higher in the column that was inoculated with 6^{th} and 7^{th} days old yeast biomass.

4.7.1. To keep the concentration of yeast biomass in SMTTW biosedimentation at a minimum, a 1:9 ratio of yeast suspension to mine tailing water was used.

4.7.2. Turbidity reductions in mine tailing water up to 1 L indicated a considerable reduction in turbidity within an hour after the yeast was added. The strain reduced the

turbidity from 780 to 73 NTU in one hour, demonstrating a 90% reduction in turbidity from initial. and 61% when compared to control under laboratory conditions.

4.7.3. Heat-treated yeast suspensions were not as effective at reducing turbidity than live yeast cells (Fig.3.36). This results gives an idea, that polysaccharide mediated biosedimentation is occurring .

4.7.4. As shown in Fig.3.48, volume of sediment was higher in the cone when yeast suspension was added, compared to the control cone (with only SMTTW containing 3.8 gm/L suspended solids but no yeast suspension). When just 100 ml of yeast suspension with 0.005gm/L was added to 900 ml of deionized water, the sediment volume was low (Positive control).

The turbidity in the decant of the bell jar and the 100L tank following the biosedimentation operation was reduced by about 76 and 88 NTU respectively, from 746 and 810 NTU. When the test and control cones decanted water were passed through the sand bed filter, the turbidity decreased by 0.1 NTU 40 NTU for the test and control cone respectively. The presence of Extracellular polysaccharide of yeast on the wall of the bell jar proves the mobilation of yeast in the container. Sarkaret al. (2018) conducted a comparative study of turbidity removal using biological and chemical agents and discovered that chemical coagulants were more effective for turbidity removal than natural coagulants, despite the fact that chemical coagulants increase TDS in treated water while natural coagulants decrease TDS.

4.7.5. Furthermore, agitating or stirring the water column resulted in a significant decrease in turbidity, thus provides a proper interaction of colloids and flocculants. The speed of agitation is suggested as 30-100 rpm for 10 min by Saritha et al., (2014), which is almost similar with that of the present study 50 rpm for higher scale biotreatment of turbidity removal.

Other parameters, such as phosphate and nitrate, were significantly reduced after yeast treatment in the SMTTW. Moreover, after passing through the sand bed filter, the mine water was colourless. Moreover, after plating of sand bed filter effluent on malt extract and nutrient agar media, no yeast colony was observed.

It can be concluded that yeast cells were caught by sand since nutrient agar plates revealed over the expansion of bacterial colony while yeast colony was lacking in plates. As a result, tertiary treatment is required to obtain turbid-free water in a short period of time. In addition, a disinfection system can be installed in the water treatment system.

For near-complete elimination of colloidal turbidity, the active cellular biocoagulant and the biotreatment process parameters can be tweaked. This could lead to the development of an environmentally friendly biotreatment technology to address the challenges created by turbidity in drinking water supplies in Goa's mining zones during monsoon.

4.7.6.Yeast combination study for biosedimentaion test

A single yeast strain reduced turbidity better than a pair of yeast strains. Tubes and bottles with mixed cultures revealed massive inner wall deposition (Fig.3.59,3.56). And, in comparison to single culture's bottles, sedimentation rate was modest after forcefully agitation in bottles of mixed culture eg. As a result, it was discovered that a single culture was capable of significantly reducing turbidity in SMTTW

4.8. Biosedimentation studies performed at lab scale upto the scale of 500 L to 1000L

Aluminum sulphate, chitin, and sago were utilised in coagulation studies, and the results showed that the method efficiently eliminated turbidity from water using 0.1–0.4 g L^{-1} of the coagulants (Saritha et al., 2017).

In present study the pH was observed similar to the other reported for efficient turbidity removal. The best pH range for removing turbidity was discovered to be 7 for alum and 6-8 for chitin, yielded the greatest turbidity removal (Saritha et al., 2017; Quasim et al., 2018). So this technique does not require pH maintenance.

4.8.1. Even after 1 hours, the turbidity of natural mine tailing water and SMTTW prepared from freshly collected (mine tailing soil) and processed remained between 400 and 300 NTU. It's possible that protracted storage has caused the soil to oxidize.

4.8.2. Significant turbidity reduction was observed in an efficient time in the 1000L test tank (in which yeast was added) when compared to the 1000L control tank (in which

yeast was not added), with sedimentation rate constants of 0.000067 and 0.000396 NTU^{-1} min^{-1} for control and test, respectively.

4.8.3. The 1000L turbidity test showed a bigger reduction than the 500L test, possibly because to the higher tank height, which provided more surface area for clay colloids and yeast cells to interact.

4.8.4. Plates (spread plated with water sample taken after experiment) did not reveal yeast growth on MEA plate, spread plated with test tank effluent, which was passed through the sand bed. Microbes grown in the same pattern in wastewater collected directly from the control and test tanks. A microscopic image of a microbial colony on a nutrient rich plate indicates that it was a bacterial colony. As a result, it can be assumed that the additional yeast was not present in the sand bed's outflowing contents.

4.8.5. The water tank was stirred 50 times in one minute for 10 minutes. The hydraulic retention time of water in the sedimentation tank may be 30-40 minutes because maximal turbidity reduction occured within 30-40 minutes. The tank and sand bed had a flow rate of 41 minutes per litre. The turbidity of the sand bed effluent after 60 minutes was 50 NTU (for the control tank effluent) and 1.5 to 0.1 NTU (for the test sand bed effluent). There was no pressure applied during the hydraulic loading. As a result, by using pressure filters, multimedia filters with charcoal, and a further disinfection system, the best grade of treated water can be obtained from this process.

4.8.6. The flocculant cost associated with the proposed technique is comparatively very less than that of existing methods. In particular, the cost of coagulant for the treatment of 1000 m^3 of turbid water with alum, raw soybean, and defatted soybean is approximately 2441.48 INR, 939.03 INR, and 305.9 INR, respectively, as per the study shown by (Hussain.G., 2020). However, the flocculants cost associated with the proposed technique is only 63.38 INR for treatment of the same amount of turbid water. Yeast based flocculation is not only cost-effective but also has the potential of marketability. The daily costs of doses of coagulating the coagulation – flocculation process using the coagulants PACl + $FeCl_3$ and PACl were 55.87.3 INR and 6612.34 INR, respectively (Zafra, M et al., 2020)**.** So the proposed technique is considered cost effective.

In this study the most challenging work was to prepare simulated turbid mine water of desired turbidity. Sediment concentrations cannot be determined easily or quickly in the field, and transportation to a laboratory for analysis is time-consuming and can be costly (Thackston and Palermo, 2000). As a result, these traditional methods are increasingly being replaced in favor of accurate, continuously-collected surrogate data for quantification of suspended solids that may be safer and less expensive to obtain, such as turbidity measurements.

Biosedimentation was almost reproducible at every scale in present work, it is suggested tank should be loner for more efficient biosedimentation.

4.9. Yeast imobilization study in agar

Beads were made in a cylinder shape. Beads that were not treated with yeast turned yellow, whereas those treated with yeast retained in their original colour after biosedimentation test, can be concluded that yeast retained the colour of beads.

Clays surface deposition was not seen in control beads, while test beads had both surface deposition and embedded clay. As a result, it can be inferred that immobilization and binding of clay with yeast surface occurred; however, because the turbidity of the water did not decrease much, this immobilization approach cannot be used to reduce turbid water turbidity.

4.10. Molecular Study of only selected yeast Strains

Candida orthopsilopsis was discovered to be the aquatic yeast utilising the PCR technique. This is the first time in the world that *C.orthopsilosis* has been isolated in a freshwater system. Earlier this species was isolated from other sources but not from freshwater. The characterization of this species also revealed that it is resistant to high pH, heat, and can thrive on modified media made from mine soil transported from the mining location. Moreover, among all studied cultures this strain displayed the best phosphorus solubilization property. Conclusion can be made that this wild type yeast can be investigated further for biotechnological uses.

After considering the phylogenetic tree of the yeast isolate, which was created by blasting with known species sequences, it revealed that it is genetically identical to

Ireland's isolate, whose gene bank number is (FN812686). Further, *C.orthopsilosis* species isolated from other environments, such as clinical, vegetation, soil, and air, beverage (Wong et al., 2020), isolated from naturally fermented Brazilian table olives (Simoes et al., 2021), can be compared to this isolate for its genetic variation.

Additionally, *C.orthopsilosis* isolated from olive oil was evaluated for safety, survival in gastrointestinal and digestion conditions, antimicrobial activity, cellular hydrophobicity, auto aggregation ability, adhesion to epithelial cells, co-aggregation, and inhibition of pathogenic bacteria adhesion and found to be good for probioactivity of the same strain (Simoes et al., 2021).

Candida tropicalis was identified from rain water utilizing the PCR technique. This is the first report ever to link yeast to rainwater from the southwest monsoon. This is the first report of *Candida sp.* and *Candida tropicalis* specifically in rainwater because *Candida tropicalis* has never been suspected of penetrating the southwest monsoon airborne cloud environment. The relationship between meteorological variables and yeast concentration can be studied further. At different sea levels, the variety of microbes in clouds can be examined, as well as their function in ambient settings and ecology. Since it has been proposed (Sarah et al., 2017) that cloud formation originates cloud condensation nuclei due to the presence of air particles and microbes, such as bacteria, fungi, and phytoplankton, their function in cloud nucleation could be examined.

4.14 Future scope

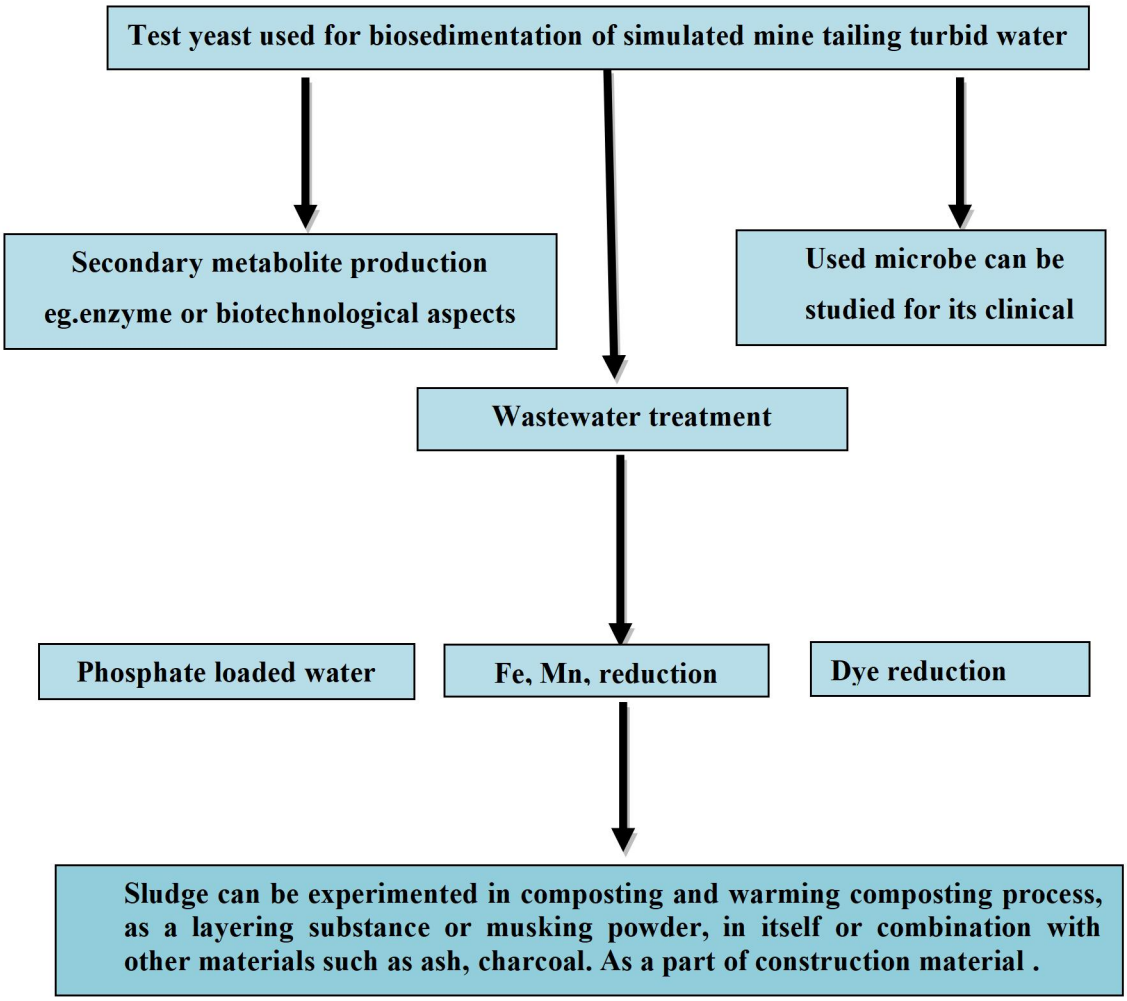


Figure.4.3: Diagram depicting the potential of the promising yeast in the future

SUMMARY

SUMMARY

The broad objective of this study was to evaluate promising yeast strain for the removal of colloidal turbidity from mine tailing water. It has been reported that colloidal turbidity was 4gm/l in the surface water reservoir of mining area in Goa. The key finding of this study is outlined as follows;

- First time in the world, freshwater yeast is used for the removal of turbidity of simulated mine tailing turbid water.
- Pioneer use of JFRAD in public domain for fractal analysis have performed. This image analysis based technique holds excellent potential for rapid screening of a large number of yeast strains required in different applications.
- Goa's freshwater bodies, revealed a great diversity of aquatic yeast. Freshwater ecosystem for yeast is not well explored .
- Culture was maintained on artificial media for long time without any change in morphological and turbidity removal character.
- Wild type yeast showed consistency in bioclarification property over long period of time.
- All studied water parameters were found to be similar in monsoon and non monsoon season and were below the permissible level.
- Yeast mediated biosedimentation is scale independent.
- Further, *C. orthopsilosis* species isolated from other environments, such as clinical, vegetation, soil, and air, can be compared to this isolate as it is isolated from nutrient poor freshwater body.
- Presence of yeast in rain water gives idea of circulation of microbes in the environment.

The study has several suggestions for further research work on the basis of research findings.

BIBILOOGRAPHY

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Abdel-raouf, N. (2012). Microalgae and wastewater treatment. *Saudi Journal of Biological Sciences*, *19*(3), 257–275. http://doi.org/10.1016/j.sjbs.2012.04.005.

Abdi O, & Kazemi M. (2015). A review study of biosorption of heavy metals and comparison between different biosorbents, J. Mater. Environ. Sci. 6 (5) (2015) 1386-1399.

Abdullah, Mohammad & Roslan, Azmi & Kamarulzaman, Mohd Hasrul & Erat, Muhammed. (2017). Colloids removal from water resources using natural coagulant: Acacia auriculiformis. AIP Conference Proceedings. 1885. 020243. 10.1063/1.5002437

Acquabella. (2006). Coagulation and Flocculation Process Fundamentals, 199–206. Retrieved from http://www.acquabella.net/coagulation and flocculation.pdf

Afzal, A. M., Rasool, M. H., Waseem, M., & Aslam, B. (2017). Assessment of heavy metal tolerance and biosorptive potential of *Klebsiella variicola* isolated from industrial effluents. *AMB Express*. http://doi.org/10.1186/s13568-017-0482-2

Agunbiade, M. O., Pohl, C. H., & Ashafa, A. O. T. (2016). A Review of the Application of Biofloccualnts in Wastewater Treatment, *25*(4), 1381–1389. http://doi.org/10.15244/pjoes/61063

Ailamaki, A., Faloutos, C., Fischbeck, P. S., Small, M. J., & Van Briesen, J. (2003). An environmental sensor network to determine drinking water quality and security. *ACM SIGMOD Record*, *32*(4), 47. http://doi.org/10.1145/959060.959069.

Ajao,V., Bruning, H., Rijnaarts, H.,& Temmink, H. (2018). Natural flocculants from fresh and saline waste water: Comparative properties and flocculation performances, Chemical Engineering Journal 349(March), 622–632.

Aibeche, C., Selami, N., El, F., Zitouni, H., Khadidja, H., & Amira, O. (2021). Bioremediation potential and lead removal capacity of heavy metal - tolerant yeasts isolated from Dayet Oum Ghellaz Lake water (northwest of Algeria). *International Microbiology*, (123456789). http://doi.org/10.1007/s10123-021-00191-z.

Akhtar S., Arzan A.,. Yaqub M., Shah, A.M. (2005.) Ecoloical Study of Khusalsar lake Kashmir: Foutrth Fungal Community.

Alez, I. N. E. G., Lach-hab, M., & Blaisten-barojas, E. (1999). On the Concentration Dependence of the Cluster Fractal Dimension in Colloidal Aggregation, *127*, 119–120.

Alfonso, V., & Losada, R. (2018). Removal of Chromium in Wastewater from Tanneries Applying Bioremediation with Algae, Orange Peels and Citrus Pectin, Contemporary Engineering Sciences11(9), 433–449.

Alhamed, Y. A. (2013). Kinetics and Performance Analysis of Batch Electrocoagulation Unit Used for the Removal of a Mixture of Phosphate and Nitrate Ions from Industrial Effluents, *8*, 3176–3185.

Al-Homaidan, Ali & Al-Qahtani, Hussein & Al-Ghanayem, Abdullah & Ameen, Fuad & Ibraheem, Ibraheem. (2018). Potential use of green algae as a biosorbent for hexavalent chromium removal from aqueous solutions. Saudi Journal of Biological Sciences. 25. 10.1016/j.sjbs.2018.07.011.

Ali, E. N., Muyibi, S. A., Salleh, H. M., Alam, Z., & Salleh, M. R. M. (2010). Production of Natural Coagulant from Moringa Oleifera Seed for Application in Treatment of Low Turbidity Water, *2010*(March), 259–266. http://doi.org/10.4236/jwarp.2010.23030.

Ali, Esam & Hashem, M.. (2007). Removal Efficiency of the Heavy Metals Zn(II), Pb(II) and Cd(II) by Saprolegnia delica and Trichoderma viride at Different pH Values and Temperature Degrees. Mycobiology. 35. 135-44. 10.4489/MYCO.2007.35.3.135.

Al-mamun, A., Alam, Z., & Raus, R. A. (2016). Fungal Coagulant for Reduction of Water Turbidity, ARPN Journal of Engineering and Applied Sciences10–12.

Aloysius, R., M.I.A. Karim and A.B. Ariff. 1999. The mechanisms of cadmium removal from aqueous solution by nonmetabolizing free and immobilized liv e biomass of Rhizopus oligosporus. World J. Microbiol. Biotech., 15 (5): 571-578.

Al-wasify, R. S. (2015). Bacterial Exopolysaccharides as New Natural Coagulants for Surface Water Treatment, International Journal of Pharm Tech Research 8(9), 198–207.

Al-Sameraiy, M. (2017) A new approach using coagulation rate constant for evaluation of turbidity removal. *Appl Water Sci* **7,** 1439–1448 . https://doi.org/10.1007/s13201-015-0341-8.

Alzate, E. (2018). Potential of microalgae in the bioremediation of water with chloride content, Brazilian Journal of Biology 1–5.

Amato, P., Joly, M., Besaury, L., Oudart, A., Taib, N., Deguillaume, L. Mone, I. (2017). Active Active microorganismsthrive among extremely diverse communities in cloud water. PLoS ONE, Public Library of Science,

Amin, M. T., Alazba, A. A., & Shafiq, M. (2016). Adsorption of copper (Cu) from aqueous solution using date palm trunk fibre: isotherms and kinetics, *3994*.

Amosa, M. K., Jami, M. S., Alkhatib, F. R., Tajari, T., Jimat, D. N., Owolabi, R. U., Alkhatib, F. R. (2016). Turbidity and suspended solids removal from high-strength wastewater using high surface area adsorbent: Mechanistic pathway and statistical analysis T*Cogent Engineering*, *5*(1). http://doi.org/10.1080/23311916.2016.1162384

Ani JU, Nnaji NJ, Okoye COB, Onukwuli OD (2012) The coagulation performance of Okra Mucilage in an industrial effluent by Turbidimetry. Int J Chem Sci 10(3):1293–1308https://doi.org/10.3389%2Ffbioe.2022.775058

Antov, M.G., Sciban, M.B. and Prodanovic, J.M. (2012) Evaluation of the Efficiency of Natural Coagulant Obtained by Ultrafiltration of Common Bean Seed Extract in Water Turbidity Removal. Ecological Engineering, 49, 48-52. http://dx.doi.org/10.1016/j.ecoleng.2012.08.015

A.S. Sheoran, Sushil Bhandari. (2005) Treatment of Mine Water by a Microbial Mat: Bench-scale Experiments. Mine Water and the Environment 24: 38–42 IMWA Springer-Verlag 2005.

Annamalai, H., Slingo, J. Active/break cycles: diagnosis of the intra-seasonal variability of

The Asian Summer Monsoon. *Climate Dynamics* 18, 85–102 (2001). https://doi.org/10.1007/s003820100161.

Ansari, M. I., & Malik, A. (2007). Biosorption of nickel and cadmium by metal resistant bacterial isolates from agricultural soil irrigated with industrial wastewater, Journal of Water Resource,98, 3149–3153.

Anusha, S. U., Sundar, S. K., & Williams, P. G. (2014). Original Research Article Studies on the isolation and characterization of marine yeast, glucan production, and immunostimulatory activity on Carassius auratus, Int.J.Curr.Microbiol.App.Sci. *3*(9), 230–240.

Arastehfar, A., Fang, W., Pan, W., Liao, W., Yan, L., & Boekhout, T. (2018). Identification of nine cryptic species of Candida albicans, C. glabrata, and C. parapsilosis complexes using one-step multiplex PCR, 1–9.

Aravantinou, A. F., Theodorakopoulos, M. A., & Manariotis, I. D. (2013). Bioresource Technology Selection of microalgae for wastewater treatment and potential lipids production. *Bioresource Technology*, *147*, 130–134. http://doi.org/10.1016/j.biortech.2013.08.024.

Areawide environmental quality management (AEQUM) plan for the mining belt of Goa. (1997) Report by TERI NEW DELHI and Goa.

Ariya, P. A., Nepotchatykh, O., Ignatova, O., & Amyot, M. (2002). Microbiological degradation of atmospheric organic compounds. https://doi:10.1029/2002GL015637, 2002.Asian Summer Monsoon. Clim. Dyn. 18: 85–102. https://doi.org/10.1007/s003820100161.

A.S. Sheoran, Sushil Bhandari. (2005) Treatment of Mine Water by a Microbial Mat: Bench-scale Experiments. Mine Water and the Environment 24: 38–42 IMWA Springer-Verlag 2005.

Attori, H. (1970). Adhesion between cells of E. coli particles, 359, 351–359.

Azeredo, J., Ramos, I., Rodrigues, L., & Oliveira, R. (1997). Yeast Flocculation: New Method for Characterising Cell Surface Interactions. Journal of the Institute of Brewing, 103(December), 359–361.

Aziz, S. Q., & Ali, S. M. (2016.) Performance of Biological Filtration Process for Wastewater Treatment: A review, Journal of pure and applied science, 28, 554–563.

Oluwatosin Gbemisola OladipoOlusegun Olufemi AwotoyeAkinyemi OlayinkaCornelius Carlos BezuidenhoutMark Steve Maboeta (2018) Heavy metal tolerance traits of filamentous fungi isolated from gold and gemstone mining sites. Braz. J. Microbiol. 49 Jan-Mar 2018 • https://doi.org/10.1016/j.bjm.2017.06.003

BacteriaIntheclouds.Eniscuola,http://www.eniscuola.net/wpcontent/uploads/2017/03/batteri_nuvole_EN.pdf.

Balaban, V., Lim, S., Gupta, G., Boedicker, J., & Bogdan, P. (2018). Quantifying emergence and self-organization of Enterobacter cloacae microbial communities. *Scientific Reports*, (January), 1–9. http://doi.org/10.1038/s41598-018-30654-9

Barry, D., & Mcgee, S. (2009). Relating Fractal Dimension to Branching Behaviour in Filamentous Microorganisms, 71–76. http://doi.org/10.21427/D77325.

Bauer, H.; Kasper,Giebl, A.; Löflund, M.; Giebl, H.; Hitzenberger, R.; Zibuschka, F.; Puxbaum, H.(2002) The contribution of bacteria and fungal spores to the organic carbon content of cloud water, precipitation and aerosol. Atmospheric Research, Volume 64, Issue 1, p. 109-119. https://doi.org/10.1016/S0169-8095(02)00084-4.

Bayramoglu, G..Tuzun, I.,Celik, G., Yilmaz, M..(2006). Arica, M.Y. Biosorption of mercury (II), cadmium (II) and lead (II) ions from aqueous system by microalgae Chlamydomonas reinhardtii immobilized in alginate beads.Int. J. Miner. Process. 81, 35–43.

Becker, D., Rodriguez-mozaz, S., Insa, S., Schoevaart, R., Barcelo, D., Cazes, M. De, Wagner, M. (2017). Removal of Endocrine Disrupting Chemicals in Wastewater by Enzymatic Treatment with Fungal Laccases.JCA

Belzer, R. B. (2020). Achieving Economically Feasible Drinking Water Regulation, *11*(2), 294–318. http://doi.org/10.1017/bca.2019.21.

Berman, J., and Hadany, L. (2012). Does stress induce (para) sex? Implications for Candida albicans evolution. Trends Genet. 28, 197–203.http:// doi: 10.1016/j.tig.2012.01.004.

Bevilacqua, A., Petruzzi, L., Corbo, M., & Sinigaglia, M. (2013). Bioremediation of Olive Mill Wastewater by Yeasts–A Review of the Criteria for the Selection of Promising Strains. Applied Bioremediation.

Bellon-fontaine, M., & J, C. (1996). Colloids and Microbial adhesion to solvents : a novel method to determine the electron-donor / electron-acceptor or Lewis acid-base properties of microbial cells.

Beuchat LR (1979) Comparison of acidified and antibiotic-supplemented potato dextrose agar from three manufacturers for its capacity to recover fungi from foods. J food protect 42: 427-428.

Bhattacharya, S., Saha, I., Mukhopadhyay, A., Chattopadhyay, D., & Chand, U. (2013). Role of nanotechnology in water treatment and purification: Potential applications and implications, ISSN 2249-8532 Original Article *3*(3), 59–6.

Bhatti, Z. A.; Mahmood, Q.; Raja, I. A. (2009). Sewage Water Pollutants Removal Efficiency Correlates to the Concentration Gradient of Amendments. J. Chem. Soc. Pakistan 2009, 31, 665.

Bharathi S, Saravanan D, Radhakrishnan M & Balagurunathan R (2011) Bioprospecting of marine yeast with special reference to inulinase production. Int J ChemTech Res 3: 1514-1519.

Bhunia, B., S, M. D. G., Mandal, T., & Dey, A. (2012). Improved production, characterization, and flocculation properties of poly (γ) -glutamic acid produced from Bacillus subtilis, *3*, 389–394.

Bilal, M., Rasheed, T., & Eduardo, J. (2018). Biosorption: An Interplay between Marine Algae and Potentially Toxic Elements — A Review, Marine drug.,1–16.

Bilotta, G. S., & Brazier, R. E. (2008). Understanding the influence of suspended solids on water quality and aquatic biota, *42*, 2849–2861. http://doi.org/10.1016/j.watres.2008.03.018.

Bellon-fontaine, M., & J, C. (1996). Colloids and g Microbial adhesion to solvents : a novel method 2013 to determine the electron-donor / electron-acceptor or Lewis acid-base properties of microbial cells.

Bonaly, R., Nancy, I., & Cedex, N. (2016). Ifp 206, (May), 1279–1284...

Boddy L, Wells JM, Culshaw C, Donnelly DP. (1999) Fractal analysis in studies of mycelium in the soil. Geoderma 1999, 88:301-328.

Boer, D. H. De, Stone, M., & Le, L. M. J. (2000). Fractal dimensions of individual and populations in streams, 667(June 1999).

Boekhout, T., Amend, A.S., El Baidouri, F. Yourkov (2022).Trends in yeast diversity discovery. *Fungal Diversity* 114, 491–537. https://doi.org/10.1007/s13225-021-00494-6.

Borkar, R. P., Gulhane, M. L., & Kotangale, A. J. (2013). Moving Bed Biofilm Reactor – A New Perspective in Wastewater Treatment, Marine drug, *6*(6), 15–21. Borowitzka, M. A. . Limits to Growth.

Boundy-mills, K. L. (2014). Methods for the Isolation and Investigation of the Diversity of Cold-Adapted Yeasts and Their Ex-Situ Preservation in Worldwide Collections. http://doi.org/10.1007/978-3-642-39681-6

Box, P. (2014). Bioremediation by using of microbes and algae with special reference to Coastline Environment, *1*(6), 130–140.

Brandão, L.R., Libkind, D., Vaz, A.B., Espírito Santo, L.C., Moliné, M., de Garcia, V., van Broock, M.R., & Rosa, C.A. (2011). Yeasts from an oligotrophic lake in

Patagonia (Argentina): diversity, distribution and synthesis of photoprotective compounds and extracellular enzymes. *FEMS microbiology ecology, 76 1*, 1-13 .

Brim, H., S. C. McFarlan, J. K. Fredrickson, K. W. Minton, M. Zhai, L. P. Wackett, and M. J. Daly. (2000). Engineering *Deinococcusradiodurans* for metal remediation in radioactive mixed waste environments. Nat. Biotechnol.18**:**85-90.

Brooks, S. D., Thornton, D. C. O. (2018). Marine Aerosols and Clouds. Annual Review of Marine Science 2018 10:1, 289-313.

Buthelezi, S. P., Olaniran, A. O., & Pillay, B. (2009). Turbidity and microbial load removal from river water using bioflocculants from indigenous bacteria isolated from wastewater in South Africa. African Journal of Biotechnology, 8(14), 3261–3266.

Buthelezi, S. P., Olaniran, A. O., & Pillay, B. (2010). Production and characterization of bioflocculants from bacteria isolated from the wastewater treatment plant in South Africa. Biotechnology and Bioprocess Engineering, 15(5), 874–881.

Buthelezi, S. P., Olaniran, A. O., & Pillay, B. (2012). Textile dye removal from wastewater effluents using bioflocculants produced by indigenous bacterial isolates. Molecules, 17(12), 14260–14274.

Buthelezi, S. P., Olaniran, A. O., & Pillay, B. (2010). Production and characterization of bioflocculants from bacteria isolated from the wastewater treatment plant in South Africa. Biotechnology and Bioprocess Engineering, 15(5), 874–881.

Butler, G., Rasmussen, M. D., Lin, M. F., Santos, M. A., Sakthikumar, S., Munro, C. A.et a;. (2009). Evolution of Pathogenicity and Sexual Reproduction in Eight Candida Genomes. Nature, 459, 657-662. http://dx.doi.org/10.1038/nature08064

C. Neal, (2001) “The potential for phosphorus pollution remediation by calcite precipitation in UK freshwaters,” *Hydrology and Earth System Sciences*, vol. 5, no. 1, pp. 119–131, 2001.

C. Y., Budiman, P. M., Pui, K., Shak, Y., & Wu, T. Y. (2016). Recent Advancement of Coagulation − Flocculation and Its Application in Wastewater Treatment.Ind. Eng. Chem. Res. 55, 16, 4363-4389.

Cai, C., Xu, J., Deng, N., Dong, X., Tang, H., & Liang, Y. (2016). A novel approach of the utilization of the fungal conidia biomass to remove heavy metals from the aqueous solution through immobilization. *Nature Publishing Group*, 1–12. http://doi.org/10.1038/srep36546.

Camarillo, M. K., & Stringfellow, W. T. (2018). Biological treatment of oil and gas produced water: a review and meta-analysis. http://doi.org/10.1007/s10098-018-1564-9.

Centre, R. (1989). FUNGI ISOLATED FROM THE EEZ OF INDIAN COAST, *46*, 37–46.Handbook

Chang, S. Y., Li, C. T., Hiang, S. Y., and Chang, M. C., (1995). Intraspecific protoplast fusion of Candida tropicalis for enhancing phenol degradation. Appl. Microbiol. Biotech., 43: 534-538. https://doi:10.1007/BF00218462.Chapter 1 44Pview of Literature.

Chaudhary P, Beniwal V, Sharma P, Goyal S, Kumar R, Ahmed A.(2022) Unloading of hazardous Cr and Tannic Acid from real and synthetic waste water by novel fungal consortia,Environmental Technology & Innovation,https://doi.org/10.1016/j.eti.2021.102230.

Chen, L., Noorbakhsh, J., Adams, R. M., Samaniego-evans, J., & Agollah, G. (2014). Two-Dimensionality of Yeast Colony Expansion Accompanied by Pattern Formation, *10*(12). http://doi.org/10.1371/journal.pcbi.1003979.

Chen, Y. (2003). Immobilized Isochrysis galbana (Haptophyta) for long-term storage and applications for feed and water quality control in clam (Meretrix lusoria) cultures, 439–444.

Cheng, K. K., Zhang, J. A., Ling, H. Z., Ping, W. X.,Wei, H., Ge, J. P. (2009). Optimization of pH and acetic acid concentration for bioconversion of hemicellulose from corncobs to xylitol by Candida tropicalis. Biochem. Eng. J., 43: 203-207. https://doi:10.1016/j.bej.2008.09.012.

Choo, K. K., Chong, P. P., Siong, A., Ho, H., Voon, P., & Yong, C. (2015). Effect of Inoculum Size and Culture Age on the Cellular Properties and Host-Pathogen Interactions of Cryptococcus neoformans, *7*(2), 100–108. http://doi.org/10.9734/BMRJ/2015/16464.

Chow, J., Notaro, M., Prabhakar, A., Free, S. J., & Cullen, P. J. (2018). Impact of Fungal MAPK Pathway Targets on the Cell Wall. http://doi.org/10.3390/jof4030093.

Christ, R.H., Oberholser, K., Shank, N. & Nguyen, M. (1981). Nature of binding between metal ions and algal cell walls. Environmental Science and Technology 15, 1212.

Christner, B. C., Morris, C. E., Foreman, C. M., Cai, R., and Sands, D. C. (2008). Ubiquity of biological ice nucleators in snowfall. *Science* 319:1214. https://doi:10.1126/science.1149757.

Chukwudi MM, Nnaji PC, Onukwuli OD (2009) Coag-flocculation kinetics and functional parameters response of Periwinkle shell coagulant (PSC) to pH variation in organic rich coal effluent medium. Nat Sci 7(6):1–18.

Composition, G., & Indices, D. Chapter 3 Isolation and characterization of yeasts from sediments of the slope.

Comparative studies on physical chemical and biological coponents of some freshwater bodies goa and Maharashtra Vikrant et al., 2015 https://shodhganga.inflibnet.ac.in/handle/10603/35673?mode=full

Cosa, S., & Okoh, A. (2014). Bioflocculant Production by a Consortium of Two Bacterial Species and Its Potential Application in Industrial Wastewater and River Water Treatment, *23*(3), Polish Journal of Environmental Studies. 689–696.

Costanza, R., J, R. A., Groot, R. De, Farberll, S., Grassot, M., Hannon, B., Belt, M. Van Den.

(1997).Value of the world ecosystem services and natural capital, *387*253–260.

Craven PA, Hayasaka SS (1982) Inorganic phosphate solubilization by rhizosphere bacteria in a Zostera marina community. Can J Microbiol 28:605–610.

Council, W. W., On, D., Programmes, O. U. R., Of, O., Energies, R., & Forum, W. W. (2022.).

Curtis., E.J.C., 1969. Sewage fungus: its nature and effects. *Water Research*, 3, 289-311.

Dan, N. P., Visvanathan, C., & Basu, B. (2003). Comparative evaluation of yeast and bacterial treatment of high salinity wastewater based on biokinetic coefficients. Bioresource Technology, 87(1), 51–56.

Das N, Chandran P (2011). Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview. Biotech. Res. Int. ID 941810:1-13.

Das, S., Roy, G., Nabi, I., Mingma, N., Sherpa, T., & Thakur, N. (2021). Diversity and composition of the North Sikkim hot spring mycobiome using a culture - independent method, 737102.

Davis, T. A., Volesky, B., & Vieira, R. H. S. F. (2000). Sargassum Seaweed As Biosorbent For Heavy Metals, Water Resources,34(17), 4270–4278.

De la Nou¨ e, J., Basseres, (1989). Biotreatment of anaerobically digested swine manure with microalgae. BiologicalWastes 29, 17–31.

De la Nou¨ e, J., Chevalier, P., Proulx, D., (1990). Effluent treatment with immobilized microalgae and cyanobacteria: a critical assessment.In: Tyagi, R.D., Vembuk (Eds.), Wastewater Treatment by Immobilized Cells. CRC Press, Boca Raton, pp. 143–152.

De la Nou¨ e, J., De Pauw, N., (1988). The potential of microalgal biotechnology. A review of production and uses of microalgae. Biotechnol. Adv. 6, 725–770.

De la Nou¨ e, J., Laliberete , G., Proulx, D., (1992). Algae and wastewater. J. Appl. Phycol. 4, 247–254.

De la Nou¨ e, J., Proulx, D., (1988). Tertiary treatment of urban wastewater by chitosan-immobilized Phormidium sp. In: Stadler,T., Mollion, J., Verdus, M.C., Kamaranos, Y., Morvan, H.,Christaien, D. (Eds.), Algal Biotechnology. Elsevier Applied Science, New Yourk, pp. 159–168.

De Pauw, N., Van Vaerenbergh, E., (1983). Microalgal wastewater treatment systems: Potentials and limits. In: Ghette, P.F. (Ed.), Phytodepuration and the Employment of the Biomass Produced. Centro Ric. Produz, Animali, Reggio Emilia, Italy, pp. 211–287.

De Pauw, N., Verlet, H., De Leenheer, L.(1980). Heated and unheated outdoor cultures of marine algae with animal manure. In: Shelef,G., Soeder, C.J. (Eds.), Algae Biomass. Elsevier North Holland Biomedical Press, Amsterdam, pp. 315–341.

Dehghani, M., & Alizadeh, M. H. (2016). The effects of the natural coagulant Moringa oleifera and alum in wastewater treatment at the Bandar Abbas Oil Refinery. *Kerman University of Medical Sciences*, *3*(4), 225–230. http://doi.org/10.15171/EHEM.2016.24.

Delvaux, F., Verstrepen, Æ. K. J., & Delvaux, F. R. (2006). Immobilized yeast cell systems for continuous fermentation applications, (July 2015). http://doi.org/10.1007/s10529-006-9132-5.

Deng, L., Su, Y., Su, H., Wang, X., Zhu, X. (2006). Sorption and desorption of lead (g) from wastewater by green algae Cladophora fascicularis. J. Hazard. Mater., Dio:10.1016.

Devendran K, Sundararaj V, Chandramohan D, Krishnamurthy K (1974) Bacteria and primary production. Indian J Mar Sci 3:139–141.

Dhankhar, R., & Hooda, A. (2011). Fungal biosorption – an alternative to meet the challenges of heavy metal pollution in aqueous solutions, Environ Technol, *3330*(May).

Dhivya, S., Ramesh, S. T., & Gandhimathi, R. (2017). Performance of Natural Coagulant Extracted from Plantago ovata Seed for the Treatment of Turbid Water, An International Journal of Environmental Pollution. http://doi.org/10.1007/s11270-017-3592-1.

Dhote. J., Ingole .S., Chavan, A. (2012) Review on waste water treatment technology.

International Journal of Engineering Research and Technology.(IJERT) Vol.1 .

Dietrich H. Nies, Efflux-mediated heavy metal resistance in prokaryotes, *FEMS Microbiology Reviews*,Volume 27, Issue 2-3, June 2003, Pages 313–339, https://doi.org/10.1016/S0168-6445(03)00048-2.

Ding, Z., Bourven, I., Guibaud, G., Hullebusch, E. D. Van, Panico, A., Pirozzi, F., & Esposito, G. (2015). Role of extracellular polymeric substances (EPS) production in bioaggregation: application to wastewater treatment. *Applied Microbiology and Biotechnology*, 9883–9905. http://doi.org/10.1007/s00253-015-6964-8.

Doi, M., Homma, M., Chindamporn, A., and Tanaka, K. (1992). Estimation of chromosome number and size by pulsed-field gel electrophoresis (PFGE) in medically important Candida species. J. Gen. Microbiol. 138, 2243–2251.https://doi:10.1099/00221287-138-10-2243.

Duniya, D. A., Maikaje, D. B., Umar, Y. A., Abba, D., & Omokunmi, P. (2018). Research Isolation, Characterization and Bioremediation Potentials of Spent Engine Oil Degrading Fungi from Contaminated Soil, Journal of Environmental Science and Pollution 4(1), 253–255.

Dupres, V., & Dufre, Y. F. Measuring Cell Wall Thickness in Living Yeast Cells Using Single Molecular Rulers, *4*(9).

Durrant AE, Scrimshaw MD, Stratful I, Lester JN (1999) Review of the feasibility of recovering phosphate from wastewater for use as a raw material by phosphate industry. Environ.

Durak, T., & Depciuch, J. (2020). E ff ect of plant sample preparation and measuring methods on ATR-FTIR spectra results. *Environmental and Experimental Botany*, *169*(October 2019), 103915. http://doi.org/10.1016/j.envexpbot.2019.103915.

Dwivedi, S., A. Mishra, and D. Saini. (2012). Removal of heavy metals in liquid media through fungi isolated from wastewater. Int. J. Sci.Res.,1 (3):181-185.

Elaine . Soh, Shee Kit Wayne Chew, Xin Wei Phuang, Victoria M.V. Ho, Kevin Y.H. Chu, Rui Rui (2002).Valorization of spent brewery yeast biosorbent with sonication-assisted adsorption for dye removal in wastewater treatment,

Environmental Research, Volume 204https://doi.org/10.1016/j.envres.2021.112385.

E. Choi, J. Choi, S. Moon, An ED model for determination of the optimal current density, Desalination 153 (1–3) (2003) 399–404.

Effect of dissolved organic carbon and salinity on flocculation process of heavy metals during mixing of the Navrud River water with Caspian Seawater. Desalination and Water Treatment, 55(4), 926–934.

Elaine . Soh, Shee Kit Wayne Chew, Xin Wei Phuang, Victoria M.V. Ho, Kevin Y.H. Chu, Rui Rui (2002) Valorization of spent brewery yeast biosorbent with sonication-assisted adsorption for dye removal in wastewater treatment,Environmental Research,Volume 204https://doi.org/10.1016/j.envres.2021.112385.

Elias, Firew & Woyessa, Delelegn & Muleta, Diriba. (2016). Phosphate Solubilization Potential of Rhizosphere Fungi Isolated from Plants in Jimma Zone, Southwest Ethiopia. International Journal of Microbiology. 2016. 1-11. 10.1155/2016/5472601.

Elimelech MJ, Gregory J, Jia X, Williams RA (1995) Particle deposition and aggregation: measurement, modeling, and simulation. Butterworth-Heinemann Ltd, Oxford https://www.infona.pl/resource/bwmeta1.element.elsevier-d5f29fff-a989-38ef-9fa2-b1ef1689097b/tab/jContent.

Emila D' Costa D' Suza (1979). Studies on eustrine yeast III hydrocarbon degraded. Mahasagar bulletin, National Institute of Oceanography. 12, (3), 1979.

Environ_Geochem_1_97.pdf. (nd). Escola, R. R., & Gerais, M. (2016). Mining, http://doi.org/10.1590/0370-44672014680202.

Erniglia, C. & Baalen, C. & Gibson, David. (1980). Metabolism of Naphthalene by the Cyanobacterium Oscillatoria sp., Strain JCM. Microbiology-sgm. 116. 485-494. 10.1099/00221287-116-2-485.WA.

Everett (1972) Manual of Symbols and Terminology for Physicochemical Quantities and Units, Appendix II: Definitions, Terminology and Symbols in Colloid and Surface Chemistry Pure Appl. Chem., 1972, Vol. 31, No. 4, pp. 577-638.

Fang T. Chioun T. (1996). Batch cultivation and astaxanthin production by a mutant of the red yeast, Phaffia rhodozyma NCHU-FS501. Journal of Industrial Microbiology 16, 175-181.

Evans, Ivor H. (1996). *Yeast Protocols Volume 53 || Isolation and Identification of Yeasts from Natural Habitats. , 10.1385/0896033198(), 1–4.* doi:10.1385/0-89603-319-8:1.

F.N. Arroyo López. 2006., Use of molecular methods for the identification of yeast associated with table olives. Food microbiol 1–22.https://doi:10.1016/j.fm.2006.02.008.

Fabricius, K. E. (2005). Effects of terrestrial runoff on the ecology of corals and coral reefs: Review and synthesis. *Marine Pollution Bulletin*, *50*(2), 125–146. http://doi.org/10.1016/j.marpolbul.2004.11.028

Fabricius, K. E. (2005). Effects of terrestrial runoff on the ecology of corals and coral reefs: Review and synthesis. *Marine Pollution Bulletin*, *50*(2), 125–146. http://doi.org/10.1016/j.marpolbul.2004.11.028.

Fang, R., Cheng, X., & Xu, X. (2010). Bioresource Technology Synthesis of lignin-base cationic flocculant and its application in removing anionic azo-dyes from simulated wastewater. Bioresource Technology, 101(19), 7323–7329.

Fell JW (2001) Collection and identification of marine yeasts. Methods in Microbiology, Vol. 30 (John HP ed.), pp. 347-356. Academic Press, Burlington.

Feiz, L., Irshad, M., Pont-lezica, R. F., Canut, H., & Jamet, E. (2006). Evaluation of cell wall preparations for proteomics: a new procedure for purifying cell walls from Arabidopsis hypocotyls, *13*, 1–13. http://doi.org/10.1186/1746-4811-2-10.

Fotedar, R., Chatting, M., Kolecka, A. *et al.* Communities of culturable yeasts and yeast-like fungi in oligotrophic hypersaline coastal waters of the Arabian Gulf

surrounding Qatar. *Antonie van Leeuwenhoek* **1**15, 609–633 (2022). https://doi.org/10.1007/s10482-022-01722-y

Fiore, M.F., Moon, D.H., Trevors, J.T. (1998). Metal Resistance and Accumulation in Cyanobacteria. In: Wong, YS., Tam, N.F.Y. (eds) Wastewater Treatment with Algae. Biotechnology Intelligence Unit. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-10863-5_7

Fractal dimension and porosities of sacchromycies.pdf. (n.d.).

Freedman, D. E., (1987). No Title.from industrial effluents: Its potential use in wastewater treatment [Thesis]. Departmentfrom http://www.ijs.nio.org/index.php/msagar/article/view/2282/2258.

Fukudome, K. & Sato, M. & Takata, Yoshihiro & Kuroda, H. & Watari, J. & Takashio, M. (2002). Evaluation of yeast physiological state by Alcian blue retention. Journal of the American Society of Brewing Chemists. 60. 149-152.

Gadd GM, White C, De Rome L (1988). Heavy metal and radionuclide uptake by fungi and yeasts. In: Norris PRand Kelly DP (Eds). Biohydro metallurgy A. Rowe, Chippenham, Wilts., U.K.

Gadd, M., Ramsay, L., Crawford, J. W., & Ritz, K. (2001). Nutritional in £ uence on fungal colony growth and biomass distribution in response to toxic metals, *204*, 311–316.

Gad, P. Kamat N. (2013) M.Sc. Dissertation Diversity of Fresh Water Yeast in mining areas of Goa.

Garc, J. (2000). High-rate algal pond operating strategies for urban wastewater nitrogen removal, 331–339.

Gates, M. A., Rogerson, A., Berger, J., Gates, M. A., Rogerson, A., & Berger, J. (1982). Dry to Wet Weight Biomass Conversion Constant for *Tetrahymena elliotti* (Ciliophora, Dry to Wet Weight Biomass Conversion Constant for Tetrahymena of Y / (Ciliophora, Protozoa), *55*(2), 145–148.

Gates, W. P., Wilkinson, H. T., & Stlrcki, J. W. (1993). Swelling Properties of Microbially Reduced Ferruginous smectite, Journal of Clays and Clay minerals 41(3), 360–364.

Gaur, R., Singh, R., Gupta, M., & Gaur, M. K. (2010). Aureobasidium pullulans , an economically important polymorphic yeast with special reference to pullulan, *9*(47), 7989–7997. http://doi.org/10.5897/AJB10.948.

Gattullo CE, Bährs H, Steinberg CE, Loffredo E. (2012) Removal of bisphenol A by the freshwater green alga Monoraphidium braunii and the role of natural organic matter. Sci Total Environ. Feb 1;416:501-6. doi: 10.1016/j.scitotenv.2011.11.033. Epub 2011 Dec 29. PMID: 22209372.

Gbemisola, O., Olufemi, O., Olayinka, A., Carlos, C., & Steve, M. (2017). Heavy metal tolerance traits of filamentous fungi isolated from gold and gemstone mining sites. Brazilian Journal of Microbiology, 49(1), 29–37.

Ghomi, A. G., Asasian-kolur, N., & Sharifian, S. (2020). Journal of Environmental Chemical Engineering Biosorpion for sustainable recovery of precious metals from wastewater. *Journal of Environmental Chemical Engineering*, *8*(4), 103996. http://doi.org/10.1016/j.jece.2020.103996.

Ghose, M. K., & Sen, P. K. (2000). Characteristics of the Iron Ore Tailing Pond Effluent in India and its Management, *59*(October), 822–828.

Ghulam Hussain & Sajjad Haydar (2020) Comparative Evaluation of Glycine max L. and Alum for Turbid Water Treatment. *Water Air Soil Pollut* 231:57 https://doi.org/10.1007/s11270-020-4423-3.

Gonçalves AL, Pires JCM, Simões M (2016). A review on the use of microalgal consortia for wastewater treatment. Journal of Applied Phycology.1331-1341.

Gonçalves, Ana & Alvim-Ferraz, Maria & Martins, Fernando & Simões, Manuel & Pires, J.. (2016). Integration of Microalgae-Based Bioenergy Production into a Petrochemical Complex: Techno-Economic Assessment. Energies. 9. 224. 10.3390/en9040224.

Gontar, A., Bottema, M. J., Binder, B. J., & Tronnolone, H. (2018). Characterizing the shape patterns of dimorphic yeast pseudohyphae.

Gonzalez-perez, A., Feld, K., & Ruso, J. M. (2016). Polymersomes mimic biofilms fractal growth. *Journal of Polymer Research*, 1–6. http://doi.org/10.1007/s10965-016-1085-3

Goyal, M. (2015). A Sustainable and Economical Approach to Water Treatment: a Review in Context of India, Sci. Revs. Chem. Commun.: 5(1), 2015, 29-42.

Grinn-gofro, A. 2015. Effects of meteorological factors on the composition of selected fungal spores in the air, Aerobiologia (Bologna). 31(1):63-72. https:// doi:10.1016/j.fm.2006.02.008.

Gsizaw, Birhanu & Tsegaye, Zerihun & Genene, Tefera & Aynalem, Endegena & Wassie, Misganaw & Abatenh, Endeshaw. (2017). Phosphate Solubilizing Fungi Isolated and Characterized from Teff Rhizosphere Soil Collected from North Showa and Gojam, Ethiopia. Journal of Fertilizers & Pesticides. 08. 10.4172/2471-2728.1000180.

Gupta, N., & Debnath, M. (2011). Characterization of Biosurfactant production by mutant strain of Candida tropicalis, *6*, 133–136.

Gupta, V.K. and A. Rastogi. (2008). Sorption and desorption studies of chromium (VI) from nonviable cyanobacterium Nostocmuscorumbiomass. J. Hazard. Mater., 154 (1-3): 347-354.

Gurunathan, Baskar. (2018)."Bioremediation of Industrial and Municipal Wastewater Using Microalgae." Bioremediation: Applications for Environmental Protection and Management. Springer, Singapore, 2018. 331-357.

Haan, André B. de, Eral, H. Burak and Schuur, Boelo. (2020) Sedimentation and Settling". *Industrial Separation Processes: Fundamentals*, Berlin, Boston: De Gruyter, 2020, pp. 289-324. https://doi.org/10.1515/9783110654806-009.

Hagler A N, Ahearn D G. (1987). Ecology of aquatic yeasts. In: Rose A H, Harrison Js (eds) The yeasts 2ndedn, vol.1 Academic, London, pp 181-206.

Hattori. T. (1970). Adhesion between cells of E. Coli and clay particles J.Gen. App.Microbial .16,351-359.

Hawksworth, David. (1991). The fungal dimension of biodiversity: magnitude, significance, and conservation. Mycological Research. 95. 641-655. 10.1016/S0953-7562(09)80810-1.

He XL, Song C, Li YY, Wang N, Xu L, Han X, Wei DS (2018) Efficient degradation of azo dyes by a newly isolated fungus Trichoderma tomentosum under non-sterile conditions. Ecotoxicol Environ Saf 150:232–239.

He, J.P. Chen, (2014). A comprehensive review on biosorption of heavy metals by algal biomass: materials, performances, chemistry, and modeling simulation tools, Bioresour. Technol. 160 (2014) 67–78.

Herrera, V.E. and Axcell, B.C. (1991). Induction of premature yeast flocculation by a polyssacharide fraction isolated from malt husk. J Inst Brew 97, 359–366.

Heslop, O. D., Ceulaer, K. De, Rainford, L., & Nicholson, A. M. (2015). Medical Mycology Case Reports A case of Candida orthopsilosis associated with septic

arthritis in a patient with Systemic Lupus Erythematosus. *Medical Mycology Case Reports*, *7*, 1–3. http://doi.org/10.1016/j.mmcr.2014.11.001

Hglyceride Y, Zhang Y, Yang QX, Yang M, Lu WZ. Comparison of degradation activities of fatty acids of five separated yeast strains, China Environ. Sci. 2005; 25(8):39.

Ho, Y. C., Norli, I., Alkarkhi, A. F. M., & Morad, N. (2010). Characterization of biopolymeric flocculant (pectin) and organic synthetic flocculant (PAM): A comparative study on treatment and optimization in kaolin suspension. Bioresource Technology, 101(4), 1166–1174.

Hobden, C., Teevan, C., Jones, L., & Shea, P. O. (1995). Hydrophobic properties of the cell surface of, (1 995).

Hoffmann, J. P. (1998). Minireview Wastewater Treatment with Suspended and Nonsuspended algae 1, *763*, 757–763.

Hongliang, W., Zhiming, Y. U., & Xihua, C. A. O. (2011). Fractal dimensions of flocs between clay particles and HAB organisms , *29*(3), 656–663. http://doi.org/10.1007/s00343-011-0065-z

Horikawa, Y., Terai, T., & Ogura, H. (2016). Soil Science and Plant Nutrition Mutual flocculation between selected clay minerals and some kinds of asexual spores from soil borne fungi, *768*(May).

Accessed from https://www.worldwatercouncil.org/fileadmin/wwc/About_us/HQ_Staff/WWC_-_6e_Forum_mondial_de_l_eau_-_VE.pdfhttps://www.epa.gov/report-environment/drinking-water.

https://www.epa.gov/report-environment/drinking-water

https://www.worldwatercouncil.org/en/dakar-2022

https://www.unwater.org/publications/un-world-water-development-report-2022G

Humnabadkar RP, Saratale GD, Govindwar SP (2008) Decolorization of purple 2R by Aspergillus ochraceus (NCIM-1146). Asian J Microbiol Biotechnol Environ 10:693–697.

H. Y. (1991). Studies on the binding between yeastand a malt polyssacharidethatinduces flocculation when added to fermentations in normal wort. In sodium-dodecyl-sulphate-polyacrylamide gel cerevisiae 2036 was pitched at a rate of 20 x 106 cells ml "1 and from mal, *97*,367–373.

Hussain, G., Haydar, S.(2020) Comparative Evaluation of *Glycine max L.* and Alum for
Turbid Water Treatment. *Water Air Soil Pollut* 231**,** 57.https://doi.org/10.1007/s11270-0204423.

I., Morshed, M., Re, I., Re, I., Nashir, M., & Re, I. (2016). Semi-Pilot Study of the Production of Biomass and b-D-Fructofuranosidase by Saccharomyces cerevisiae IFSTBY111 in a Fed-Batch., (February 2014). http://doi.org/10.1089/ind.2013.0021

In, M. (2010). Insights on yeast bioremediation processes Insights on yeast bioremediation processes Introduction Industrial revolution changed the way of life, increasing the scientific knowledge dynamics in soil or water and its ability to consume xenobiotics as corbon source . Applied Bioremediation., *5*(51).

Ingo Schmidt, Olav Sliekers, Markus Schmid, Eberhard Bock, John Fuerst, J. Gijs Kuenen, Mike S.M. Jetten, Marc Strous, New concepts of microbial treatment processes for the nitrogen removal in wastewater, *FEMS Microbiology Reviews*, Volume 27, Issue 4, October 2003, Pages 481–492, https://doi.org/10.1016/S0168-6445(03)00039-1

Incharoensakdi, A. and P. Kitjaharn. (2002). Zinc biosorption from aqueous solution by a halotolerant cyanobacterium *Aphanothecehalophytica*. Current Microbiol.,45 (4):261-264.

Irawati, W., Parhusip, A. J. N., Christian, S., & Yuwono, T. (2017). The potential capability of bacteria and yeast strains isolated from Rungkut Industrial Sewage in Indonesia as a bioaccumulators and biosorbents of copper, Biodiversitas.,18(3), 971–977.

Islam, Aminul & Saha, Pranesh & Iqbal, Mosud & Islam, Mohammad & Ahmed, md. (2016). Removal of Arsenic by Water Hyacinth from Arsenic Contaminated Water. International Journal of Agricultural Papers. 1. 36-41.

Island, K. (2006). Metal tolerance of yeasts isolated from water, soil, and plant environments, J. Basic Microbiol. 46 (2006) 2, 145–152.

Islands, A., Arora, P., Singh, P., Wang, Y., Yadav, A., Pawar, K., Padmavati, G. (2021.). Environmental Isolation of Candida auris from the Coastal Wetland of Andman Island India.

Isolation and Identification of Yeasts and Filamentous. *Brazilian Journal of Microbiology*, *32*, 117–122. http://doi.org/10.1590/S1517-83822001000200009.

Izabela M., Katarzyna C., Anna W.K. (2013) State of the Art for the Biosorption Process—a Review. Appl Biochem Biotechnol. 2013; 170(6): 1389 1416.doi: 10.1007/s12010-013-0269-0

Jadhav JP, Govindwar SP (2006) Biotransformation of malachite green by Saccharomyces cerevisiae MTCC 463. Yeast 23:315–323.

Jadhav JP, Parshetti GK, Kalme SD, Govindwar SP (2007) Decolourization of azo dye methyl red by Saccharomyces cerevisiae MTCC 463. Chemosphere 68:394–400.

Jadhav SU, Jadhav MU, Kagalkar AN, Govindwar SP (2008) Decolorization of Brilliant Blue G dye mediated by degradation of the microbial consortium of Galactomyces geotrichum and Bacillus sp. J Chin Inst Chem Eng 39:563.

Jagadevan, S., & Mukherji, S. (2004). Successful in situ oil bioremediation programmes – Key parameters, *3*(October), 495–501.

Jambon, I., Thijs, S., Weyens, N., & Vangronsveld, J. (2018). Harnessing plant-bacteria-fungi interactions to improve plant growth and degradation of organic pollutants, Journal of Plant Interactions*9145.*

Jarque, S., Bittner, M., Blaha, L., & Hilscherova, K. (2016). Yeast Biosensors for Detection of Environmental Pollutants: Current State and Limitations. Trends in Biotechnology, 34(5), 408–419.

Jayasingam, P., Gopinath, M., & Sampathkumar, P. (2018). Seasonal Variation in Physico-gas-chemical Parameters of Cuddalore Coastal Waters, Southeast Coast of India, .

Jannasch, H. W., Wirsen, C. O., & Winget, C. L. (1973, July). A bacteriological pressure-retaining deep-sea sampler and culture vessel. In *Deep Sea Research and Oceanographic Abstracts* (Vol. 20, No. 7, pp. 661-664). Elsevier.

Ji, Zhou (2014) "CMEIAS JFrad: A Digital Computing Tool to Discriminate the Fractal Geometry of Landscape Architectures and Spatial Patterns of Individual Cells in Microbial Biofilms." *Microbial Ecology* 69: 710-720.

Johnson, D. B. (2003). Chemical and microbiological characteristics of mineral spoils and drainage waters at abandoned coal and metal mines. *Water, Air, and Soil Pollution: Focus*, *3*(1), 47–66. http://doi.org/10.1023/A:1022107520836.

Joshi, P.K.,(2011). Bioremediation of heavy metals in liquid media through fungi isolated from contaminated sources. Indian J. Microbiol., 51(4): 482-487.

Journal, B. (2012). water quality and diversity of yeasts from tropical lakes and rivers from the Rio, 1582–1594.

Journal, I., & Sciences, P. (2014). Isolation, Screening, and identification of cefdinir degrading yeasts for the treatment of pharmaceutical wastewater.

Journals, H., & Article, R. (2016). Iron Ore Mining, Waste Generation, Environmental Problems and Their Mitigation through Phytoremediation Technology, (1).

Kacprzak, M., & Malina, G. (2005). The tolerance and Zn 2 +, Ba 2 + and Fe 3 + accumulation by Trichoderma atroviride and Mortierella exigua isolated from contaminated soil., Canadian Journal of Soil Science.Canadian Journal of Soil Science, 2005, 85(2): 283-290.

Kalsoomakhtar M. WaheedAkhtar Ahmad M.Khalid ,2008. Removal and recovery of zirconium from its aqueous solution by *Candida tropicalis* Vol 156 108117.https://doi.org/10.1016/j.jhazmat.2007.12.002.

Kamala-Kannan S, Lee KJ (2008). Metal tolerance and antibiotic resistance of Bacillus species isolated from suncheon bay sediments, South Korea. Biotechnology. 7: 149-152.

Kamat, N.M., Kolte, R.R., & Dabolkar, S.(2014). Do unique stratospheric life forms get a piggy backride inside SW monsoon clouds to leave signatures in locally sampled

rainwater.Posterpresentation.https://www.researchgate.net/publication/260852193_rainwater_final_poster.

Kamel, S, Zaki, Z. Z. M, Kassim, J (2017) The Effectiveness of Psophocarpus Tetragonolobus's Seed as Turbidity Removal International Journal of Engineering & Technology, 7 (3.11) (2018) 144-146.

Kamal k.R., Singh,G, (2015). Assesment of ground water quality in the minng areas of Goa,

India. Indian Journal Of science and Technology,Vol 8(6), 588-595.

Kandasamy, K., Alikunhi, N. M., & Subramanian, M. (2012). Yeasts in marine and estuarine environments. *Journal of Yeast and Fungal Research*, *3*(December), 74–82. http://doi.org/10.5897/JYFR12.003.

Kang, L. (1994). Flocculation kinetics using Fe (III) coagulant in water treatment: the effects of sulfate and temperature, (Iii).

Kanmani, P. (2017). Exopolysaccharide from Bacillus sp. YP03: its properties and application as a flocculating agent in wastewater treatment. *International Journal of Environmental Science and Technology*. http://doi.org/10.1007/s13762-017-1416.

Kapoor, A., T. Viraraghavan and D.R. Cullimore. (1999). Removal of heavy metals using fungus*Aspergillusniger*. *Bioresour. Tech.*, 70 (1): 95-104.

Karbassi, A. R., Heidari, M., Vaezi, A. R., Samani, A. R. V., Fakhraee, M., & Heidari, F. (2014). Effect of pH and salinity on flocculation process of heavy metals during mixing of Aras River water with Caspian Sea water. *Environmental Earth Sciences*, *72*(2), 457–465. http://doi.org/10.1007/s12665-013-2965.

Karman, S. B., Diah, S. Z. M., & Gebeshuber, I. C. (2015). Raw Materials Synthesis from Heavy Metal Industry Effluents with Bioremediation and Phytomining: A Biomimetic Resource Management Approach, *2015*.

Katayon, S., M. J. Megat Mohd Noor, M. Asma, L. A. Abdul Ghani, A. M. Thamer, I. Azni, J. Ahmad, B. C. Khor, and A. M. Suleyman (2006).Effects of storage conditions of Moringa oleifera seeds on its performance in coagulation. Bioresour. Technol. 97: 1455-1460 https://doi.org/10.1016/j.biortech.2005.07.031.

Kato, M., & Iefuji, H. (2011). Breeding of a new wastewater treatment yeast by genetic engineering. AMB Express, 1(1), 7.

Kazmi A. A. (2003). Yeast yields treatment success. Magazine of the International Water Association, Water 21, February 2003.

Kazy, S.K., Sar, P., Singh, S. Sen Ashish. (2002). Extracellular polysaccharides of a copper-sensitive and a copper-resistant *Pseudomonas aeruginosa* strain: Synthesis, chemical nature, and copper binding. world J. Microbiol. Biotech., 18: 583-588.

Keeley, j., Jarvis P., Judd S. J., (2014). Coagulant Recovery from Water Treatment Residuals: A Review of Applicable Technologies, Critical Reviews in Environmental Science and Technology, 44:24, 2675-2719, DOI: 10.1080/10643389.2013.829766.

Keiluweit, M., Bougoure, J. J., Zeglin, L. H., Myrold, D. D., Weber, P. K., Pett-ridge, J., Nico, P. S. (2012). Nano-scale investigation of the association of microbial nitrogen residues with iron oxides in a forest soil O-horizon. Geochimica et Cosmochimica Acta *95* 213–226.https: //doi.org/10.1016/j.gca.2012.07.001.

Kellogg, C. A., & Griffin, D. W. (2006). Aerobiology and the global transport of desert dust, *21*(11). j. tree.2006.07. 004.https:// doi: 10.1016/j.tree.2006.07.004.

Khan, M.S., Zaidi, A. & Wani, P.A. Role of phosphate-solubilizing microorganisms in sustainable agriculture — A review. *Agron. Sustain. Dev.* 27, 29–43 (2007). https://doi.org/10.1051/agro:2006011.

Kiran Aftab, Kalsoom Akhtar, Abida Kausar, Shazia Khaliq, Numrah Nisar, Huma Umbreen, Munawar Iqbal,(2017). Fungal strains isolation, identification and application for the recovery of Zn(II) ions, Volume 175,Journal of Photochemistry and Photobiology B: Biology,Volume 175,https://doi.org/10.1016/j.jphotobiol.2017.08.028.

Klassen, I., Hillebrand, G., Olsen, N. R. B., Vollmer, S., & Lehmann, B. (2013). Flocculation processes and sedimentation of fine sediments in the open annular flume – experiment and numerical modeling, 437–481. http://doi.org/10.5194/esurfd-1-437-2013

Klymenko, N. Y., Siora, I. V, Novikova, E. A., Golovan, A. P., Krupskaya, T. V, Suvorova, L. A., & Turov, V. V. (2017). Destruction of Hydrocarbons by the Composite System Based on the Nanosilicas and Yeast Cells Mixture in Aqueous Medium, *39*(4), 209–213. http://doi.org/10.3103/S1063455X17040051.

Komarkova, E., Paca, J., Klapkova, E., Stiborova, M., Soccol, C. R., and Sobotka, M., (2003). Physiological changes of *Candida tropicalis* population degrading phenol in a fed batch reactor. Brazilian Archi. Biol. Tech., 46: 537-543..https://doi.10.1590/S1516-89132003000400007.

Koohestanian, A., Hosseini, M., & Abbasian, Z. (2008). The Separation Method for Removing of Colloidal Particles from Raw Water, American-Eurasian J. Agric. & Environ. Sci., 4 (2): 266-273, 2008*4*(2), 266–273.

Kostka, J. E., Stucki, J. W., Nealson, K. H., & Jun, W. U. (1996). Reduction of structural Fe(III) in smectite by a pure culture of Shewanella putrefaciens strain MR-1. Clays and Clay Minerals, 44(4), 522–529.

Kostka, J. E., Wu, J., Nealson, K. H., & Stucki, J. W. (1999). The impact of structural Fe(III) reduction by bacteria on the surface chemistry of smectite clay minerals. Geochimica et Cosmochimica Acta, 63(22), 3705–3713.

Kregiel, D., & Berlowska, J. (2012). Novel permittivity test for determination of yeast surface charge and flocculation abilities, 1881–1886. http://doi.org/10.1007/s10295-012-1193-y

Krein, A., Petticrew, E., & Udelhoven, T. (2003). The use of fine sediment fractal dimensions and colour to determine sediment sources in a small watershed, Science Direct 53, 165–179.
Krein, A., Petticrew, E., & Udelhoven, T. (2003). The use of fine sediment fractal dimensions and colour to determine sediment sources in a small watershed, Science Direct 53, 165–179.
Krein, A., Petticrew, E., & Udelhoven, T. (2003). The use of fine sediment fractal dimensions and colour to determine sediment sources in a small watershed, *53*, 165–179. http://doi.org/10.1016/S0341-8162(03)00021-3.

Krishan, G., Kumar, C. P., Purandara, B. K., & Singh, S. (2016). Assessment of Variation in Water Quality Index (WQI) of Groundwater in North Goa, India, *11*(1), 39–46.

Kristemann, T., Claßen, T., Koch, C., Dangendorf, F., Gebel, J., Vacata, V. Fischeder, R. (2002). Microbial Load of Drinking Water Reservoir Tributaries during Extreme Rainfall and Runoff *Applied and Environmental Microbiology*, *68*(5), 2188–2197. http://doi.org/10.1128/AEM.68.5.2188.

Ksheminska H, Fedorovych D, Babyak L, Yanovych D, Kaszycki P, Koloczek H.(2005) Chromium (III) and (VI) tolerance and bioaccumulation in yeast: A survey of cellular chromium content in selected strains of representative genera. Process Biochemistry.; 40:1565-1572.

Kuiran, Y. A. N., Ying, Z., & Zhenming, C. H. I. (2010). Distribution and Diversity of *Candida tropicalis* Strains in Different Marine Environments, J. Ocean Univ. China*9*(2), 139144.https://doi10.1007/s11802010-0139-0.

Kumar, G., Kumar, S., & Srivastava, A. (2018). Possible bioremediation of arsenic toxicity by isolating indigenous bacteria from the middle Gangetic plain of Bihar, India. Biotechnology Reports, 17, 117–125.

Kumari, A. R., & Sobha, K. (2015). International Journal of ChemTech Research, *8*(4), 1769–1782.

Kurtzman, C. P., and Fell, J. W., (2000). The Yeasts−A taxonomic Study. Fourth revised and enlarged edn. Elsevier, Amsterdam, Lausanne, New York, Oxford, Shannon, Singapore, Tokyo.77-947. https://doi.org/10.1016/B978-0-444-81312-1.X5000-X.

Kurtzman, C. P., Fell, J. W., Boekhout, T., & Robert, V. (2011). *Methods for Isolation , Phenotypic Characterization and Maintenance of Yeasts*. *The Yeasts, A Taxonomic Study*. Elsevier B.V. http://doi.org/10.1016/B978-0-444-52149-1.00007-0Science and Pollution Research International 16545–16559.

Lacerda, F., Alencar, S. De, & Navoni, J. A. (2017). The use of bacterial bioremediation of metals in aquatic environments in the twenty-first century: a systematic review, 16545–16559. http://doi.org/10.1007/s11356-017-9129-8.

Laliberte´ G, Olguı´n EJ, de la Noue J. (1997). Mass cultivation and wastewater treatment using Spirulina. In: VonshakA, editor. Spirulina platensis—physiology, cell biology, and biotechnology. London (UK): Taylor and Francis; 1997. p. 159–73.

Lange, O. L., Budel, B., Heber, U., Meyer, A., Zellner, H., & Green, T. G. A. (1993): Temperate rainforest lichens in New Zealand: High thallus water content can : severely limit photosynthetic CO_2 exchange. Oecologia 95: 303-313.

Laurent J, Casellas M, Dagot C. (2009) Heavy metals uptake by sonicated activated sludge: relation with floc surface properties. J Hazard Mater. 2;162(2-3):652-60. doi: 10.1016/j.jhazmat.2008.05.066. Epub 2008 May 21. PMID: 18584956.

Lee, C., & Kramer, T. A. (2004). Prediction of three-dimensional fractal dimensions using the two-dimensional properties of fractal aggregates, *112*, 49–57. http://doi.org/10.1016/j.cis.2004.07.001.

Leeuwen, S. H. J. Van, & Sarkar, R. A. B. (2018). Removal of organic matter from reservoir water : mechanisms underpinning surface chemistry of natural adsorbents. *International Journal of Environmental Science and Technology*, *15*(4), 847–862. http://doi.org/10.1007/s13762-017-1447-3.

Lestari, V. D., Mardawati, E., & Nurhadi, B. (2018). Fractal Dimension Analysis of Texture Formation of Whey Protein-Based Foods, International Journal of Food Science Volume 2018, Article ID 7673259, 17.

Leung, R. P. C. (2005). Determination of the Fractal Dimension of Microbial Flocs from the Change in Their Size Distribution after Breakage, Environ.Sci. Technol. 39(8), 2731–2735.

Li, B. (2000). Fractal geometry applications in description and analysis of patch patterns and patch dynamics, *132*, 33–50.

Li, D., & Ganczarczyk, J. (1989). Fractal Geometry of Particle Aggregates Generated in Water and Wastewater Treatment Processes, *23*(11), 1385–1389. http://doi.org/10.1021/es00069a009

Li, X., Liu, S., Na, Z., Lu, D. and Liu, Z. (2013) Adsorption, Concentration, and Recovery of Aqueous Heavy Metal Ions with the Root Powder of Eichhorniacrassipes. Ecological Engineering, 60, 160-166.

Li, Y., Wang, H., Wang, W., Yang, L., & Zu, Y. (2013). Ectomycorrhizal Influence on Particle Size , Surface Structure , Mineral Crystallinity , Functional Groups , and Elemental Composition of Soil Colloids from Different Soil Origins, *2013*.

Libkind, D., Russo, G., Broock, M. Van, & Scientific, N. (2014). Yeasts from extreme aquatic environment : hyperacidic freshwaters 20 Yeasts from extreme aquatic environments : hyperacidic freshwaters, (February 2015).

Libkind, D., Vaz, A. B. M., Branda, L. R., Garc, V. De, Broock, M. Van, & Rosa, C. A. (2011). Research article. http://doi.org/10.1111/j.1574-6941.2010.01030.x.

Libkind, Diego & Buzzini, Pietro & Turchetti, Benedetta & Rosa, Carlos. (2017). Yeasts in Continental and Seawater. 10.1007/978-3-319-62683-3_1.

Lima, D. P. De, Santos, A., Marques, M. R., Giannesi, G. C., Beatriz, A., Yonekawa, M. K. A., & Montanholi, A. S. (2018). Fungal Bioremediation of Pollutant Aromatic Amines. Current Opinion in Green and Sustainable Chemistry.

Lin, C.C. and Y.T. Lai. (2006). Adsorption and recovery of lead (II) from aqueous solutions byimmobilized *Pseudomonas aeruginosa* PU21beads. J. Hazard. Mater., 137 (1): 99-105.

Lin, J., & Harichund, C. (2011). Isolation and characterization of heavy metal removing bacterial bioflocculants. Journal of Microbiology, 5(6), 599–607.

Linda, F., Ogawa, M., Bisson, L. F., García-martínez, T., Mauricio, J. C., & Moreno-garcía, J. (2019).

Liu, G. (2005). An investigation of UV disinfection performance under the influence of turbidity & particulates for drinking water applications.

Liu, Junying, (2016) "Yeast as a Bioremediation Nanoparticle Agent in Piggery-Digested Wastewater Treatment." *Environmental Engineering Science 33.5:* 317-323.

Liu, Y., Chen, Z., Li, J., Zhu, Z., Pang, S., & Xu, J. (2022). Extensive Diversity and Prevalent Fluconazole Resistance among Environmental Yeasts from Tropical China.

Logan, B. E. (n.d.). Fractal Coagulation Kinetics.

López Errasquín E, Vázquez C (2003). Tolerance and uptake of heavy metals by Trichoderma atroviride isolated from sludge. Chemosphere 50: 137-143.

Loukidou, M.X. (2000). Diffusion kinetic study of cadmium (II) biosorption by *Aeromonascaviae*. J. Chem. Tech. Biotech., 79 (7): 711-719.

Luan, L., Sun, Y., Chen, S., Wu, C., & Hu, Y. (2018). A study of fractal dimension as a quality indicator of hairtail (Trichiurus haumela) samples during frozen storage. *Scientific Reports*, (December 2017), 1–8. http://doi.org/10.1038/s41598-018-33880-3

Lucas MS, Dias AA, Sampaio A, Amaral C, Peres JA (2007) Degradation of a textile reactive azo dye by a combined chemical–biological process: Fenton's reagent-yeast. Water Res 41:1103–1109.

Luck, I. (2004). Fractal dimension as a tool for detection of morphological changes caused by the impact of mechanical waves on mushroom mycelium, *13*, 101–107.

Lundy, S. D., Payne, R. J., Giles, K. R., & Garrill, A. (2001). Heavy metals on mycelial morphology of Achlya bisexualis as determined by fractal geometry, FEMS Microbiology Letters 201 201, 259–263.

Lv, W., Hesham, A. E. L., Zhang, Y., Liu, X., & Yang, M. (2011). Impacts of cell surface characteristics on population dynamics in a sequencing batch yeast reactor treating vegetable oil-containing wastewater. Applied Microbiology and Biotechnology, 90(5), 1785–1793.

Ma, F., Zheng, L. N., & Chi, Y. (2008). Applications of Biological Flocculants (BFs) for Coagulation Treatment in Water Purification: Turbidity Elimination, Chem Biochem Engineering, *22*(3), 321–326.

Ma, M. (2011). Enhancement of Hematite Flocculation in the Hematite À Starch À (Low-Molecular-Weight) Poly (acrylic acid) System, 11950–11953.http://doi.org/10.1021/ie2013373.

Ma, Z., Qin, J., Liou, C., Zhang, L., & Valiyaveettil, S. (2012). Effects of Coagulation pH and Mixing Conditions on Characteristics of Flocs in Surface Water Treatment. Advances in Civil, Environmental, and Materials Research 26-30.

Mabinya L.V., Cosa S., Nwodo U., OkohA.I.(2012). Studies on bioflocculant Production by Arthrobacter sp. Raats, a freshwater bacteria isolated from Tyume River, South Africa. Int. J. Mol. Sci. 13, 1054.

Machado, M. D., Santos, M. S. F., Gouveia, C., Soares, H. M. V. M., & Soares, E. V. (2008). Removal of heavy metals using a brewer's yeast strain of Saccharomyces cerevisiae: The flocculation as a separation process. Bioresource Technology, *99*(7), 2107–2115.

Madden, D. (2007). Immobilised yeast. http://www.ncbe.reading.ac.uk/PRACTICALS/PDF/ ImmobilisedYeast2.1_UK_eng.pdf

Mahmood, M. N. (2006). Applications of Fractal Dimension, (10), 54–73.

Mahmood, M. N. (2006). Applications of Fractal Dimension, Iraqi Journal of Statistical Science (10), 54–73.

Mahvi, A. H. and L. Diels. (2004). Biological removal of cadmium by *Alcaligenes eutrophus* CH34. *Int. Env. Sci. Tech., 1 (3):* 199-204.

Maini, H., & Shukla, A. (2016). Freshwater Fungal Richness, Their Assessment and Impact on Human Welfare: A Review, *5*(4), International Journal of Current Research,2013–2016.

Maiti. S. (2011) Handbook of Methods in Environmental Studies Vol. 1: Water and Wastewater Analysis. Centre of Mining Environment Indian School of Mines Dhanbad-826 004, India.

Malekani, K., Rice, J. A., & Lin, J. (1996). Comparison Of Techniques for Determining The Fractal Dimensions Of Clay Minerals, *44*(5), 677–685.

Malik A. Metal bioremediation through growing cells. Environ Int. 2004 Apr;30(2):261-78. doi: 10.1016/j.envint.2003.08.001. PMID: 14749114.

Malik, Q. H. (2018). Performance of alum and assorted coagulants in turbidity removal of muddy water. *Applied Water Science*, *8*(1), 1–4. http://doi.org/10.1007/s13201-018-0662-5.

Mamvura, T. A. (2010). Yeast cell immobilisation on carbon nanotubes for fermentation processes.

María L.R., Libkind, D., Vaz, A.B., Espírito , L.C., Moliné, M., de Garcia, V., van Broock, M.R., & Rosa, C.A. (2011). Yeasts from an oligotrophic lake in Patagonia (Argentina): diversity, distribution and synthesis of photoprotective compounds and extracellular enzymes. *FEMS microbiology ecology, 76 1*, 1-13 . anes, Carlos Andrés Martínez-Garay, Juan Carlos Igual.

Markx, G. H., & Davey, C. L. (1999). The dielectric properties of biological cells at radio frequencies: Applications in biotechnology, *25*, 161–171.

María Angeles Ju Brandão, L.R., Libkind, D., Vaz, A.B., Espírito Santo, L.C., Moliné, M., de Garcia, V., van Broock, M.R., & Rosa, C.A. (2011). Yeasts from an oligotrophic lake in Patagonia (Argentina): diversity, distribution and synthesis of photoprotective compounds and extracellular enzymes. *FEMS microbiology ecology, 76 1*, 1-13 . anes, Carlos Andrés Martínez-Garay, Juan Carlos Igual, María Carmen Bañó

Massaccesi, G., et al. (2002). Cadmium removal capacities of filamentous soil fungi isolated from industrially polluted sediments, La Plata, Argentina. World J. Microbio. Biotech., 18 (9): 817

Matsuyama, T., & Matsushita, M. (1992). Self-Similar Colony Morphogenesis by Gram-Negative Rods as the Experimental Model of Fractal Growth by a Cell Population, Applied and Environmental Microbiology*58*(4), 1227–1232.

Matsuyama, T., & Matsushita, M. (1992). Self-Similar Colony Morphogenesis by Gram-Negative Rods as the Experimental Model of Fractal Growth by a Cell Population, Applied and Environmental Microbiology*58*(4), 1227–1232.

Matsuyama, T., & Matsushita, M. (1992). Self-Similar Colony Morphogenesis by Gram-Negative Rods as the Experimental Model of Fractal Growth by a Cell Population, *58*(4), 1227–1232.

Mc Hardy, B.M., George, J.J., (1990). Bioaccumulation and toxicity of zinc in green alga Cladophora glomerata. Environ. Pollut. 66, 55–66.

Me, A., Donoso, M. G., & Amparo, M. (2006). Ultrastructural and Physico-chemical heterogeneities of yeast surfaces were revealed by mapping lateral-friction and normal-adhesion forces using an atomic force microscope Antonio Me, 495–509. http://doi.org/10.1007/s10482-005-9048-4.

Meghdad Pirsaheb, Samira Mohamadi, Sama Rahmatabadi, Hooshyar Hossini & Fabrício Motteran (2018) Simultaneous wastewater treatment and biogas production using integrated anaerobic baffled reactor granular activated carbon from baker's yeast wastewater, Environmental Technology, 39:21, 2724-2735, DOI: 10.1080/09593330.2017.1365939.

Meiburg, E., & Kneller, B. (2010). Turbidity Currents and Their Deposits. *Annual Review of Fluid Mechanics*, *42*, 135–156. http://doi.org/10.1146/annurev-fluid-121108-145618

Melamane, X. L., Strong, P. J., & Burgess, J. E. (2007). Treatment of Wine Distillery Wastewater: A Review with Emphasis on Anaerobic Membrane Reactors, *28*(1), 25–36.

Melamane, X. L., Strong, P. J., & Burgess, J. E. (2007). Treatment of Wine Distillery Wastewater: A Review with Emphasis on Anaerobic Membrane Reactors, South African Journal of Enology and Viticulture. *28*(1), 25–36.

Meng, X., Yang, J., Xu, X., Zhang, L., Nie, Q., and Xian, M., (2009). Biodiesel production from oleaginous microorganisms. Renew. Energy, 34: 1-5. https://doi: 10.1016/j.renene.2008.04.014.

Mandrekar, A., & Chachadi, A. G. (2012). Evaluation of water quality in an intense iron ore mining

watershed in Goa. International Conference SWRDM-2012.

Microbial Load of Drinking Water Reservoir Tributaries during Extreme Rainfall and Runoff. *Applied and Environmental Microbiology*, *68*(5), 2188–2197. http://doi.org/10.1128/AEM.68.5.2188

Microbienne, L. D. B., I, U. D. N., Lebrun, A., & Cedex, F.-N. (2009). Yeast Flocculation: Influence of Nutritional Factors on Cell Wall Composition, (1982), 2001–2009.

Microbienne, L. D. B., I, U. D. N., Lebrun, A., & Cedex, F.-N. (2018). Yeast Flocculation: Influence of Nutritional Factors on Cell Wall Composition.

Microbiology and Molecular Genetics University of the Punjab, Lahore.microorganisms thrive among extremely diverse communities in cloud water, PLoS ONE 12(8):

Mietta, F., Chassagne, C., Verney, R., & Winterwerp, J. C. (2011). On the behavior of mud floc size distribution: model calibration and model behavior, 257–271. http://doi.org/10.1007/s10236-010-0330-2

Mill, B. Y. P. J., Court, S., & Poges, S. (2016). Non-Flocculent Walls of Saccharomyces cerevisiae, J. gen. Microbiol. 1966, 329–341.

Mittapalli, G. V. S. S. (2017). A Study on the Use of Alum for Turbidity Removal in Synthetic Water A Study on the Use of Alum for Turbidity Removal in Synthetic Water, (June 2016), 4–8.

Mona EmMabrouk.(2014). Production of bioflocculant by the marine actinomycete Nocardiopsisaegyptia sp. nov. Life Sci. Journal. 11 (12), 27. 32.

Monapathi, M. E., Bezuidenhout, C. C., Howard, O., & Rhode, J. (2020). Aquatic yeasts : diversity, characteristics and potential health implications. http://doi.org/10.2166/wh.2020.270.

Moreira, S. R., Schwan, R. F., Carvalho, E. P. De, Wheals, A. E., & Lavras, U. F. De. (2001). Isolation and Identification of Yeasts and Filamentous. *Brazilian Journal of Microbiology*, *32*, 117–122. http://doi.org/10.1590/S1517-83822001000200009.

Moreno-garcía, J., García-martinez, T., Moreno, J., Mauricio, J. C., Ogawa, M., Luong, P., & Bisson, L. F. (2018). Impact of Yeast Flocculation and Biofilm Formation on Yeast-Fungus Co-Adhesion in a Novel Immobilization System. http://doi.org/10.5344/ajev.2018.17067.

Morrow, C. A., and Fraser, J. A. (2013). Ploidy variation as an adaptive mechanism in human pathogenic fungi. Semin. Cell Dev. Biol. 4, 339–346.https://doi:10.1016/j.semcdb.2013.01.008.

Mota, M. (1997). recognized synonym for *Saccharomyces cerevisiae*, *103*(April), 93–98.

Mousa, K. M., & Hadi, H. J.(2016). Coagulation / Flocculation Process for Produced Water Treatment, International Journal of Current Engineering and Technology*6*(2), 551–555.

Murtey, M. Das, & Ramasamy, P. (n.d.). Sample Preparations for Scanning Electron Microscopy – Life Sciences.

Muter, O., Patmalnieks, A., & Rapoport, A. (2001). Interrelations of the yeast Candida utilis and Cr (VI): metal reduction and its distribution in the cell and medium, *36*, 963–970.

Myers, Jessica E. (2016). Untangling algae-bacteria consortia: The ecological, phylogenetic, and bioremediation properties of freshwater microbiota. The University of Tulsa, .

Nagahama T, Abdel-Wahab MA, Nogi Y, Miyazaki M, Umatsu K, Hamamoto M and Horikoshi K. (2008). Dipodascus tetrasporeus sp. nov., an ascosporogenous yeast isolated from deep-sea sediments in the Japan Trench. Int J Syst Evol Micr 58: 1040–1046.

Nagahama T, Hamamoto M and Horikoshi K. (2006). Rhodotorula pacifica sp. nov. a novel yeast species from sediment collected on the deep-sea floor of the northwest Pacific Ocean. Int J Syst Evol Micr 56: 295–299.

Nagahama T, Hamamoto M, Nakase T and Horikoshi K. (1999). Kluyveromyces nonfermentans sp. nov. a new yeast species isolated from the deep sea. Int J Syst Evol Micr 49: 1899–1905.

Nagahama T, Hamamoto M, Nakase T, Takami H and Horikoshi K. (2001). Distribution and identification of red yeasts in deepsea environments around the northwest Pacific Ocean. Anton van Leeuwen 80: 101–110.

Nagahama T, Hamamoto M, Nakase T, Takaki Y and Horikoshi K. (2003). Cryptococcus surugaensis sp. nov. a novel yeast species from sediment collected on the floor of Suruga Bay. Int J Syst Evol Micr 53: 2095–2098.

Nagahama T. (2006). Yeast biodiversity in freshwater, marine and deep-sea environments. Biodiversity and Ecophysiology of Yeasts (The Yeast Handbook series) (Rosa CA & Peter G, eds), Springer, Berlin & London. 241–262.

Nakari-seta, T., Azeredo, J., Henriques, M., Linder, M., & Penttila, M. (2002). Expression of a Fungal Hydrophobin in the Saccharomyces cerevisiae Cell Wall: Effect on Cell Surface Properties and Immobilization, *68*(7), 3385–3391. http://doi.org/10.1128/AEM.68.7.3385

Nan, J., Yao, M., Chen, T., Wang, Z., Li, Q., & Zhan, D. (2016). Experimental and numerical characterization of floc morphology: role of changing hydraulic retention time under flocculation mechanisms. *Environmental Science and Pollution Research*, *23*(4), 3596–3608. http://doi.org/10.1007/s11356-015-5539-7.

Nan, J., Yao, M., Chen, T., Wang, Z., Li, Q., & Zhan, D. (2016). Experimental and numerical characterization of floc morphology: role of changing hydraulic retention time under flocculation mechanisms. *Environmental Science and Pollution Research*, *23*(4), 3596–3608. http://doi.org/10.1007/s11356-015-5539-7.

Nan, J., Yao, M., Li, Q., Zhan, D., Chen, T., Wang, Z., & Li, H. (2016). The role of shear conditions on floc characteristics and membrane fouling in

coagulation/ultrafiltration hybrid process – the effect of flocculation duration and slow shear force. *RSC Adv.*, *6*(1), 163–173. http://doi.org/10.1039/C5RA18328F.

Nan, J., Yao, M., Li, Q., Zhan, D., Chen, T., Wang, Z., & Li, H. (2016). The role of shear conditions on floc characteristics and membrane fouling in coagulation/ultrafiltration hybrid process – the effect of flocculation duration and slow shear force. *RSC Adv.*, *6*(1), 163–173. http://doi.org/10.1039/C5RA18328.

Nandini, G. K. M., & Sheba, M. C. (2016). Emanating Trends in the Usage of Bio-coagulants in Potable Water Treatment: a Review. Nakajima, A., *et al.* (2001). Copper biosorption by chemically treated *Micrococcus luteus* cells.World J. Microbio. Biotech., 17: 343-347.

Nandini, G. K. M., & Sheba, M. C. (2016). Emanating Trends in the Usage of Bio-coagulants in Potable Water Treatment: a Review.

Nasnolkar, Chanda, M Shirodkar, PV Singbal , SYS (1996).

Naqvi, S. W. A., Lam, P., Narvenkar, G., Sarkar, A., Naik, H., Pratihary, A., Kuypers, M. M. M. (2018). Methane stimulates massive nitrogen loss from freshwater reservoirs in India. *Nature Communications*, . http://doi.org/10.1038/s41467-018-03607

Nautiyal, C. S. (1999). An ancient microbiological growth medium for screening phosphate solubilizing microorganisms, 170(436), 265–270.

Nayak G.N. (2002) Impact of Mining on Environment in Goa .

Nayyar, A., Walker, G., Canetta, E., Wardrop, F., & Adya, A. K. (2017). Influence of Cell Surface and Nanomechanical Properties on the Flocculation Ability of Industrial Saccharomyces cerevisiae Strains, *6*(5), 1–10. http://doi.org/10.5539/jfr.v6n5p1.

Nayak G.N. et al.,(1995). Thesis, chapter one Description of study area and characteristics of iron ore

minehttps://shodhganga.inflibnet.ac.in/bitstream/10603/31564/9/09_chapter%201.pdf

Nazareth. Kowshik, M. (2001). Biosedimentation of Mine Tailings by Fusarium Solani, Jr." of Industrial Pollution Control 7(2) pp .41 - 3467(2).

Neisi, A., Esteresh, A., Takdastan, A., & Orooji, N. (2015). Removal of Turbidity and Coliform Bacteria from Karoon River water by natural Coagulants Aid (Bread Yeast) With PAC.

New insights on yeast and filamentous fungus adhesion in a natural co-immobilization system: proposed advances and applications in the wine industry. http://doi.org/10.1007/s00253-019-09870-4

Ng, W. (n.d.). Identifying microbes from environmental water samples in a discovery-based learning module, *14*(1), 103–106.

Nie, M., Yin, X., Jia, J., Wang, Y., Liu, S., Shen, Q. Wang, Z. (2011). Production of a novel bioflocculant MNXY1 by *Klebsiella pneumoniae* strain NY1 and application in precipitation of cyanobacteria and municipal wastewater treatment, 547–558. http://doi.org/10.1111/j.1365-2672.2011.05080.

Nidheesh P.V, Thomas, Nair P., Kishore & Joju, Jones & Aswathy, P. & Jinisha, R. & Varghese, George & Gandhimathi, R.(2017). Potential Use of Hibiscus Rosa-Sinensis Leaf Extract for the Destabilization of Turbid Water. Water, Air, & Soil Pollution. 228. 51. 10.1007/s11270-016-3232-1.

Ningthoujam, D. (2011). Studies on Bioactive Actinomycetes in a Niche Biotope , Nambul River in. http://doi.org/10.4172/1948-5948.S6-001. (2011), *4*(10).

Noie, J. De, Lalibert, G., & Proulx, D. (1992). Algae and waste water, 247–248.

Nomura, T., Miyazaki, J., Miyamoto, A., Kuriyama, Y., Tokumoto, H., & Konishi, Y. (2013). Exposure of the Yeast Saccharomyces cerevisiae to Functionalized Polystyrene Latex Nanoparticles: Influence of Surface Charge on Toxicity. http://doi.org/10.1021/es400053x.

Northocote, D.H. (1952). The chemical composition and structure of the yeast cell wall. Biochem. j,51920,232-236.

Novak Babič, Monika & Zalar, Polona & Gunde-cimerman, Nina. (2016). Opportunistic black yeasts in drinking water.

Ntsaluba l., Nwodo U., Mabinya V., Okoh I. (2013). Studies on bioflocculant production by a mixed culture of Methylobacterium sp. Obi and Actinobacterium sp. Mayor. BMC. Biotechnol. 13, 62, 2013.

Nwodo .U., agunbiadem.O., Green e., Nwamadi m., Rumbold K., Okoh A.I. (2013). Characterization of an exopolymeric flocculant produced by a *Brachybacterium* sp. Materials. 6, 1237.

Nykter, M., Yli-harja, O., Shmulevich, I., & Dudley, A. M. (2014). Quantitative analysis of colony morphology in yeast, *56*(1), 18–27. http://doi.org/10.2144/000114123.

Odegaard, H., Grutle, S., Ratnaweera, H. (1992). An Analysis of Floc Separation Characteristics in Chemical Wastewater Treatment. In: Klute, R., Hahn, H. (eds) Chemical Water and Wastewater Treatment II. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-77827-8_7

O.P. Abioye, E.O. Afolayan and S.A. Aransiola, (2015). Treatment of Pharmaceutical Effluent by Saccharomyces cerevisiae and Torulasporadelbrueckii Isolated from Spoilt Water Melon. Research Journal of Environmental Toxicology, 9: 188-195.

Obert M, (1993) Microbial Growth Pattern: Fractal and Kinetics Characteristics of Pattern Generated by a Computer Model to Stimulate Fungal Growth.

Obert M, Pfeifer, P., Sernetz M. Giessen J. (1990). Microbial Growth Patterns Described by Fractal Geometry, *172*(3), 1180–1185.

Obert. M., (1993) microbial growth patterns: fractal and kinetic characteristics of patterns generated by a computer model to simulate fungal growth.

Ogbonna JC, Yoshizawa H, Tanaka H. (2000). Treatment of high strength organic wastewater by a mixed culture of photosynthetic microorganisms. J ApplPhycol;12(3–5):277 –84.

Ogden, L. E. 2014. Life in the Clouds, BioScience *64*(10), 861867.https://doi:10.1093/biosci/biu144.

Oh SE, Hassan SHA, Joo JH. (2009). Biosorption of heavy metals by lyophilized cells of *Pseudomonas stutzeri*. World J MicrobBiot 25:1771-1778.

Okolo, C.C., Oyedotun, T.D.T. & Akamigbo, (2018). Open cast mining: threat to water quality in rural community of Enyigba in south-eastern Nigeria. *Appl Water Sci* 8**,** 204 https://doi.org/10.1007/s13201-018-0849-9.

Olgu ı, E. J. (2003). Phycoremediation: key issues for cost-effective nutrient removal processes, *22*, 81–91. http://doi.org/10.1016/j.biotechadv.2003.08.009.

Oluwatosin Gbemisola OladipoOlusegun Olufemi AwotoyeAkinyemi OlayinkaCornelius Carlos BezuidenhoutMark Steve Maboeta (2018) Heavy metal tolerance traits of filamentous fungi isolated from gold and gemstone mining sites. Braz. J. Microbiol. 49 Jan-Mar 2018 • https://doi.org/10.1016/j.bjm.2017.06.003

Oswald, W. J. &Gotaas, H. B. (1957). Photosynthesis in sewage treatment. Trans. Am. Soc. Civ. Eng. 122:73–105.

Oteborg, C. G. (2007). Standardization of Yeast Growth Curves from Several Curves with Different Initial Sizes Md. Asaduzzaman. master thesis submitted to Goteborg University.

Oyegbile, B., Ay, P., & Satyanarayana, N. (2016). Flocculation kinetics and hydrodynamic interactions in natural and engineered flow systems: A review, *49*(0).

Ozdemir, G. (2003). Heavy metal biosorption by biomass of Ochrobactrumanthropi producing exopolysaccharide in activated sludge.*Bioresour. Tech.,* 90 (1): 71-74.

Ozturk, A. (2007). Removal of nickel from aqueous solution by the bacterium *Bacillusthuringiensis*. J. Hazard. Mater., 147 (1-2): 518-523.

Palacio Manuel L. B. and Bhushan Bharat (2012) Bioadhesion: a review of concepts and applications*Phil. Trans. R. Soc. A*.3702321–2347http://doi.org/10.1098/rsta.2011.0483.

Pertile, G., Lamorski, K., Bieganowski, A., Boguta, P., Sas-paszt, L., & Fr, M. (2021). Immediate effects of the application of various fungal strains with urea fertiliser on microbiome structure and functions and their relationships with the physicochemical parameters of two different soil types, *163*. http://doi.org/10.1016/j.apsoil.2021.103972.

Phulpoto, A. H., & Kanhar, M. A. M. N. A. (2021). Culture dependent to culture independentapproaches for the bioremediation of paints : a review. *International Journal of EnvironmentalScience and Technology*, *18*(1), 241–262. http://doi.org/10.1007/s13762-020-02801-1.

Ponnusamy, S. K., Subramaniam, R., Senthamarai, C., Niranjanaa, M. P., Vijayalakshmi & Sivanesan, S. (2010). Adsorption of dye from aqueous solution by cashew nut shell: Studies on equilibrium isotherm, kinetics and thermodynamics of interactions. Desalination. 261. 52-60. 10.1016/j.desal.2010.05.032.

Papagianni M: (2004). Fungal morphology and metabolite production in submerged mycelial processes. Biotechnol Adv 2004, 22:189-259.

Papagianni, M. (2006). Quantification of the fractal nature of mycelial aggregation in Aspergillus niger submerged cultures, *13*, 1–13. http://doi.org/10.1186/1475-2859-5-5

Parajo, J. C., Domínguez, H., Domínguez, J. M. (1998). Biotechnological production of xylitol. Part 1: Interest of xylitol and fundamentals of its biosynthesis. Bioresour Technol. 65, 191–201. https://doi.org/10.1016/S0960-8524(98)00038-8.

Pariolkar (2014) Comparative studies on physical chemical and biological components of some selected temple tanks in Goa. Thesis http://hdl.handle.net/10603/134683.

Paripurnanda Loganathan, Saravanamuthu Vigneswaran, Jaya Kandasamy & Nanthi S. Bolan (2014) Removal and Recovery of Phosphate from Water Using Sorption, Critical Reviews in Environmental Science and Technology, 44:8, 847-907, DOI: 10.1080/10643389.2012.741311.

Park, J. B. K., Craggs, R. J., & Shilton, A. N. (2011). Bioresource Technology Wastewater treatment high rate algal ponds for biofuel production. *Bioresource Technology*, *102*(1), 35–42. http://doi.org/10.1016/j.biortech.2010.06.158.

Paulina A. Kobielska, Ashlee J. Howarth, Omar K. Farha, Sanjit Nayak,(2018)Metal–organic frameworks for heavy metal removal from water,Coordination Chemistry Reviews,Pages 92-107,ISSN 0010-8545,https://doi.org/10.1016/j.ccr.2017.12.010.

Pereira, A.R.B. (2014). Removal of trace elements by isolates of *Aspergillus brasiliensis*EPAMIG 0084 and *Penicillium cirtinum*EPAMIG0086 in biofilters. *African J. Biotech.,* 13 (37):3759-3773.

Physiological changes of Candida tropicalis population degrading phenol in a fed batch reactor. Brazilian Archi. Biol. Tech., 46: 537-543.https://doi: 10.1590/S1516-89132003000400007.

Porman, A. M., Alby, K., Hirakawa, M. P., and Bennett, R. J(2011). Discovery of a phenotypic switch regulating sexual mating in the opportunistic fungal pathogen Candida tropicalis. Proc. Natl. Acad. Sci. U.S.A. 108, 21158–21163. https:// doi: 10.1073/pnas.1112076109.

Potential Applications in Industrial Biotechnology. Journal of microbiology and biotechnology. 26. 10.4014/jmb.1605.05074.

Prado, E. G. De, Rivas, E., Silóniz, M. De, Diezma, B., & Barreiro, P. (2014). Quantitative analysis of morphological changes in yeast colonies growing on solid medium: the eccentricity and Fourier indices, 431–440. http://doi.org/10.1002/.

Priolkar, K. D. (2014). Comparative Studies on Physical, Chemical and Biological components of some selected temple tanks in goa, a thesis submitted to Goa University.

Prakash, P. Sengupta, A.K. (2003). Selective coagulant recovery from water treatment plant residuals using Donnan membrane process, Environ. Sci. Technol. 37 (19) 4468–4474.

Prasad, M.N.V. (2004). Metallothioneins, Metal Binding Complexes and Metal Sequestration in Plants. In: Prasad, M.N.V. (eds) Heavy Metal Stress in Plants. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-07743-6_3.

Priyatharishini, M., Mokhtar, N. M., & Kristanti, R. A. (2019). Study on the Effectiveness of Banana Peel Coagulant in Turbidity Reduction of Synthetic Wastewater, *6*(June), 82–90.

Promod KC, Dhevendaran K (1987) Studies on phosphate bacteria in Cochin backwater. J Mar Biol Assoc India 29:297–305.

Puchkov, E. (2016). Image Analysis in Microbiology: A Review, 8–32. Quantifying the dominant growth mechanisms of dimorphic yeast using a lattice-based model.

R. K. Tiwary. (2001). Environmental Impact of Coal Mining Onwater Regime and Its Management. *Water, Air, and Soil Pollution*, (132), 185. http://doi.org/10.1023/A:1012083519667.

R.Yang, H. Li M. Huang, H. Yang , A Li, (2016). A review based on chitosan-based flocculant and their application in watertreatment., Water Res.95, 59-89.

Radwan S.S., Al-Hasan R.H., Salamah S. (2002). Bioremediation of oily sea water by bacteria immobilized in biofilms coating microalgae. Int BiodetBiodeg. 50:55–59.

Rahman A, Farooq H, Shakoori AR. (2007). Copper tolerant yeast, Candida tropicalis, isolated from industrial effluent: its potential use in wastewater treatment *Pakistan J. Zool., vol. 39(6), pp. 405-412, 2007.*

Raja, S., et al. (2014). "Bioremediation by using of microbes and algae with special reference to coastline environment." Int. J. Biosci. Nanosci 1.6: 130-140.

Rajasundaram, K., & Wright, C. J. (2018). A Biosensing Technique through a Coadhesion Study between *Saccharomyces cerevisiae* and *Lactobacillus Plantarum, 7*(7), 2330–2339.

Rajeevan, M., Unnikrishnan, CK, Bhate, J., Niranjan, K., & Sreekala, PP 2012. Nort east monsoon over India: variability and prediction, Meteorol. App 236, 226–236. https://doi: 10.1002/met.1322.

Ramalho PA, Cardoso MH, Cavaco-Paulo A, Ramalho MT (2004) Characterization of azo reduction activity in a novel ascomycete yeast strain. Appl Environ Microbiol 70:2279–2288.

Ramavandi, B. (2014). Treatment of water turbidity and bacteria by using a coagulant extracted from *Plantago*ovate. Water Resources and Industry 36-35.

Rani, C. Rajashaker Talikoti (2013). Adsorption isotherm studies of the simultaneous removal of turbidity and hardness by natural coagulants. Water Practice & Technology.495-502. 10.2166/wpt.2013.053.

Ren, G. N. A., Babu, D. R. A., & Anoharachary, C. M. (1992). Studies on the extra aquatic fungi associated with two polluted water bodies , andhra pradesh , india.

Rao, R. S., Jyothi, Ch. P., Prakasham, R. S., Sarma, P. N., and Rao, L. V., (2006). Xylitol production from corn fiber and sugarcane bagasse hydrolysates by Candida tropicalis. Bioresour. Technol., 97: 1974-1978. https://doi.org/10.1016/j.biortech.2005.08.015.

Rawat, R., Tewari, L. (2011) Effect of Abiotic Stress on Phosphate Solubilization by Biocontrol Fungus *Trichoderma* sp. *Current Microbiol* 62**,** 1521–1526. https://doi.org/10.1007/s00284-011-9888-2.

Rapoport, A., Borovikova, D., Kokina, A., & Patmalnieks, A. (2011). Immobilisation of yeast cells on the surface of hydroxyapatite ceramics Immobilisation of yeast cells on the surface of hydroxyapatite ceramics. *Process Biochemistry*, *46*(3), 665–670. http://doi.org/10.1016/j.procbio.2010.11.009.

Ren, P., Nan, J., Zhang, X., & Zheng, K. (2016). Analysis of floc morphology in a continuous-flow flocculation and sedimentation reactor, Science Direct *52*, 1–8.

Road, L. (2014). The National Academy of Sciences, In 84 India Abstracts of the Accepted Research Papers the Annual Session to held.http://www.nasi.nic.in/1st%20Circular%20_84th%20Annual%20Session_.pdf.

Robinson P.K. (1998). Immobilized Algal Technology for Wastewater Treatment Purposes. In: Wong YS., Tam N.F.Y. Wastewater Treatment with Algae. Biotechnology Intelligence Unit. Springer, Berlin, Heidelberg.

Robinson, P. K. (1998). for Wastewater Treatment Purposes, 1–2.

Robl, D., Thimoteo, S. S., Souza, G. C. C. F. De, Beux, M. R., Dalzoto, P. R., Básica, D. D. P., & Federal, U. (2014). Occurrence of Candida orthopsilosis in Brazilian tomato fruits (*Lycopersicum esculentum* Mill .), *109*, 105–109.

Rodriguez, D., Almirante, B., & Pahissa, A. (2008). Prevalence and Susceptibility Profile of *Candida metapsilosis* and *Candida orthopsilosis*: Results from Population-Based Surveillance of Candidemia in Spain, *52*(4), 1506–1509. http://doi.org/10.1128/AAC.01595-07.

Roane TM, Pepper IL (2000) In: Maier RM, Pepper IL, Gerba CB (eds) Microorganisms and metal pollution, in environmental microbiology, vol 55. Academic, London, pp 403–423R

Ross IC, Townsley CC (1986). The uptake of heavy metals by filamentous fungi. Immobilization of Ions by Biosorption Ellis Horwood. eds. H. Eccles and S. Hunt, Ellis Horwood Lid. Publishers, Chichester. Pp: 49-58.

Russo, G., LibkId, D., Ulloa, R. J., Garcı, V. De, Sabbah, I., Baransi, K., Massalha, N., Dawas, A., Saadi, I., & Nejidat, A.(2013). Efficient ammonia removal from wastewater by a microbial biofilm in tuff-based intermittent biofilters. Ecological Engineering, 53, 354–360.

Russo, G., Libkind, D., Ulloa, R. J., Garcı, V. De, Sampaio, J. P., & Broock, R. Van. (2010). Cryptococcus agrionensis sp . nov ., a basidiomycetous yeast of the acidic rock drainage ecoclade , isolated from an acidic aquatic environment ofvolcanic origin, , 996–1000. http://doi.org/10.1099/ijs.0.012534-0.

Ruta L, Paraschivescu C, Matache M, Avramescu S, Farcasanu IC. Removing heavy metals from synthetic effluents using "kamikaze" *Saccharomyces cerevisiae* cells. Appl Microbiol Biotechnol. 2010 Jan;85(3):763-71. doi: 10.1007/s00253-009-2266-3. PMID: 19795117.

Ruta, L. L., Kissen, R., Nicolau, I., Neagoe, A. D., Petrescu, A. J., Bones, A. M., & Farcasanu, I. C. (2017). Heavy metal accumulation by Saccharomyces cerevisiae cells armed with metal binding hexapeptides targeted to the inner face of the plasma membrane, 5749–5763. http://doi.org/10.1007/s00253-017-8335-0.

S Hussain1 J. van Leeuwen1, R. Aryal1 B. Sarkar, C. W. K. Chow, S. Beecham. (2018) Removal of organic matter from reservoir water: mechanisms underpinning surface chemistry of natural adsorbents. Int. J. Environ. Sci. Technol. 15:847–862

S. A. Parsons & S. J. Daniels (1999) The Use of Recovered Coagulants in Wastewater Treatment, Environmental Technology, 20:9, 979-986, DOI: 10.1080/09593332008616893

Sabtie, H. A., Ali, I. N., & Baqer, N. N. (2014). Evaluate the efficiency of the two species yeast *Candida dubliniensis* and *Candida glabrata* in reducing pollutants from wastewater using the batch system. Materials and Methods: *2*(9), 546–550.

Saha, P., Shinde, O., & Sarkar, S. (2017). Phytoremediation of industrial mines wastewater using water hyacinth. *International Journal of Phytoremediation*, *19*(1), 87–96. http://doi.org/10.1080/15226514.2016.1216078.

Sahin, Y. and A. Ozturk. (2005). Biosorption of chromium (VI) ions from aqueous solution by the bacterium *Bacillus thuringiensis*. Process Biochem., 40 (5): 1895-1901.

Sakhawoth, Yasine & Michot, Laurent Levitz, Pierre & Malikova, Natalie. (2017). Flocculation of Clay Colloids Induced by Model Polyelectrolytes: Effects of Relative Charge Density and Size. Chemphyschem : a European journal of chemical physics and physical chemistry. 18. 10.1002/cphc.201700430.

Salehizadeh, H. and S.A. Shojaosadati.(2003). Removal of metal ions from aqueous solution by polysaccharide produced from B*acillus firmus*.Water Res., 37 (17): 4231-4235.

Salehizadeh, H., & Shojaosadati, S. A. (2001). Extracellular biopolymeric flocculants: Recent trends and biotechnological importance.*Biotechnology Advances, 19(5), 371–385.*

Samani, A. R. V., Karbassi, A. R., Fakhraee, M., Heidari, M., Vaezi, A. R., & Valikhani, Z. (2015). Effect of dissolved organic carbon and salinity on flocculation process of heavy metals during mixing of the Navrud River water with Caspian Seawater. *Desalination and Water Treatment*, *55*(4), 926–934. http://doi.org/10.1080/19443994.2014.920730

Samarth, D.P., C.J. Chandekar and R.K.Bhadekar. (2012). Biosorption of heavy metals from aqueous solution using Bacillus licheniformis. Int. J. Pure and Appl. Sci. Tech.,10 (2): 12-19.

Sameh S. Ali, Abd El-Fatah Abomohra, Jianzhong Sun,(2017)Effective bio-pretreatment of sawdust waste with a novel microbial consortium for enhanced biomethanation,Bioresource Technology,Volume 238, 425-432,ISSN 09608524,https://doi.org/10.1016/j.biortech.2017.03.187.

Sánchez, E. (2014). The role of algae in bioremediation of organic pollutants, International Research Journal of Public and Environmental Health Vol.1 (2), pp. 19-32.

Saratale RG, Saratale GD, Chang JS, Govindwar SP (2009a) Ecofriendly degradation of sulphonated diazo dye reactive green 19A using Int Microbiol *Micrococcus glutamicus* NCIM 2168. Bioresour Technol 100: 3897–3905.

Sarkar, A., & Rao, K. V. B. (2018). Marine yeast : A potential candidate for biotechnological applications - A review marine yeast : a potential candidate for biotechnological applications- a review.

Sati, S.C., Pant, P. (2019) Evaluation of phosphate Solubilization by root endophytic aquatic Hyphomycete *Tetracladium setigerum*. *Symbiosis* **77,** 141–145. https://doi.org/10.1007/s13199-018-0575-y.

Satyawali, Y., & Balakrishnan, M. (2008). Wastewater treatment in molasses-based alcohol distilleries for COD and color removal: A review. *Journal of Environmental Management*, *86*(3), 481–497.

Sarupria, Manan & Manjare, Sampatrao & Girap, Mohan. (2018). Environmental impact assessment studies for mining area in Goa, India, using the new approach. Environmental Monitoring and Assessment. 191. 10.1007/s10661-018-7135-z.

Sawaiker, R. U., & Rodrigues, B. F. (2016). Physico-Chemical Characteristics and Phytoplankton Diversity in Physico-Chemical Characteristics and Phytoplankton Diversity in Some Fresh Water Bodies of goa.

Sciban, M., Klasnja, M., Antov, M. and skrbic, B. (2009) Removal of Water Turbidity by Natural Coagulants Obtained from Chestnut and Acorn. Bioresource Technology, 100, 6639-6643.http://dx.doi.org/10.1016/j.biortech.2009.06.047.

Science, E. (2016). The use of Moringa Oleifera Seed Powder as Coagulant to Improve the Quality of Wastewater and Ground Water. IOP Conf. Series: Earth and Environmental Science 31 12033.

Science, E. (2018). Bioremediation of petroleum-contaminated soil: A Review Bioremediation of petroleum-contaminated soil: A Review. IOP Conf. Series: Earth and Environmental Science 118 (2018) 012063.

Science, E. (n.d.). The use of Moringa Oleifera Seed Powder as Coagulant to Improve the Quality of Wastewater and Ground Water The use of Moringa Oleifera

Seed Powder as Coagulant to Improve the Quality of Wastewater and Ground Water. http://doi.org/10.1088/1755-1315/31/1/012033.

Sciences, A. (2017). Aggregation and fractal dimension of aggregates formed in sand dunes stabilized by PistachioPAM and PistachioPVAc, 783–791.

Sciences, A. (2017). Aggregation and fractal dimension of aggregates formed in sand dunes stabilized by PistachioPAM and PistachioPVAc, (September), 783–791. http://doi.org/10.1111/ejss.12458.

Sciences, P. (2007). Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. sedimentology.

Seervai, R. N. H., Jones, S. K. Jr., Hirakawa, M. P., Porman, A. M., and Bennett, R. J. 2013. Parasexuality and ploidy change in Candida tropicalis. Eukaryot. Cell 12, 1629–1640. https://doi:10.1128/EC.00128-13.

Selvi, A., and N. Das. (2014). "Isolation, screening, and identification of cefdinir degrading yeasts for the treatment of pharmaceutical wastewater." Int J Pharm Pharmaceut Sci 6: 382-386.

Semple, K.T. and Cain, R.B. (1996). Biodegradation of phenolics by Ochromonasdanica. Appl. Environ. Microbiol. 62, 1265^1273.

Sen, A. M. (2001). 'Acidophilic Sulfate Reducing Bacteria: Candidates for Bioremediation of Acid Mine Drainage Pollution', *Ph.D. Thesis*, School of Biological Sciences, Bangor, University of Wales, 298 pp.

Senan RC, Abraham TE (2004) Bioremediation of textile azo dyes by aerobic bacterial consortium aerobic degradation of selected azo dyes by bacterial consortium. Biodegradation 15:275–280.

Sessitsch, A., Weilharter, A., Gerzabek, M. H., Kirchmann, H., & Kandeler, E. (2001). Microbial Population Structures in Soil Particle Size Fractions of a Long-Term Fertilizer Field Experiment, *67*(9), 4215–4224. http://doi.org/10.1128/AEM.67.9.4215.

Seyedeh Zana Mahallati . Ebrahim Pazira ; 2 Fariborz Abbasi ; (2018), (1990). Estimation of Soil Water Retention Curve Using Fractal Dimension. J. Appl. Sci. Environ. Manage.

S, D. K., Karthik, L., Kumar, G., & V, B. R. K. (2011). ewsletter Dinesh et al . Biosynthesis of Silver anoparticles from Marine Yeast and Their Antimicrobial Activity Against Multidrug Resistant Pathogens Dinesh et al ., *1111*, 1100–1111.

Sharma, K. (2011) “Inorganic Phosphate Solubilization by Fungi Isolated from Agriculture Soil”. *Journal of Phytology*, Vol. 3, no. 4,Apr., http://updatepublishing.com/journal/index.php/jp/article/view/2264.

Sharma, R., & Sharma, M. (2011). Keratinase activity of dermatophytes and yeast species for poultry waste and waste water treatment. IIOAB Journal, 2(3), 19–22.

Shene C, Chisti Y, Vergara D, Burgos-Díaz C, Rubilar M, Bustamante M (2016) Production of eicosapentaenoic acid by *Nannochloropsisoculata*: effects of carbon dioxide and glycerol. J Biotech 239:47–56.

Sheoran, A. S., & Bhandari, S. (2005). Treatment of Mine Water by a Microbial Mat: Bench-scale Experiments, 38–42.

Shepardson, K. M., & Cramer, R. A. (2013). Fungal cell wall dynamics and infection site microenvironments: signal integration and infection outcome. *Current Opinion in Microbiology*, *16*(4), 385–390. http://doi.org/10.1016/j.mib.2013.03.003.

Shilpa, B. S., & Girish, P. (2012). Evaluation of Cactus and Hyacinth Bean Peels as Natural Coagulants, International Journal of Chemical and Environmental Engineering. Vol. 3, No. 3, 2012, pp. 187-191.

Shimofuruya H., koide A., shirota A., tsuji T., nakamura M., suzukiJ.(1996).The production of flocculating substance(s) by Streptomyces griseus. Biosci. Biotechnol. Biochem; 60, 498,

Shirali, S. A. (2014). Fractal Dimension and the Cantor Set, (November), 1000–1004.

Shourian M, Noghabi KA, Zahiri HS et al (2009). Efficient phenol degradation by a newly characterized Pseudomonas sp. SA01 isolated from pharmaceutical wastewaters. Desalination 246:577–594.

Siddiquee, S., Rovina, K., Azad, S. Al, Naher, L., Suryani, S., & Chaikaew, P. (2015). Heavy Metal Contaminants Removal from Wastewater Using the Potential Filamentous Fungi Biomass: A Review, Microbial & Biochemical Technology *7*(6), 384–393.

Siddiquee, S., Rovina, K., Azad, S. Al, Naher, L., Suryani, S., & Chaikaew, P. (2015). Microbial & Biochemical Technology Heavy Metal Contaminants Removal from Wastewater Using the Potential Filamentous Fungi Biomass: A Review, *7*(6), 384–393. http://doi.org/10.4172/1948-5948.100024.

Silkina, A., Nelson, G. D., Bayliss, C. E., Pooley, C. L., & Day, J. G. (2017). Bioremediation efficacy comparison of nutrient removal from an anaerobic digest waste-based medium by an algal consortium before and after cryopreservation, 1331–1341. http://doi.org/10.1007/s10811-017-1066.

Silkina, Alla, et al. (2017). "Bioremediation efficacy—comparison of nutrient removal from an anaerobic digest waste-based medium by an algal consortium before and after

Simpson. 1921.Origin of southwest monsoon. Nature. https://doi.org/10.1038/107154.

Singh, K. K., & Vaishya, R. (2017). Bioremediation of Heavy Metal Using Consortia Developed from, chromate resistant bacteria. Chemosphere. 48 (4); 427-435. http://doi.org/10.18090/samriddhi.v9i01.8339.

Singh, R., & Prashant, R. (2017). A review on characterization and bioremediation of pharmaceutical industries wastewater: an Indian perspective. *Applied Water Science*, 1–12. http://doi.org/10.1007/s13201-014-0225-3.

Silva-Bedoya LM, Ramírez-Castrillón M, Osorio-Cadavid E. (2014) Yeast diversity associated with sediments and water from two Colombian artificial lakes. Braz J Microbiol. 19;45(1):135-42. doi: 10.1590/S1517-83822014005000035.

Sizing, F. (1967). Floc Sizing, *15*(1), 125–134

Skinder B.M., Uqab B., Ganai B.A. (2020) Bioremediation: A Sustainable and Emerging Tool for Restoration of Polluted Aquatic Ecosystem. In: Qadri H., Bhat R., Mehmood M., Dar G. (eds) Fresh Water Pollution Dynamics and Remediation. Springer, Singapore. https://doi.org/10.1007/978-981-13-8277-2_9.

Smith, D. J. 2014. Opportunities for Astrobiology Research, Astobiology. https://doi:10.1089/ast.2013.1074.

Smoluchowski MV (1917) Verucheiner Mathemtischen Theorie der Koagulations Kinetic Kolloider Lousungen. Int J Res Phys Chem Phys 92:29–168.

Smith, Kevin & Raghavan, Rajeev & Dahanukar, Neelesh & Molur, Sanjay & Holland, Robert & Hughes, Adrian & Allen, David. (2011). Freshwater Biodiversity Assessment in the Western Ghats, India: Synthesis for all taxa.

Smirnov N. (1964) pleuroxus (Chydoridae): field observations and growth. Hydrobiologia, 23: 305-320.

Solani, F. (2001). Biosedimentation of mine tailings by *Fusarium solani*, *7*(2).

Sohrabi, Y., Rahimi, S., Nafez, A. H., & Mirzaei, N. (2018). Chemical Coagulation Efficiency in Removal of Water Turbidity, *10*(3), 188–194.

Sonali, P., and Banwari, L., (2008). Investigation of the potential of agro-industrial material as low-cost substrate for ethanol production by using *Candida tropicalis* and *Zymomonas mobilis.* Biomass Bioenergy, 32: 596 https://doi.org/10.1016/j.biombioe.2007.12.008

Song ZG, Wang GY, Yue XP, Li XQ, Biodegradation of Phenol and m-Cresol by *Candida maltosa,* China Water and wastewater. (; 29(7):97-99.

Sonnleitner, B., Locher, G., & Fiechter, A. (1992). Biomass determination, *25,* 5-22.

Souza, R. A. D., & Kamat, N. M. (2017). Potential of FTIR spectroscopy in chemical characterization of Termitomyces Pellets. http://doi.org/10.7324/JABB.2017.50412.

Souza, A. C. De, Ferreira, I., Melo, D. S., Lopes, L. A. A., & Magnani, M. (2017). Probiotic properties of yeasts isolated from Brazilian fermented table olives, 1–15. http://doi.org/10.1111/jam.15065.

Spina, F., Tigini, V., Romagnolo, A., & Varese, G. C. (2018). Bioremediation of Landfill Leachate with Fungi : AutochthonousAllochthonousStrains.

Srivastava, A., Seo, S., Ko, S., Ahn, C., & Oh, H. (2018). Critical Reviews in Biotechnology Bioflocculation in natural and engineered systems: current perspectives, *8551*. http://doi.org/10.1080/07388551.2018.1451984.

Stephenson, T., (1990). Substrate inhibition of phenol oxidation by a strain of Candida tropicalis. Biotech. Lett., 12: 843-846. https://doi.org/10.1007/BF01022607.

Stone, M. (2003). Floc morphology and size distributions of cohesive sediment in steady-state flow Floc morphology and size distributions of cohesive sediment in steady-state flow, *1354*(July 2018). http://doi.org/10.1016/S0043-1354(03)00082-4.

Studies on organic carbon, nitrogen, and phosphorous in the sediments of Mandovi estuary, Goa. Niscair-Csir, india. http://nopr.niscair.res.in/handle/123456789/37101.

Su X., Shen X., Ding L., Yokota A (2012). Study on the flocculability of the *Arthrobacter* sp., an actinomycete resuscitated from the VBNC state. World J Microbial Biotechnol. 28, 91.

Subramanian, R. S. (n.d.). Non-Circular Conduits, 1–12.

Suh h., Kwon H., Lee C.h., Kim H.S., Oh H.M., Yoon B.D (1998). Characterization of bioflocculant produced by Bacillus sp. DP-152. J. Ferment. Bioeng. 84, 108, 1998. 33.

Sun, J., Qin, L., Li, G., & Kang, Y. (2013). Effect of hydraulic conditions on flocculation performances and floc characteristics in Chinese herbal extracts by chitosan and chitosan hydrochloride. *chemical engineering* Journal, 225, 641–64http://doi.org/10.1016/j.cej.2013.03.108.

Sun S, Weber-Shirk M, Lion LW. Characterization of Flocs and Floc Size Distributions Using Image Analysis. Environ Eng Sci. 2016 Jan 1;33(1):25-34. doi:10.1089/ees.2015.0311. PMID: 26909006; PMCID: PMC4752185.

Surface Treatment of Concrete by Calcium Carbonate Biodeposition Using Candida orthopsilosis. (2022), *16*(1), 2022.

Swaikar R., Rodrigues, B. (2016). physico-chemical Characteristics and Phytoplankton Diversity in some Fresh Water Bodies of Goa, India. 10.

T chobanoglous, G.; Burton, F. L.; Stensel, H. D. (2003). Wastewater Engineering: Treatment and Reuse; McGraw-Hill: New York,.

T. Li, Z. Zhu, D.S. Wang, C.H. Yao, H.X. Tang, Characterization of floc size, strength and structure under various coagulation mechanisms, Powder Technol. 168 (2006) 104–110.

Talvitie, A., Jules Dupuit and benefit-cost analysis: Making past to be the present, Transport Policy (2017), https:// doi.org/10.1016/j.tranpol.2018.01.013.

Tambe, M. (n.d.). Understanding the southwest monsoon and its influence on ancient trade between India, the West, and South East Asia.

Teh, C. Y., Budiman, P. M., Pui, K., Shak, Y., & Wu, T. Y. (2016). Recent Advancement of Coagulation − Flocculation and Its Application in Wastewater Treatment.*I*nd. Eng. Chem. Res.*,* 2016, 55 (16), pp 4363–4389http://doi.org/10.1021/acs.iecr.5b04703.

Terry, Leigh Gilmore, "Organic Matter Removal via Biological Drinking Water Filters: Removal Efficiency Based on Quantifiable System Factors" (2017). Civil Engineering Graduate Theses & Dissertations. 164. https://scholar.colorado.edu/cven_gradetds/164.

Tesson SVM and Šantl-Temkiv T (2018). Ice Nucleation Activity and Aeolian Dispersal Success in Airborne and Aquatic Microalgae. *Front. Microbiol.* 9:2681. https:// doi: 10.3389/fmicb.2018.02681.

Thanh, N., & Simard, R. (1973). Biological Treatment of Wastewater by Yeasts. *Journal (Water Pollution Control Federation), 45*(4), 674-680. www.jstor.org/stable/25037807.

The, L. (2009). Homework # 6. Coagulation, Flocculation, Sedimentation, 1–5.

Thackston, E.L., and Palermo, M.R. (2000). Improved methods for correlating turbidity and suspended solid for monitoring. DOERTechnical Notes collection .U.S. Army Engineer Research and Development Centre. www.wes.arrmy.mile/el/dots/doer.

Tina Santl Temkiv, Kai Finster and Ulrich Gosewinkel Karlson.(2015). Cloud and Atmosphere Metagenomics. https://doi.org/10.1007/978-1-4899-7475-4 98.

Tomankova, K. (2006). Use of Image Analysis to Study Growth and Division of Yeast Cells, *6*(June 2014). http://doi.org/10.2352/J.ImagingSci.Technol.(2006)50.

Torres, L., Kr, A., Csibra, E., Gianni, E., & Pinheiro, V. B. (2016). Synthetic biology approaches biological containment: pre-emptively tackling potential risks, *0*(October), 393–410. http://doi.org/10.1042/EBC20160013.

Touhami, A., Hoffmann, B., Vasella, A., Denis, F. A., Duf, Y. F. (2003). Aggregation of yeast cells: Direct measurement of discrete lectin-carbohydrate interactions. Microbiology, 149(10), 2873–2878.

Tsuyoshi, N., Fudou, R., Yamanaka, S., & Kozaki, M. (2005). Identification of yeast strains isolated from marcha in Sikkim , a microbial starter for amylolytic fermentation, *99*, 135–146. http://doi.org/10.1016/j.ijfoodmicro.2004.08.011

Trapping, C. (2011). Dynamic Live Cell Imaging of Yeast, 3–6.

Treasury Her Majesty's,(2010) Economic and Fiscal Strategy Report and Financial Statement and Budget Report, 2.128, Her Majesty's Stationery Office, .

The trick, W. E., & Jarvis, W. R. (1998). Epidemiology of nosocomial fungal infection in the 1990s, 2–6.

Tripathi, A. K., Sudhir, N., Harsh, K., & Gupta, N. (2007). FungN. (Treatment of industrial effluents: a mini-review, 2–5. https://nepis.epa.gov/Exe/ZyNET.exe/94001WWN.TXT.

Tripathy, A., Sen, P., Su, B., & Briscoe, W. H. (2017). Tripathy, A., Sen, P., Su, B ., & Briscoe, W . H. (2017). Natural and bioinspired nanostructured bactericidal surfaces. Advances in Colloid and Publisher â€TM s PDF, also known as Version of record License: 85–104. http://doi.org/10.1016/j.cis.2017.07.030.

Tronnolone, H., Gardner, J. M., Sundstrom, J. F., Jiranek, V., Oliver, S. G., & Binder, B. J. (2017).*C.* tropicalis, providing new insights into adaptation and fungal sexual evolution. Eukaryot. Cell 11, 773.

Tsekova, K. and G. Petrov. (2002). Removal of heavy metals from aqueous solution using *Rhizopusdelemar* mycelia in free andpolyurethaneboundform. *Zeitschriftfuer Naturforschung*. C: J. Biosci., 57 (7-8): 629-633.

Turbidity removal for small public system.pdf.
https://nepis.epa.gov/Exe/ZyPDF.cgi/94001WWN.PDF?Dockey=94001WWN.PDF

Udayashankara, T. H., Swetha, D. (2015) "Water Quality Indices and Plankton Diversity in Two Lakes of Mysore City", International Journal for Scientific Research and Development, Vol. 3, Issue 6, pp 381-385.

Ukrit, R., Sutipa T., Lily, E., and Verawat, C., (2009). Simultaneous non-thermal saccharification of cassava pulp by multi-enzyme activity and ethanol fermentation by Candida tropicalis. J. Biosci. Bioeng., 107: 488-493.https:// doi: 10.1016/j.jbiosc.12.024.

Valderrama LT, Del Campo CM, Rodrı´guez CM, de-Bashan LE, Bashan Y (2002). Treatment of recalcitrant wastewater from ethanol and citric acid production using the microalga *Chlorella vulgaris* and the macrophyte Lemnaminuscule. Water Res 2002;36(17):4185– 92.

Varma, R. J., and Gaikwad, B. G., (2009). Biodegradation and phenol tolerance by recycled cells of Candida tropicalis NCIM 3556. Int. Biodeterior. Biodegrad.,63:539-542.

Vatilingom, M., Deguillaume, L., Vinatier, V., Sancelme, M., & Amato, P.(2012). Potential impact of microbial activity on the oxidant capacity and organic carbon budget in clouds. PNAS,561. https://doi.org/10.1073/pnas.1205743110.

Vazquez, P., Holguin, G., Puente, M. (2000). Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. *Biol Fertil Soils* 30**,** 460–468. https://doi.org/10.1007/s003740050024.

Vella, D., Ajdari, A., & Vaziri, A. (2012). The indentation of pressurized elastic shells: from polymeric capsules to yeast cells, (August 2011), 448–455.

Verlencar, X. (1987). Distribution of nutrients in the coastal and estuarine waters of Goa. *Mahasagar, 20*(4), 205-215. Retrieved

Verspagen, J. M. H., Visser, P. M., &Huisman, J. (2006). Aggregation with clay causes sedimentation of the buoyant cyanobacteria Microcystis spp. Aquatic Micribial Ecology,44- 165.

Verstrepen, K. J., & Klis, F. M. (2006). MicroReview Flocculation, adhesion and biofilm formation in yeasts, *60*, 5–15. http://doi.org/10.1111/j.1365-2958.2006.05072.

Veselá, M., Veselý, M., & Nežádal, M. (2014). The Use of Fractal Analysis for the Determination of Yeast Cell Diameter the Use of Fractal Analysis for the Determination of Yeast Cell Diameter, 9–21.

Vigezzi, C., Icely, P. A., Dudiuk, C., Rodríguez, E., Miró, M. S., Castillo, G. D. V, Sotomayor, C. Distribution, virulence factors and antifungal susceptibility patterns of *Candida parapsilosis* cryptic species isolated from patients with candidemia from Argentina's Central region. *International Journal of Infectious Diseases*, *73*(2018), 282–283. http://doi.org/10.1016/j.ijid.2018.04.4059.

Vijay Raghav K. Jegan J.R.., Palanivelu and velan.(2004) copper removal from aqueous by marine green alga ulva reticula. Electronic journal of Biotechnology, 22p.61-71.

Villegas, L. B., & Ferna, Æ. P. M. (2008). Chromate removal by yeasts isolated from sediments of a tanning factory and a mine site in Argentina, 591–600. http://doi.org/10.1007/s10534-008-9145-8.

Volesky B (1990). Biosorption of Heavy Metals. CRC Press. Bora Ratan, Ann. Arbor, Boston, USA. pp. 83-90.

Vymazal, J., (1984). Short-term uptake of heavy metals by periphyton algae. Hydrobiologia 119, 171–179.

Wagner M, Loy A, Nogueira R, Purkhold U, Lee N &Daims H (2002) Microbial community composition and function in wastewater treatment plants. Antonie van Leeuwenhoek 81: 665–680.

Wagner Artifon, Luciana Prazeres Mazur, Antônio Augusto Ulson de Souza, Débora de Oliveira,(2022) Production of bioflocculants from spent brewer's yeast and its

application in the treatment of effluents with textile dyes,Journal of Water Process Engineering,Volume 49102997,ISSN 2214-7144,https://doi.org/10.1016/j.jwpe.2022.102997.

Walker, G. M., & Adya, A. K. (2009). Nanoscopic Morphological Changes in Yeast Cell Surfaces Caused by Oxidative Stress: An Atomic Force Microscopic Study, *19*(January), 547–555. http://doi.org/10.4014/jmb.0809.515.

Walker, L., Sood, P., Lenardon, M. D., Milne, G., Olson, J., Jensen, G., Gow, A. R. (2018). crossm The Viscoelastic Properties of the Fungal Cell Wall Allow Traffic of AmBisome as Intact Liposome Vesicles, *9*(1), 1–15.

Walker, J., C, R., vaituzis, Z. (1975) Petroleum-degrading achlorophyllous alga *Prototheca zopfii*. *Nature* 254, 423–424. https://doi.org/10.1038/254423a

Wall, J. D., & Krumholz, L. R. (2006). Uranium reduction. Annual Review of Microbiology, 60,149–166.

Walmsley, R. M., & Keenan, P. (2000). The Eukaryote Alternative: Advantages of Using Yeasts in Place of Bacteria in Microbial Biosensor Development, Biotechnol. Bioprocess Eng. 2000, 5: 387-394.

Wang J, Chen C (2009) Biosorbents for heavy metals removal and their future. Biotechnol Adv 27(2):195–226.

Wang JS, Hu XJ, Liu YG, Xie SB and Bao ZL. (2010). Biosorption of uranium (VI) by immobilized Aspergillus fumigatus beads. J. Environ. Radioact., 101(6): 504-508.

Wang l., Ma f., Qu y., Sun D., Li A., Guo J., Guo J., Yu b. (2011). Characterization of a compound bioflocculant produced by mixed culture of *Rhizobium radiobacter* F2 and *Bacillus sphaeicus* F6. World J. Microbiol. Biotechnol. 10, 1007, 2011.

Wang, D., Wu, R., Jiang, Y., & Chow, C. W. K. (2011). Colloids and Surfaces A: Physicochemical and Engineering Aspects Characterization of floc structure and strength: Role of changing shear rates under various coagulation mechanisms. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *379*(1–3), 36–42. http://doi.org/10.1016/j.colsurfa.2010.11.048.

Waterburyt, J. B., & Stanier, R. Y. (1978). Patterns of Growth and Development in Pleurocapsalean Cyanobacteria, *42*(1), 2–44.

Water Specialist Technology (2003) About coagulation and flocculation. Information Bulletins, Sanford, pp 1–10.

White, G. N., & Berkheiser, V. E. (1982). Thin-Film Analysis of Clay Particles Using Energy Dispersive X-ray Analysis 1, Clay and Clay Minerals *30*(5), 375–382.

WHO, & WHO. (1997). Guidelines for drinking water quality, *1*. Retrieved from http://www.who.int/water_sanitation_health/dwq/gdwqvol32ed.pdf

Wong, H. S., & Buenfeld, N. R. (2006). Euclidean Distance Mapping for computing microstructural gradients at interfaces in composite materials, *36*, 1091–1097.

Woo, C., An, C., Xu, S., & Yamamoto, N. 2018. Taxonomic diversity of fungi deposited from the atmosphere. *The ISME Journal*, 2051–2060. http://doi.org/10.1038/s41396-018-0160-7.

Woodward, J. D. (1968). diameter is desirable., *74*, 427–429.

Woriax, H. E. (2009). A Study on Fractal Morphogenesis in Bacteria as a Response to Environmental Stress Hannah E. Woriax Honors College Thesis UNC Pembroke, 1–18.

Xerosis, C. (1999). Flocculation of Fine Fluorite Particles with, 225–230.

Xie, J., Du, H., Guan, G., Tong, Y., Kourkoumpetis, T. K., Zhang, L. 2012. N-acetylglucosamine induces white-to-opaque switching and mating in CandidaJun;11(6):773-82. doi: 10.1128/EC.00047-12.

Xu, W., Gao, B., Yue, Q., & Bo, X. (2011). Influence of pH on Flocs Formation, Breakage, and Fractal Properties — The Role of Al 13 Polymer, *1*(1), 45–57. http://doi.org/10.11912/jws.1.1.45-57

Y S. (2019). Yeast Colonies on Solid Media, (1988), 3061–3069.

Yadav, K. K., Gupta, N., Kumar, V., & Singh, J. K. (2017). Bioremediation of Heavy Metals from Contaminated Sites Using Potential Species: A Review, IJEP (2017)*37*(1), 65–84.

Yan, G. and T. Viraraghavan. (2001). Heavy metal removal in a biosorption column by immobilized *Mucor rouxii*biomass. Bioresour. Tech., 78 (3).

Yao, M., Nan, J., & Chen, T. (2014). Effect of particle size distribution on turbidity under various water quality levels during flocculation processes. Desalination, 354, 116–124.. http://doi.org/10.1016/j.desal.2014.09.029.

Silva-Bedoya LM, Ramírez-Castrillón M, Osorio-Cadavid E.(2014)Yeast diversity associated with sediments and water from two Colombian artificial lakes. Braz J Microbiol. 19;45(1):135-42. doi: 10.1590/S1517-83822014005000035.

Yellishetty, M., Ranjith, P. G., & Kumar, D. L. (2009). Resources, Conservation and Recycling Metal concentrations and metal mobility in unsaturated mine wastes in mining areas of Goa, India, *53*, 379–385. http://doi.org/10.1016/j.resconrec.2009.02.005.

Yeast diversity associated to sediments and water from two Colombian artificial lakes. (2014), *142*, 135–142.

Yi, Yanmei & Huang, Weiyi & Ge, Ying. (2008). Exopolysaccharide: A novel important factor in the microbial dissolution of tricalcium phosphate. World Journal of Microbiology and Biotechnology. 24. 1059-1065. 10.1007/s11274-007-9575-4.

Yokoi, H., Obita, T., Hirose, J., Hayashi, S., & Takasaki, Y. (2000). Flocculation properties of pectin in various suspensions, Bioresource Technol *84* (January), 287–290.

Yoshizawa, K., & Yamada, M. (2014). The Formation of Higher Alcohols in the Fermentation of Amino Acids by Yeast, Agricultural and Biological Chemistry 1369. http://doi.org/10.1080/00021369.1962.10858007.

Yoshizawa K. Treatment of wastewater discharged from a Sake Brewery using yeast, Ferment. Technol., 56, 389-395(1978).

Zaky, A. S., Tucker, G. A., Daw, Z. Y., & Du, C. (2014). Marine yeast isolation and industrial application. FEMS Yeast Research, 14(6), 813–825.

Zaky, A. Greetham, Darren, Louis, Edward, Tucker, Gregory Du, Chenyu. (2016). A New Isolation and Evaluation Method for Marine Derived Yeast spp with Potential Applications in Industrial Biotechnology. Journal of microbiology and biotechnology. 26. 10.4014/jmb.1605.05074.

Zalar, P., & Gunde-cimerman, N. (2014). Cold-Adapted Yeasts in Arctic Habitats. http://doi.org/10.1007/978-3-642-39681-6.

Zayed M. Abu Tawila, Salmah Ismail, Arezoo Dadrasnia, Mohammed Maikudi Usman (2018). Production and Characterization of a Bioflocculant Producedby *Bacillus salmalaya* 139SI-7 and Its Applications in Wastewater Treatment. Molecules. 23(10): 2689. doi: 10.3390/molecules23102689.

Zeng, D., Wu, J., & Kennedy, J. F. (2008). Application of a chitosan flocculant to water treatment, Carbohydrate Polymers71, 135–139.

Zhang, C., Cui, Y., & Wang, Y. (2012). Bioflocculant produced from bacteria for decolorization, Cr removal, and swine wastewater application, *22*(2), 129–134.

Zhang, C., Cui, Y., & Wang, Y. (2012). Bioflocculant produced from bacteria for decolorization, Cr removal, and swine wastewater application, Sustain. Environ. Res., 22(2), 129-134.

Zhang, S.G. Hou, S.Y. Wang, (2013) Biosorption of Cr (VI) by Immobilized Discarded Brewers Yeast, Food Research and Development 34, 14-17.

Zhang, F., Jiang, W., Wang, X., Ji, X., Wang, Y., Zhang, W., & Chen, J. (2012). Culture Condition Effect on Bioflocculant Production and Actual Wastewater Treatment Application by Different Types of Bioflocculants.http://dx.doi.org/10.5772/62114.

Zhang, W.-H., Kaur, I., Zhang, W., Shen, J., Ni, Y., (2017). Recovery of manool from evaporator condensate by induced air flotation in a kraft pulp mill-based integrated biorefinery. Separation and Purification Technology 188, 508–511. https://doi.org/10.1016/j.seppur.2017.07.063.

Zheng, Y., Ye, Z., Fang, X., Li, Y., &Cai, W. (2008). Production and characteristics of a bioflocculant produced by Bacillus sp. F19, *99*, 7686–7691.http://doi.org/10.1016/j.biortech.2008.01.068.

Zhao, Guixia & Huang, Xiubing & Tang, Zhenwu & Huang, Qifei & Niu, Fenglei & Wang, Xiang-Ke. (2018). Polymer-based nanocomposites for heavy metal ions removal from aqueous solution: A review. Polymer Chemistry. 9. 10.1039/C8PY00484F.

Zhou, Y., Liang, Z., & Wang, Y. (2008). Decolorization and COD removal of secondary yeast wastewater effluents by coagulation using aluminium sulfate. Desalination, 225(1–3), 301–311.

Zhu, F., Du, B., & Xu, B.(2016). Food Hydrocolloids A critical review on production and industrial applications of beta-glucans. Food Hydrocolloids, 52, 275–288.

Zhu, Z. (2018). Taxonomy characterization and plumbum bioremediation of novel fungi, J Basic Microbiol, 368–376.https://doi.org/10.1002/jobm.201700469.

Zuza-Alves DL, Silva-Rocha WP, Chaves GM. (2017). An Update on *Candida tropicalis* Based on Basic and Clinical Approaches. FrontMicrobiol https://doi.org/10.3389/fmicb.2017.01927.

APPENDICES

Appendix-I

Media

2% Malt Extract Agar (MEA) (Commercial, obtained from HiMedia)

1 g refined bacteriological grade malt extract (HiMedia) and 3 g bacteriological grade agar (HiMedia). Dissolve and make the total volume to 600 ml with distilled water. Heat to boiling for dissolving / digest agar completely. Sterilize by autoclaving at 121 °C at 15 lbs pressure for 20 min. Allowed to cool at room temperature.

Nutrient Agar (Commercial, obtained from HiMedia)

Suspend 2.8.0 grams in 100 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C.

Sodium Acetate Agar (Commercial, obtained from HiMedia)

Suspend 6.19 grams (the equivalent weight of dehydrated medium per litre) of dehydrated medium in 100 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool and mix well and pour into sterile Petri plates.

Corn Meal Agar (Commercial, obtained from HiMedia)

Suspend 1.7 grams in 100 ml purified/ distilled water. Heat to boiling to dissolve the medium completely.. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool and mix well and pour into sterile Petri plates.

Nitrate Agar (Commercial, obtained from HiMedia)

Suspend 2.1 grams in 100 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Dispense in tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allowed to cool and mix well and pour into sterile Petri plates.

Pikovskya Agar (Commercial, obtained from HiMedia)

Suspend 3.13 grams in 100 ml distilled water. Heat to boiling to dissolve the medium completely and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Incorporated with Bromophenol blue. Mixed well and pour into sterile Petri plates**.**

Antibiotics solutions

Stock solution of antibiotics solution was prepared by dissolving a tablet of Faropanem weighing 200mg in 50 ml sterile distilled water. Required concentration was obtained from stock solution.

Stains

Lactophenol Cotton Blue (Commercial stain obtained from HiMedia)

Ammoniacal Congo Red (ACR)

Dissolve 0.1 g of congo red powder (HiMedia) in 100 ml strong ammonia solution

Reagents

0.1 N sodium hydroxide

Dissolve 4 g NaOH and make the volume to 1000 ml in volumetric flask.

2 M sodium hydroxide

Dissolve 8 g NaOH and make the volume to 100 ml in volumetric flask.

0.1 N hydrochloric acid

0.1 N HCl by mixing 10.43 ml of conc. 35% HCl (Merck) carefully with distilled water and

0.2 make the volume to 1000 ml.

0.1 M phosphate buffer pH (7.2)

0.2 Stock solutions preparations:

A) 0.2 M di-sodium hydrogen phosphate, anhydrous (solution A) Take 1.42 g of Na HPO_4 (HiMedia) in 100 ml distilled water 2 4

B) 0.2 M sodium di-hydrogen orthophosphate, dehydrate (solution B) Take 1.56 g of NaH PO_4 . 2H O (HiMedia) in 100 ml distilled water 2 4 2 To prepare 0.1 M

phosphate buffer add 36 ml of solution A to 14 solution B (0.2 M phosphate buffer) + 50 ml distilled water. To this more 100 ml of distilled water was added.

Sterile glycerol

Take 10 ml of glycerol (HiMedia) and make the total volume to 100 ml using 90 ml sterile distilled water,autoclaved.s

Carbon dioxide free water

Prepared all stock and standard solutions and dilution water for the standardisation procedure with distilled water that has been freshly boiled for 15 minutes and cooled to room temperature.

The final pH of the water was > = 6.0 and its conductivity was < 2 *Il* Siemens/cm.

Potassium biphathalate solution (approx. O.05N)

Crush 15 to 20 g primary standard potassium biphathalate (KHCsHP4) to about 100 mesh and dry at120°C for 2 h. Cool in desiccator. Weigh 10.0 ± 0.5 g (to the nearest mg), transferto a 1000 mLvolumetric flask and make up the volume with CO2 free water.

Standard sodium hydroxide (O.02N) titrant

Dissolve 0.8 g NaOH and dilute to1000 mL using CO_2 free distilled water. Store in air-tight, mbber stopperPyrex/corning glass bottles to protect from atmospheric CO_2, Standardized against 0.05 N potassium bipathalate.

Phenolphthalein indicator

Dissolved 0.5 g of phenolphthalein indicator in 500 mL 95% ethyl alcohol. Add 500 mL distilled water. Add drop wise 0.02 N NaOH till pink colour appears.

Methyl orange indicator

Dissolve 0.5 g of methyl orange indicator and dilute to 1000 mL with CO_2 free distilled water.

Standard sodium thiosulphate, O.l N

Dissolved 25g Na_2SO_3.5 ~O in 1000 rnL of freshly boiled and cooled distilled water and standardized against potassium bi-iodate .

This initial storage is necessary to allow oxidation of bisulphite ion present. Added about 5 ml of chloroform as a preservative to minimize bacterial decomposition.

Starch indicator

Prepared slurry by adding small quantity of water to 1.0 g starch powder. Add 100 ml boiling water to it and continue boiling for a few minutes until solution becomes clear. The solution is cooled and preserved by adding 1.25 g of salicylic acid or a few drops of chloroform.

1. **Buffer solution (pH 10)**

   Dissolved 16.90 gNH_4Cl in 143 ml of conc NH_4OH. Added 1.25 g EDTA Mg salt to obtain sharp change in indicator and diluted to 25ml.

   This was titrated with standard calcium solution to avoid interference produced by EDT A to the buffer. Stored in a plastic or borosilicate glass container for no longer than I month. Stopper tightly to prevent loss of ammonia (NH_3) or pick up of carbon dioxide (CO_2) from air.

2. **Eriochrome black T indicator**

   Mix 0.5 g dye with 100 g NaCI to prepare dry powder dissolve 0.5 g of indicator in 100 rnl of ethyl alcohol.

Standard calcium solution (0.0lN) (1 g/L) Weigh accurately 1.0 g grade $CaCO_3$ (dried at 180 OC for 1 h before weighing) and transfer to 250 ml conical flask. Placed a funnel in the neck of a flask and add 1+1 HCl till $CaC0_3$ dissolved completely. Added 200 ml distilled water and boil for 30 min to remove Co_2 Cool and added methyl red indicator.

Added NH4OH (3N) till intermediate orange colour develops. Diluted to 1000 ml to obtain I ml = I mg CaC03, (1mL =0.40 I mg Ca or 0.243 mg Mg).

Standard EDTA solution (O.O1M)

Dissolved 3.723 g EDTA-disodium salt and dilute to IL. Standardised against standard calcium solution. 1mL = 1 mg $CaCO_3$

Inhibitor

Dissolved 1.5 g hydroxylamine hydrochloride in 100rnL 95% ethyl alcohol or isopropyl alcohol.

Stock nitrate solution (1 ml= 100 1mg $N0_3$-N)

Dry potassium nitrate (KNO]) in an oven at 105°C for 24 h. Dissolve 721.8 mg in water and dilute to 1000 ml. Preserve with 2 ml chloroform per L. This solution is stable for at least 6 months.

Stock potassium solution (1.907g/L) Dissolved 1.907 g of dry (110°C) KCI in 1000 ml of distilled water; 1 ml = 1 m

Permissible level of water parameters.

Parameters	**Permissible limit**		
	Drinking water IS 10500: 2012	**WHO**	**EPA**
pH	6.5 to 8.5	6.5-8.5	6.5-8.5
TDS (mg/l)	500	-	-
DO (mg/l)	-	-	-
Hardness (as CaCO3) (mg/l)	200	500	-
Alkalinity (as CaCO3) (mg/l)	200	-	-
Nitrate (mg/l)	45	50	
Sulfate (mg/l)	200	400	-
Phosphate (mg/l)	-	-	-
Fluoride (mg/l)	1		
Chloride (mg/l)	250	200	250
Turbidity (NTU)	5	0.1	-
Arsenic (mg/l)	0.01	-	-
Copper (mg/l)	0.05	1	1.5
Cadmium (mg/l)	0.003	0.003	0.005
Chromium (mg/l)	0.05	0.05	0.1
Lead (mg/l)	0.01	0.01	0.05
Iron (mg/l)	0.3	0.1	-
Zinc (mg/l)	5	5	-

Preparation of alcohol series for SEM studies

30%, 50%, 70%, 90% Ethanol Take 30 ml of absolute ethanol and make the volume to 100 ml with respective volume of distilled water to get different concentration of ethanol.

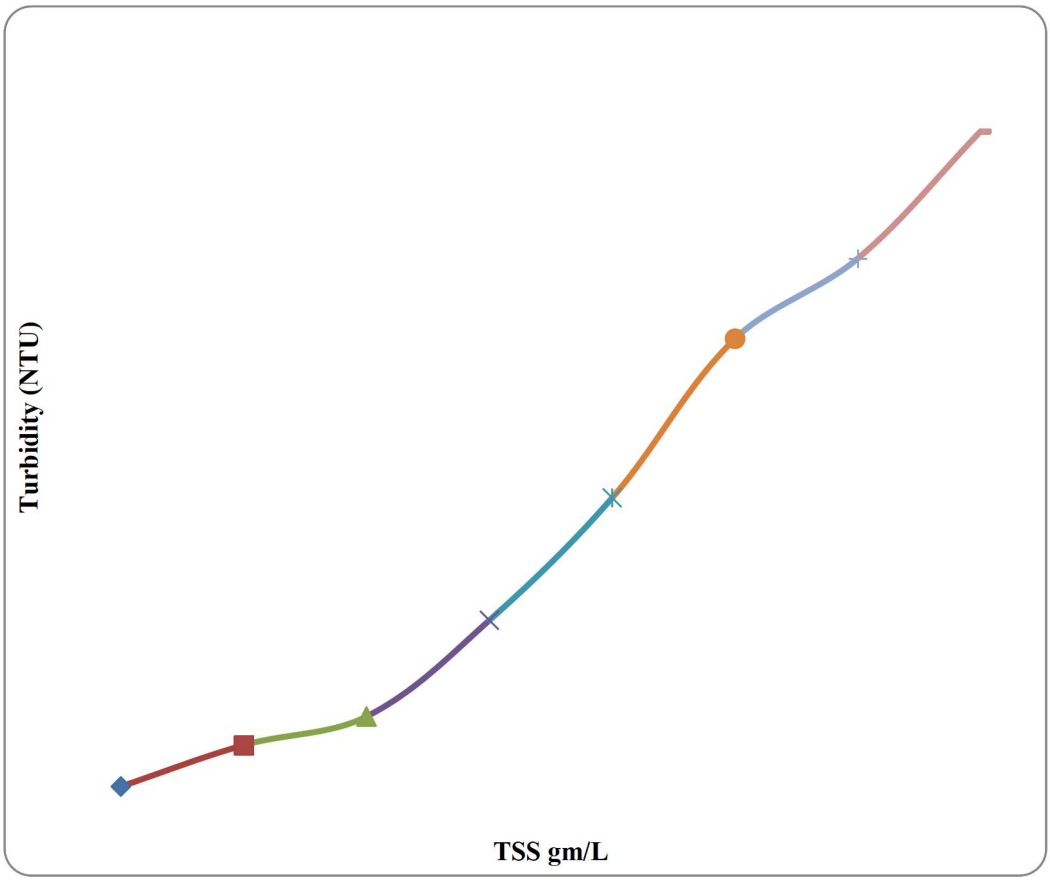

Figure 1. Turbidity vs SS graph of simulated turbid mine tailing water

Relationship between dry weight of yeast biomass and wet weight of biomass. It is not exact but approximate.

S.No.	Dry weight gm/L	Wet weight(gm/L)	Mean of wet weight
1.	0.005	0.045	0.047gm/l ~0.05gm/L
2.	0.005	0.05	
3.	0.005	0.047	

Research published

Pal, S. and Kamat, N. (2019). Fractal analysis of colony margins as an aid for screening freshwater yeast cultures for bioclarification of turbid polluted water resources. Journal of Applied and Natural Science, 11(2): 250- 256 https://doi.org/10.31018/ jans.v11i2.2028https://journals.ansfoundation.org/index.php/jans/article/download/2028/1763

(Annexure I)

Pal, S. and Kamat, N. (2020). Yeast in Southwest monsoon rainwater. (Fungal territory Journal, Sci cell Publisher). office.scicell.org/index.php/FT/article/view/95/61.

(Annexure-II)

Research under review

A Quantified Approach to Test Bioefficacy of Phosphate Solubilizing Yeasts Useful in

Phosphate Removal in Turbid water". (Studies in Fungi)

Research communicated

Potential of Novel Biotreatment Process Using Freshwater Yeast Cultures as Active Cellular Bioflocculants for Reduction of Turbidity of due to entry of Mine Tailing Water.

Research Presentations

Oral

Oral presentation on the topic, Effective use of yeast for bioclarification of turbid drinking water sources, at International Conference on Recent Innovations in Biological Research. Krishnammal college for Women. Department of Botany (DST –FIST Sponsored). PSGR . 17-18 Marc2022 **(Annexure III)**

Oral presentation on the topic,Present knowledge of sustainable bioremediation of highly turbid freshwater resources with prospects of application in mining areas of Goa, India, at National Conference on Change Environment Challenges, Solution and Strategies. Dempe college Goa. 8^{th}-9^{th} March 2018**. (Annexure IV)**

Oral presentation on the topic, Successful eco-friendly demonstration of yeast mediated bioclarification of inorganic colloidal turbidity in simulated mining wastewater at thousand litres scale, at National webinar on Technical Advances in Applied Microbiology. Department of Microbiology Goa University. 10^{th}-12^{th} November 2021. **(Annexure V)**

Oral presentation on the topic, Fractal analysis of fresh water yeast colony margins facilitates strain selection in biotreatment of turbid water, at International Symposium on Fungal Biology: Advances, Application and conservation and 45^{th}

annual meeting of mycological Society of India. At ARI Pune Maharashtra. 19-21 November 2018. **(Annexure VI)**

Poster

Poster presentation on the topic, Phosphate Solubilizing Freshwater Yeast: A Novel resource for biotreatment of turbid Phosphorous loaded polluted water. New Vistas in Botany (NSNVB, 2020) Dept. Botany Goa University 13th-14th February 2020.

(Annexure VII)

Poster presentation on the topic, Yeast in Southwest monsoon rainwater: Some ecological and biometeorological implications, National symposium on Current Trend and Future Prospects in Plant Science Research at Banaras Hindu University Varanasi.1-3rd February 2019. **(Annexure VIII)**

Poster presentation on the topic, Potential of novel biotreatment process using freashwater yeast culture as active cellular bioflocculants for reduction of turbidity of mine tailing water, National level seminar on microbial bioremediation: Novel approaches and trend. St. Xavier college Goa ,27th-28th February 2019. **(Annexure VIII)**

Poster presentation on the topic, Fractal analysis of colony margins as an aid for screening oligotrophic freshwater yeast cultures for bioclarification of turbid polluted water resources in the Iron ore mining region of Goa, International Conference on Trend in Biochemical and Biomedical Research Advances and challenges. Organized by Banaras Hindu University, Varanasi, India.13th-15th February 2018. **(Annexure IX)**

Workshop, Seminar, Conference attended

Novel Vistas in Plant Science, Department of Botany Goa University.

Attended Conference on water Management, Organized by Confederation of Indian Industry on 26july 2019.

Contributated in Successful organization of Nobel Exhibition, Nobel media, Govt. of Goa at Kala Academy, Panaji, Goa.
Attended National webinar on “Advances in Plant Sciences” Department of Botany , Goa University.

Webinar on Book writing and related topics, Webinar on Springer Nature
The Complete Step-by-step Guide to a Successful Career in Microbiology, Department of Microbiology, P.E.S's R.S.N College of Arts and Science, Farmagudi, Ponda-Goa.