International Research Journal of Engineering and Technology (IRJET) e-ISSN: 2395-0056
Volume: 07 Issue: 05 | May 2020 www.irjet.net p-ISSN: 2395-0072

Isolation, Identification and Characterization of Sacchromyces Cerevisiae Strains, Obtained from Different Sources

Er.Aarti Gautam[1], Dr. Ashish Shukla[4]

[1]Assistant Professor Department of Biotechnology FET, RBS, ETC Bichpuri Agra
[2]Assistant Professor, Department of Biotechnology, F. E. T., R. B. S. ETC, Bichpuri, Agra

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ABSTRACT

On the basis of the Morphology and biochemical characterstics to isolate fungi(Sacchromyces Cerevisiae) from different five sources. Saccharum officinarum(sugarcane), solanum tuberosum soil(potato soil), curd, whey and garden soil. Sacchromyces cerevisiae was collected from sugarcane juice, curd, whey, soil and potato soil from the local market of Dayalbagh and Raja Balwant Singh Engineering Technical Campus Bichpuri Agra (U.P) India. Most of the isolated yeast showed Hydrogen sulphide test produced brown colour means yeast is present. The ability to tolerant PH, Temperature, Ethanol and Osmotolerance test were studied. Ten strains are isolated from one sources with different concentration (10^{-1} to 10^{-9}) with serial dilution Method. Five Sample of different sources preserved at 0°c, and the sample were shifted in the laboratory at stored at 4°c for 24 hours and proceed further for study. Sacchromyces cerevisiae species was identified by applied for different tests including morphological, cultural, biochemical characterstics and physiological test, which facilitate the opportunity for identification of yeast. This sources is a good habitat for yeast.

Keywords: Sacchromyces Cerevisiae, Sugarcane, Curd, Whey, Potato Soil, Normal Soil, Biochemical Test, Qualitative Analysis.

INTRODUCTION

Sacchromyces Cerevisiae is a universal organism. Yeast are the multicellular fungi that can be classified in to two phylogenetic group i.e. teleomorphic and anamorphic ascomycetocus or teleomorphic and anamorphic basidiomycetous yeasts that reproduce by budding or fission and that form their sexual states (i.e. asci), which are not enclosed in a fruiting body. Saccharomyces strain is harmful and useful of human being **(Mayuri K. 2011).** It has long history used in the fermentation food, non-food and including cosmetic and pharmaceutical industries. This taxon consists of four yeast species, namely Sacchromyces Cerevisiae, Zygosacchromyces Species, Sacchromyces ellipsoids, , these are produced a bioethanol with different percentage , the help of fermentation process. The fermentation process involves conversion of sugars to alcohol and carbon dioxide by the yeast Saccharomycescerevisiae.

$C_6H_{12}O_6 + H_20\ C_2H_5OH + CO_2$ ⟶

The yeast has classified and isolated from sugar cane juice. The sugar cane was grown in over 120 countries in the all world. Brazil is the world's Biggest Sugarcane producing country in all over the nations with a total production of 672,157,000 tonnes, While India is the 2nd biggest producer after Brazil Sugar cane, as a raw material, is used for 60% of global ethanol production. The sugar cane is used as the source of table sugar juice, rum, fuel ethanol, sugar molasses, vinegar (Siraka) and directly as fresh sugar cane **(M. de**

Araujo Vicente *et al*. 2006) The high sugar content of sugar cane makes it an ideal source for the production of alcoholic beverages. Thus the sugarcane juice is an excellent medium for fermentation in order to elaborate alcoholic beverages as it is a rich source of sucrose, glucose and fructose **(Morimura et al., 1997 and Agrawal et al., 1998)**. Fresh sugarcane juice has been used as a thirst quenching drink in some places such as South East Asia and also in Mexico and some parts of South America The Brazilian spirit and Caribbean countries was a sugarcane spirit obtained by the distillation of cooked fermented sugar cane juice and molasses **(Kurtzman and Fell 2013).** The yeast dose rate also has effect on performance. A higher dose rate will result in a faster start of fermentation, which helps to control contamination. Dose rates must be optimized for cost effective performance. Nutrition is another important parameter to take into consideration. optimal fermentation, yeast requires building block substances (C, N, P, S, O) in optimal ratios, minerals (e.g., K, Na, Mg, Ca, Zn, Fe, Mn, Cu, Co) and vitamins (B1, B5, B6, Biotin, etc.). Oxygen is normally present at very low levels in commercial scale ethanol fermentations **(Linderholm *et al*. 2008).** In practice, the process cannot be completely anaerobic because oxygen is required for production of unsaturated fatty acids that are essential for yeast growth and ethanol production. Most substrates for commercial ethanol production have been found by to be nitrogen limited. It is therefore recommended to add to grain-based ethanol fermentations yeast nutrients containing a nitrogen source usable by yeast. High ethanol concentration also stresses yeast. Avoidable yeast stress factors, such as high temperatures, high osmotic pressure, high sodium concentration. Saccharomyces cerevisiae species is yeast belonged to Kingdome of fungi and isolated from sugar cane juice that was identified by studying specific morphological, biochemical and physiological characteristics as given by Saccharomyces cerevisiae (brewer's yeast) has been used in classical food fermentation applications such as production of beer, bread, yeast extract/vitamins, wine, sake, and distilled spirits supplements in human and animal diets as well as in the production of single cell proteins. Saccharomyces cerevisiae yeast is largely used fermentation of ethanol production using such renewable **(Boekhout & Kurtzman, 1996; Kurtzman & Fell, 1998; Querol & Belloch, 2003).**

biomass as sugarcane or sugar beet molasses as the main carbon source The Hydrogen sulphide was produced by various mechanisms of Saccharomyces cerevisiae. It can degrade sulphur-containing amino acids to utilize the nitrogen, and the release of H2S or other volatile sulphur compounds as by-products. The protoplast fusion was produced recombinant yeast negative for H2S (hydrogen sulphide) production and maintaining the flocculation trait **(Ribeiro & Horii 2004, Noroul Asyikeen2013).** These characteristics were discussed in the fermentation studies of sugarcane juice, in the quality of sugarcane spirit and alcoholic fermentation process.

The objective of this study was to isolate Saccharomyces cerevisiae from different sources like sugar cane juice ,whey ,Curd, Potato Soil, and Normal Soil and the isolated yeast strains were identified by studying specific morphological, biochemical and physiological to screen these isolates for desirable traits such as homo fermentative ability and the production of various enzymes.

MATERIAL AND METHODS

Microorganism

Sacchromyces Cerevisiae was isolated from sugarcane ,curd , Normal soil , Potato soil s and whey samples was provided by Dayalbagh and R.B.S.E.T.C Bichpuri, Agra.

Media

The culture media used in this work were YEPD (yeast extract,10g/L peptone,20g/L dextrose,20g/L agar,15g/L) i.e. the growth medium of yeast and carbohydrate fermentation test media is L.B. media(trptophan 10g/L,yeast extract 5g/L, Nacl 5g/L,1M(NaOH) 1ml/L, PH -7) with different carbon sources at the concentration of 20g/L(glucose, sucrose, lactose, starch, manitol).

Sample collection

The sample were collected randomly by different sources likesugarcane juice, curd, whey, potato soil, and normal soil from different places in Agra, Dayalbagh, Bichpuri, in sterile bottle at 4°c in ice bugs and have used different five sources to produce Sacchromyces species .

Isolation of yeast

45 samples of fresh juice, curd ,whey, potato soil and normal soil were collected from different sources that were available in local market(Dayalbagh and Bichpuri) in Agra city. One gram of fruit sample, one gram thick curd sample, one gram of soil sample, and one ml of whey sample, blended and mixed with sterilized nine milileter Distilled water then a serial of dilution was made untill 10^{-9} and every dilution was cultured by pour plate method using a YEPD medium containing yeast extract(10g/L),Peptone(20g/L),Dextrose(20g/L),Agar(15g/L), pH 5.5 .plates were incubated at 30°c for 3-5 days. These special conditions (high sugar concentration and higher temperatures) were chosen because Saccharomyces yeast strains are normally subjected to similar conditions during the fermentative process (20g/L dextrose) the medium was sterilized autoclaved at 121°c,with 15 psi. some plates have a refine colonies and some plates colonies are not identified then replicate a refine colonies plates with same method. Morphological appreances and microscopic examination of the colonies were done after staining (Lactophenol Blue).

Identification of sacchromyces cerevisiae (Morphology)

Yeast viability staining

The culture with the take off loop on slide. The culture was subjected to yeast viability staining produced and microscopically under high power objectives speherical and budding yeast were observed.

Lacto phenol cotton blue staining

Lactophenol cotton blue solution is a mounting medium. Firstly the loop picked a colony on a slidethen S. cerevisiae**(Panneerselvam A. *et al.* 2014)** culture was stained by cotton blue dye producer and observed under high power objectives. The colonies was checked with on the basis of colour and other features.

Simple staining of microbes

The isolated culture picked up with the help of loop of culture on a slide the isolated culture was subjected to sample staining produced and observed under high power objective. Speherical and budding yeast were observed.

Taxanomic identification

The isolates belonging to S. cervisiae species were identified by the carbon assimilation test as described by Vaughan-Martin and Martin(1993) and Lonner(1993)

Flocculation Test

The isolates were inoculated in 10 ml YPG; Yeast peptone glucose medium, yeast extract(10g/L),Peptone(20g/L),Glucose(20g/L)liquid in each test tube and incubated at 30°c for 72 hours. After incubation the tubes were agitated for the visualization of flocculation.

Thermotolerance Test

YPD media detecting thermotolerance & growth in liquid media of selected yeast isolated, The medium was autoclaved at 121°c,15psi & cooled. 10 ml of medium was distributed in each test tube with harfloopful of 2-3 days(old culture) yeast isolated. The initial optical density of each tubes was recorded on spectrophotometer at 600nm. Against the medium is blank. After 48 hours measure a optical density at same nm. And all culture were incubated at 26°c,29°c, 34°c,38°c,42°c and 44°c. The increase in optical density in a tube was recorded as evidence of growth. Without it, growth on YEPD agar media at 26°C, 29°C, 34°C, 38°C, 42°C and 44°C was also observed to ensure thermotolerence of the strain.

pH Tolerance Test

In YEPD liquid medium for detecting the ability to grow at different pH. The medium was autoclaved at 121°c & 15 psi ,cooled at before inoculation. YEPD browth was prepared at pH 1 to 9 and blank media was used as control , 600nm. at 30°c for 48 hours.cell density was further recorded 600nm. for growth.

Ethanol Tolerance Test

The isolates were inoculated at 10ml of liquid YPD media in each test tubes 6 to 24%v/v added different flask with same medium at 600nm. all culture were incubated 40°c for 5 days.

Osmotolerance Test

YEPD broth was prepared containing 6%, 9%, 12%, 15%, 18% and 20% of NaCl. Each Test tube contained 10 ml of YEPD liquid media with appropriate concentration of salt and blank media was used as a control. Then each was inoculated by half loopful of Yeast cell and measured the initial optical density at 600 nm and incubated at 30°C for 48 hrs. After 48 hrs cell density was further recorded at 600 nm.

H_2S Test

The YPG medium was sterilized at 121° ,15 psi for detection the yeast for producing a hydrogen sulphide gas The selection of H_2S trait was used YPG medium incubated at 30°c for 48 hours. The white colour had non- sulphide producing strains, while the H_2S producer presented various , colours that light brown to black colour i.e. test is possative.

Starch hydrolysis Test

The isolates were plated in YPGA medium was sterilized at 121°c & 15 psi, cooled the medium and inoculated a 2-3 day old yeast on plates. Plates were incubated at 25°c in invert position ,keeping in incubator for 5-7 days. After 5-7 days growth are full on plates the flooded a iodine solution for 30 sec. exaimed the disappearing the starch agar media plates of clear zone around the fungal growth.

Killer toxin Test

Yeast killer toxin are protein compound, which are active against members of same species, and the activities of these toxins are analogus to the activities of bacteriocins in bacterial species(Lowes et.al.,2000). As a rule, nutritionally rich organic media are more suitable than synthetic media. In most case, glucose yeast extract-peptone agar with sodium citrate-phosphate buffer are used. It is apparent that both the level and expression of killer toxin activity depends on a number of variables. In particular, the assay condition can be crucial for detecting killer with low activity of those organisms that are weakly sensitive (Kurtzman and Fell, 1997). It is described the action of a killer strain on a sensitive strain is easy to demonstrate in the laboratory on an agar culture medium at pH 4.2-4.7 at 20° C. The sensitive strain is inoculated into the mass of agar before it solidifies; then the strain to be tested is inoculated in streaks on the solidified medium. If it is a killer strain, a clear zone in which the sensitive strain cannot grow encircles the inoculums streaks. In this study the following was followed to observe killer toxin production by Yeast:

Result

S. Cerevisiae strains has been obtained from different sources like sugarcane juice, curd, whey, potato soil and normal soil from the agriculture field of RBSETC Bichpuri, and local market of Dayalbagh in Agra(U.P) India then dilution of this sample $1/10^{th}$ rule. Take ten test tube and then mark 10^{0}- 10^{-9} and the evey dilution have make one plate of each source. Every test tube diluted sample mixed well and take 100µl in the yeast extract peptone dextrose agar petri plates disc for 3-4 day incubation period at 30°c then I focused single colony with different concentration of serial dilution, that plate after then pouring of culture on YEPD agar plate further incubated for 3-4 days. Yeast was identify according to shape, size and individual yeast cell colourless, grown on YEPD media. In the previous study, they produce may be white ,cream colour and brownish pigment. Most of the isolated colonies have observed smooth surface with circular margin. The cells were found to be a various shapes, such as round oval. Comparative to previous study, The isolated strain grown on YEPD medium have founded smooth surface with circular margin, creamy ,creamy white. The yeast species are also determined by great extent of physiological characterstics.

S. cerevisiae strain were obtained and, identified based on the morphological characterstics.S. cerevisiae strain were identified on the basis of biochemical test and physiological test. First of all isolated yeast strain concentration with different sources, colonies were observed under compound microscope. The strain was stained by yeast viability staining(methyl blue staining) that the cell morphology was morphologically observed under a microscope. S. cerevisiae culture was subjected to lactophenol cotton blue staining observed as high power objectives. Budding S. cerevisiae cell were seen. The morphological characterization of yeast was subjected to simple staining(crystal violet staining) .

The yeast was observed possative result for urea test. S. cerevisiae has hydrolysis of urea by urease enzyme in rapid urease test browth used the urease reaction given by H. PYLORI.

The starch hydrolysis test detects the ability of fungi to produce a hydrolysis of starch no clear zone are observed around the fungal growth, S. cerevisiae observed urea negative and starch hydrolysis test is also negative

In previous study, S. cerevisiae strain was produce of hydrogen sulphide during to fermentation, determined of YPGA medium, during to H_2S production observed increase the brown colour and decreased lighter colony colour, isolated strain colour was found brown and black

In the taxanomic identification of yeast, we have total 45 samples of different concentration with different five sources, in flocculating test used only a 9 sample with a different concentration sugarcane[$10^{-8}(AS_1)$,$10^{-6}(AS_2)$]sample,curd[$10^{-6}(AC_2)$,$10^{-9}(AC_1)$]sample,whey

[$10^{-6}(AW_2)$,$10^{9}(AC_1)$]sample, potatosoil.

[$10^{-9}(APS_1)$]sample, normal soil [$10^{-4}(ANS_2)$,$10^{-6}(ANS_1)$]. In this test have AS_1 have a low flocculation and AS_2 have a intensive growth of yeast. In carbohydrate fermentation test different sugar are used like glucose, starch ,lactose , sucrose and mannitol. One test tube use as a control no inoculation occur. All nine test tube change from red to orange in glucose and sucrose. The orange colour identifie a production of acid. i.e. yeast is present. In starch fermentation test AW_2,ANS_1,AS_2,and APS_1 no colour change and remeaning test tube produce a orange colour test is possative. In manitol and starch are no colour change properly slightly change in colour. Result can be neither be possative nor negative.

In thermotolerance test after 48 hrs. the cell density is high, sample concentration AW_2,AC_2,AS_2,APS_2,AW_1,ANS_1 at 26°c . at 29°c cell density is hight, in one sampleANS_1, 34°C cell density high AS_2,ANS_1,temp 38°c high density of a cell is AC_1, temp 42°c cell density is high at AC_1,temp 44°c high density of a cell is AC_2 as compare to initial density.

pH tolerance test, yeast cell grow a specific pH with different concentration from the five sources. pH 1 yeast cell grow after 48 hrs at ANS_1 ,high cell density at pH 6 yeast cell growth high at AS_1, pH 8 yeast cell growth hight at a concentration of AW_2,AW_1,pH 9 yeast cell density high at AC_2.

In ethanol tolerance test 6% of ethanol, yeast cell density is high at 600nm. after 48 hrs AW_2,AC_1. Ethanol 9% of ethanol yeast cell density is high ,concentration is AW_2.ethanol 11% of ethanol yeast tolerate to ethanol concentration of sample is AC_1,ethanol 14 % yeast cell density is high,concentration of sample is ANS_1,ethanol 19% ANS_1 concentration of sample density is high.

In osmotolerance, 5% of NaCl cell density is high of yeast is AS_1,NaCl 8% cell density is high or tolerance of salt concentration is more and sample name is AS_2,NaCl 13% cell density is hight atAS_1, NaCl 16% cell density is high at ANS_2,NaCl 18% yeast cell density high at AS_1,NaCl 19% yeast cell density high at ANS_2.

TABLE 1 :Morphological characterstics of Sacchromyces Strain Isolated from different sources like Sugarcane Juice, Curd, whey, Normal soil and potato soil

Sugarcane juice source	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**	**10^{-6}**	**10^{-7}**	**10^{-8}**	**10-9**
Colonies	Mixed	Mixed	Mixed	Mixed	Define	Present	Present	Present	Present
Margin	Irregular	Irregular	Not define	Less Circular	Circular	Circular	Circular	Circular	Circular
Cells	Not identified	Not identified	Not identified	Not identified	Oval	Oval	**Round**	**Round**	**Round**
Colour	**Creamy**	**Brown**	**Light Brown**	**White**	**Creamy**	**Creamy**	**Creamy/White**	**White/ Creamy**	**Creamy/White**
Colony Count	**No**	**No**	**No**	**No**	**No**	**25**	**21**	**17**	**15**
Surface	**Smooth**	**Smooth**	**Rough**	**Rough**	**Smooth**	**Smooth**	**Smooth**	**Smooth**	**Smooth**

Curd Source	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**	**10^{-6}**	**10^{-7}**	**10^{-8}**	**10^{-9}**
Colonies	**Undefined**	**Undefined**	**Undefined**	**Undefined**	**Undefined**	**Define/ present**	**Define/ present**	**Define/ present**	**Define/ present**
Margin	**Irregular**	**Irregular**	**Irregular**	**Not/ define**	**Circular**	**Circular**	**Circular white**	**Circular**	**Circular**
Cells	**Not identified**	**Not identified**	**Not identified**	**Less identified**	**Round**	**Oval**	**Oval**	**Round**	**Round**
Colour	**Light brown**	**Dark brown**	**Light brown**	**Creamy**	**Creamy**	**White**	**Creamy**	**Creamy**	**Creamy/ white**
Colony Count	**No**	**No**	**No**	**33**	**25**	**20**	**17**	**15**	**12**
Surface	**Rough**	**Rough**	**Rough**	**Rough**	**Rough**	**Smooth**	**Smooth**	**Smooth**	**Smooth**

Potato soil source	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**	**10^{-6}**	**10^{-7}**	**10^{-8}**	**10^{-9}**
Colonies	**Mixed**	**Mixed**	**Mixed**	**Absent**	**Define**	**Define/ present**	**Define/ present**	**Present**	**Present**
Margin	**Irregular**	**Irregular**	**Irregular**	**Irregular**	**Circular**	**Circular**	**Circular**	**Circular**	**Circular**
Cells	**Not identified**	**Not identified**	**Not identified**	**Not identified**	**Not identified**	**Oval**	**Round**	**Oval**	**Oval**
Colour	**White**	**Creamy**	**Light brown**	**Dark brown**	**White/ creamy**	**Creamy**	**White/ creamy**	**Creamy**	**Creamy**
Colony Count	**No**	**No**	**No**	**No**	**No**	**18**	**15**	**14**	**12**
Surface	**Smooth**	**Smooth**	**Smooth**	**Smooth**	**Smooth**	**Smooth**	**Smooth**	**Smooth**	**Smooth**

Whey Source	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**	**10^{-6}**	**10^{-7}**	**10^{-8}**	**10^{-9}**
Colonies	**Mixed**	**Mixed**	**Mixed**	**Absent**	**Absent**	**Present**	**Absent**	**Mixed**	**Present**
Margin	**Irregular**	**Irregular**	**Irregular**	**Irregular**	**Irregular**	**Circular**	**Irregular**	**Circular**	**Circular**
Cells	**Not identified**	**Not identified**	**Not identified**	**Not identified**	**Not identified**	**Oval**	**Not identified**	**Oval**	**Oval**
Colour	**Creamy**	**Creamy**	**Creamy**	**Creamy**	**Creamy**	**Creamy**	**Creamy**	**Creamy**	**Creamy**
Colony Count	**No**	**No**	**No**	**No**	**No**	**33**	**No**	**No**	**15**
Surface	**Rough**	**Rough**	**Rough**	**Rough**	**Rough**	**Smooth**	**Smooth**	**Rough**	**Smooth**

Normal Soil source	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
Colonies	Mixed	Mixed	Mixed	Present	Present	Present	Absent	Absent	Absent
Margin	Irregular	Irregular	Irregular	Circular	Circular	Circular	Irregular	Irregular	Irregular
Cells	Not identified	Not identified	Not identified	Oval	Oval	Round	Round	Not identified	Not identified
Colour	Creamy	Creamy	Creamy	Creamy	Dark brown	Creamy	Creamy/ brown	Light/brown	Creamy
Colony Count	No	No	No	17	22	25	No	No	31
Surface	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth

TABLE 2: Biochemical Analysis of Sacchromyces Strain Isolated from different Sources for Different Area

S.No.	Sources	Starch hydrolysis Test	Urea Test	H_2S Test	Killer Toxin Test	Morphological Test
1	Sugarcane	+	-	Brownish ,Black	-	+
2	Curd	+	-	Brownish	-	+
3	Whey	+	-	Black	-	+
4	Normal soil	+	-	Black	-	+
5	Potato soil	+	-	Brownish	-	+

(-)- Negative Result

(+)-Positive Result

TABLE 3: Taxanomic Identification (Carbon Test) of Sacchromyces Cerevisiae Strain from different Sources with different concentration

S. No.	Concentration Of sources	Glucose	Sucrose	Lactose	Manitol	Starch
0(Control)	-	_	_	_	_	_
1(AW_2)	Whey 10^{-6}	++	_	+	_	_
2(AC_2)	Curd 10^{-6}	++	+	+	+	+
3(ANS_2)	Normal soil 10^{-4}	+	+	_	_	+
4(AS_2)	Sugarcane 10^{-6}	+	++	++	++	++
5(APS_1)	Potato soil 10^{-8}	++	++	+	+	+
6(AW_1)	Whey 10^{-9}	++	+ ++	+	_	++
7(ANS_1)	Normal soil 10^{-6}	_	+	+	+	+
8(AC_1)	Curd 10^{-9}	+	+	+	+	+
9(AS_1)	Sugarcane 10^{-8}	_	_	+	+	+

(-)- no acid production, (yeast absent)

(++)- shows a orange colour(acid production, yeast present)

(+)- shows a orange/red colour(less acid production,less growth of yeast)

(+++)- shows a yellow colour(more acid production, intensive growth are present of yeast)

TABLE 4: Taxanomic Identification(Flocculation Test) of Sacchromyces Cerevisiae Strain From different sources with different concentrations

S. No.	Concentration of sources	Flocculating Test
0(Control)	-	-
1 (AW_2)	Whey 10^{-6}	+
2 (AC_2)	Curd 10^{-6}	++
3 (ANS_2)	Normal soil 10^{-4}	++
4 (AS_2)	Sugarcane 10^{-6}	+++
5 (APS_1)	Potato soil 10^{-8}	+
6 (AW_1)	Whey 10^{-9}	-
7 (ANS_1)	Normal soil 10^{-6}	-
8 (AC_1)	Curd 10^{-9}	++
9 (AS_1)	Sugarcane 10^{-8}	+

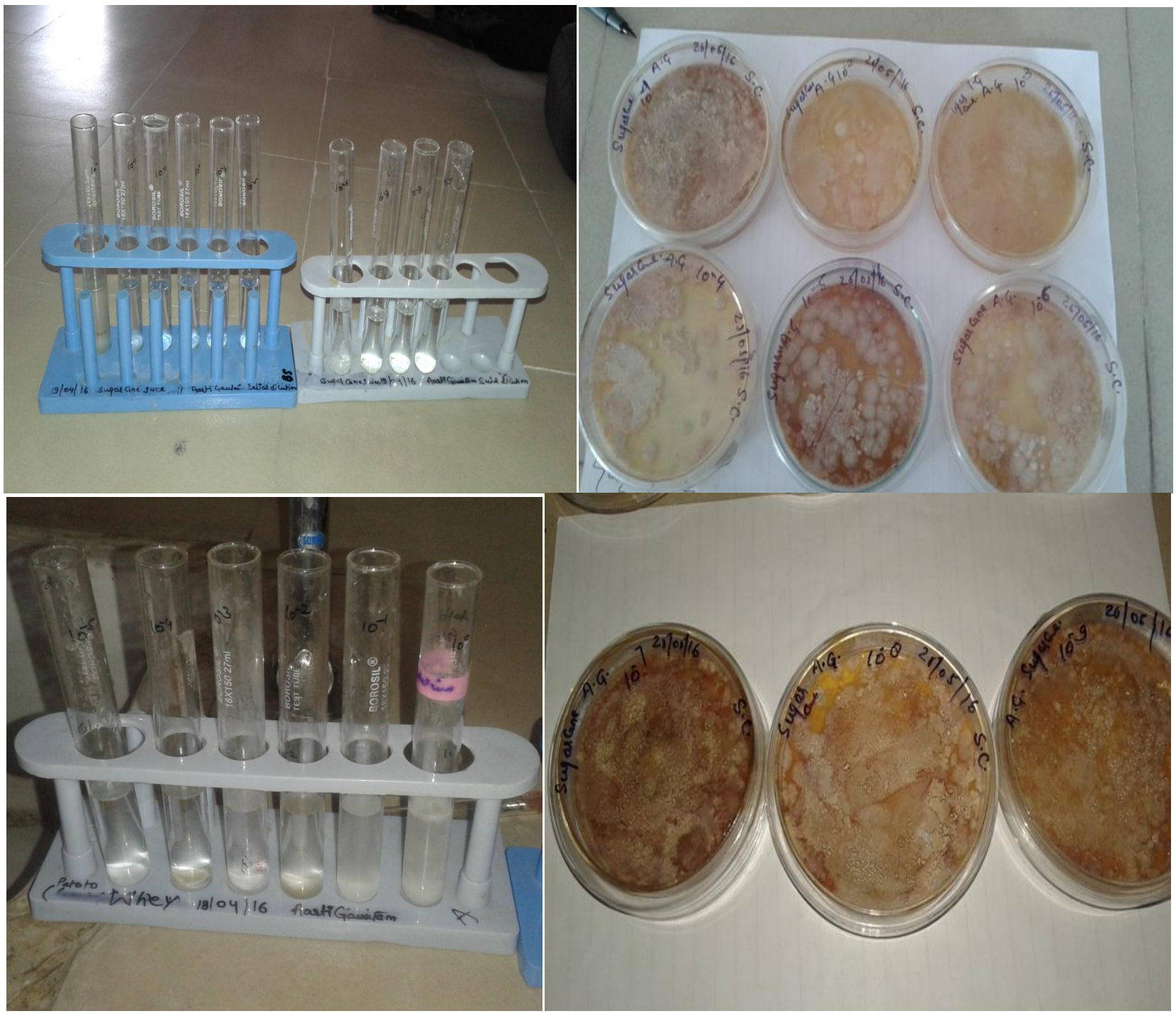
(-)- No flocculation

(+)- Low flocculation

(++)- Moderate flocculation

(+++)- Intensive flocculation

Fig 1: Serial dilution of sugarcane , curd, whey, normal soi and potato soil Colony isolation from different sources



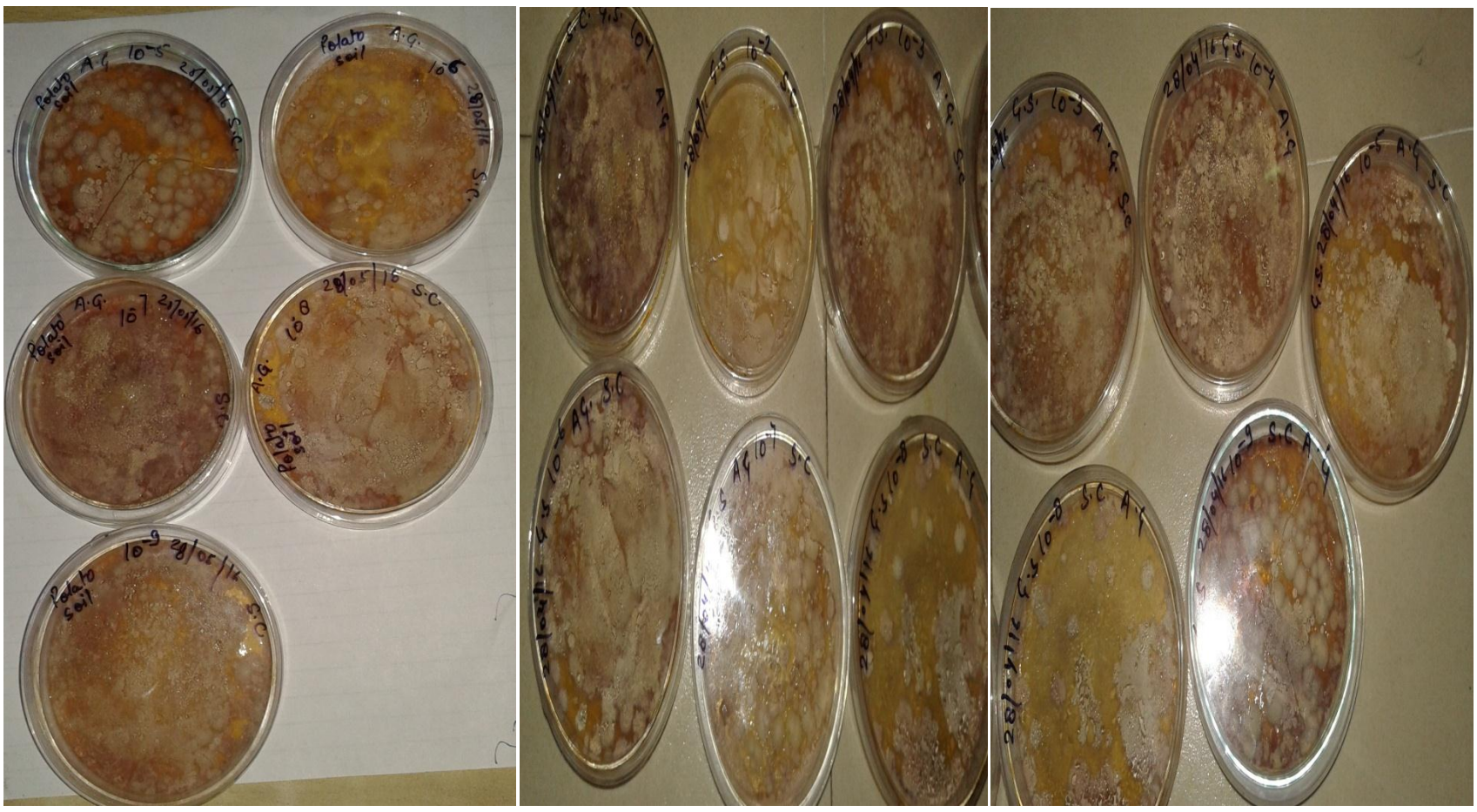


Fig 2: Replica plates isolated strains from previous plates from different sources of different concentration

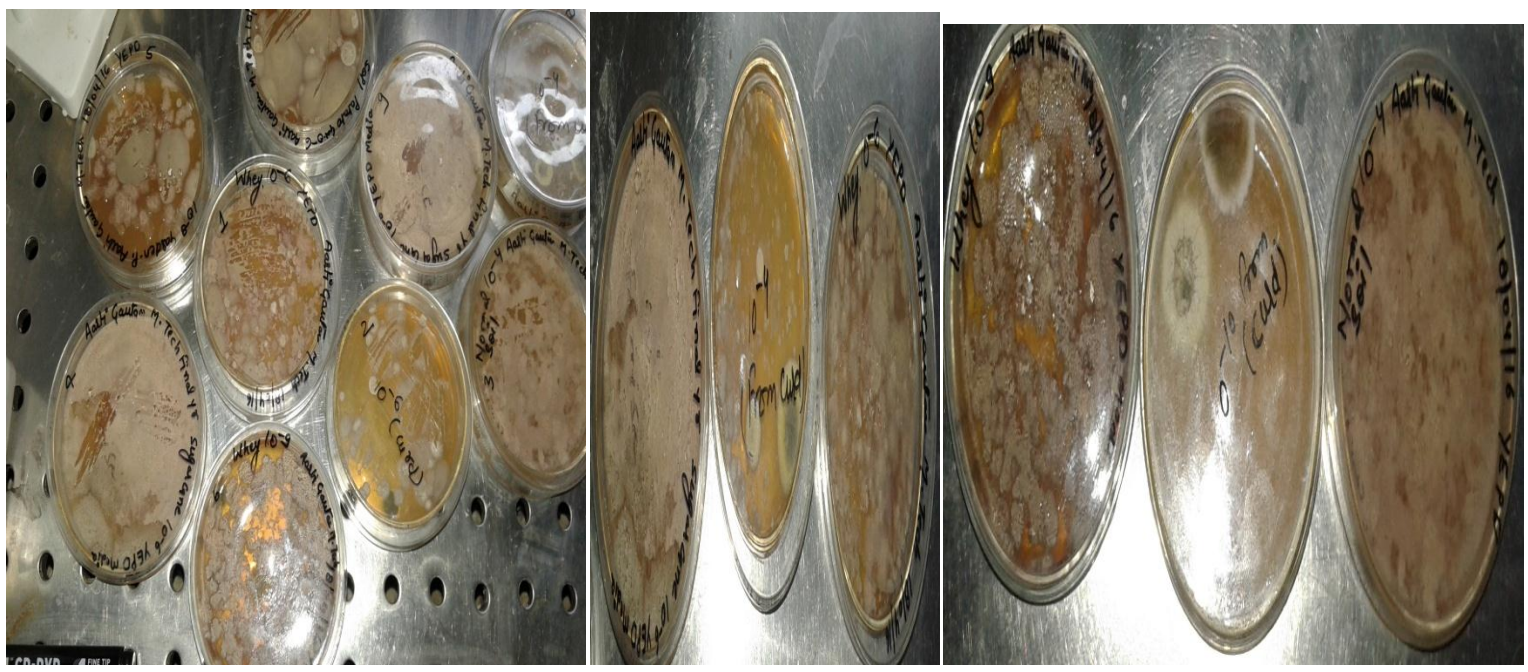


Fig 3: Morphological test(Lactophenol blue) Fig 4: Hydrogen sulfide Test

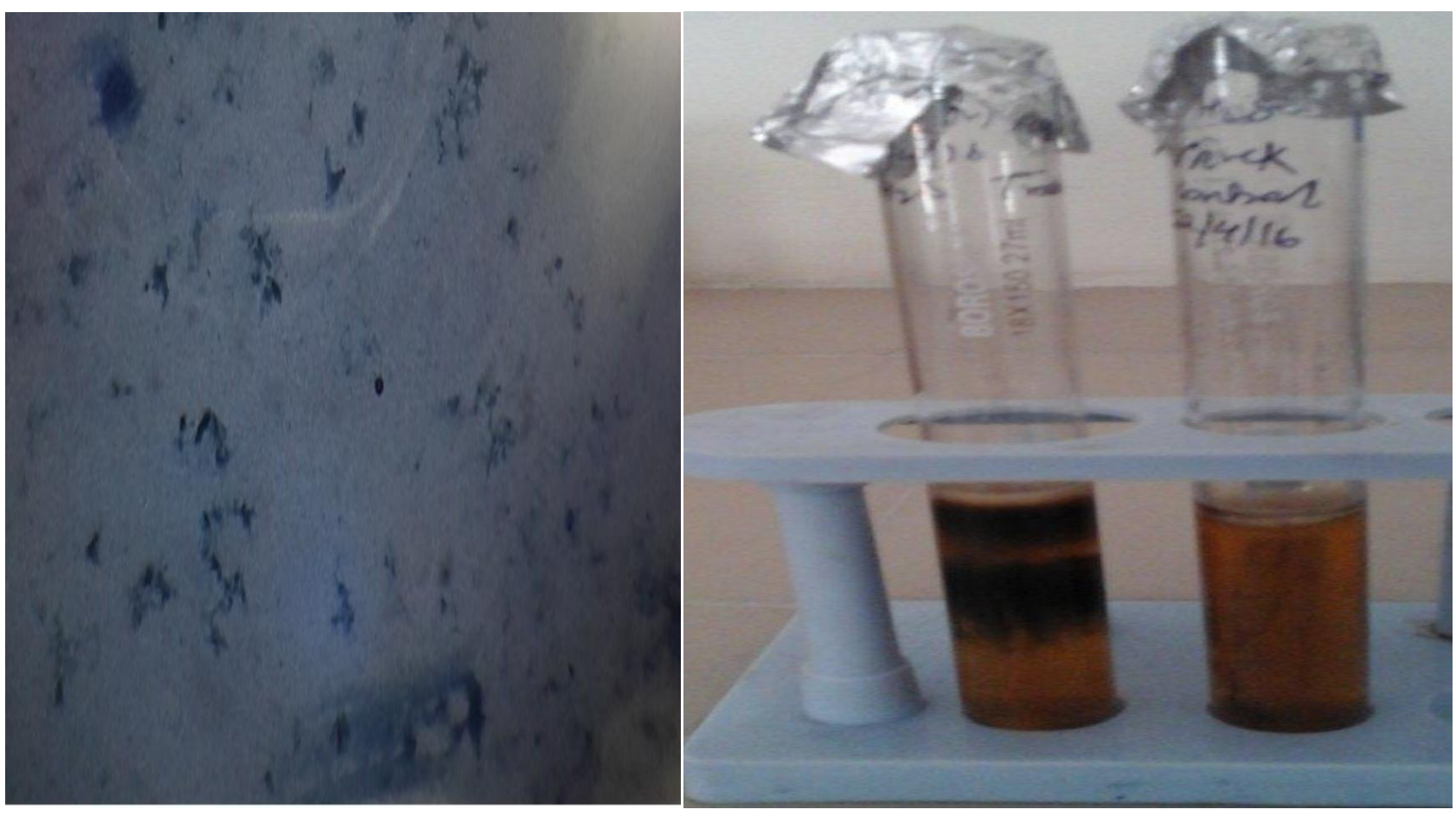


Fig 5:Carbohydrate Fermentation Test(glucose)

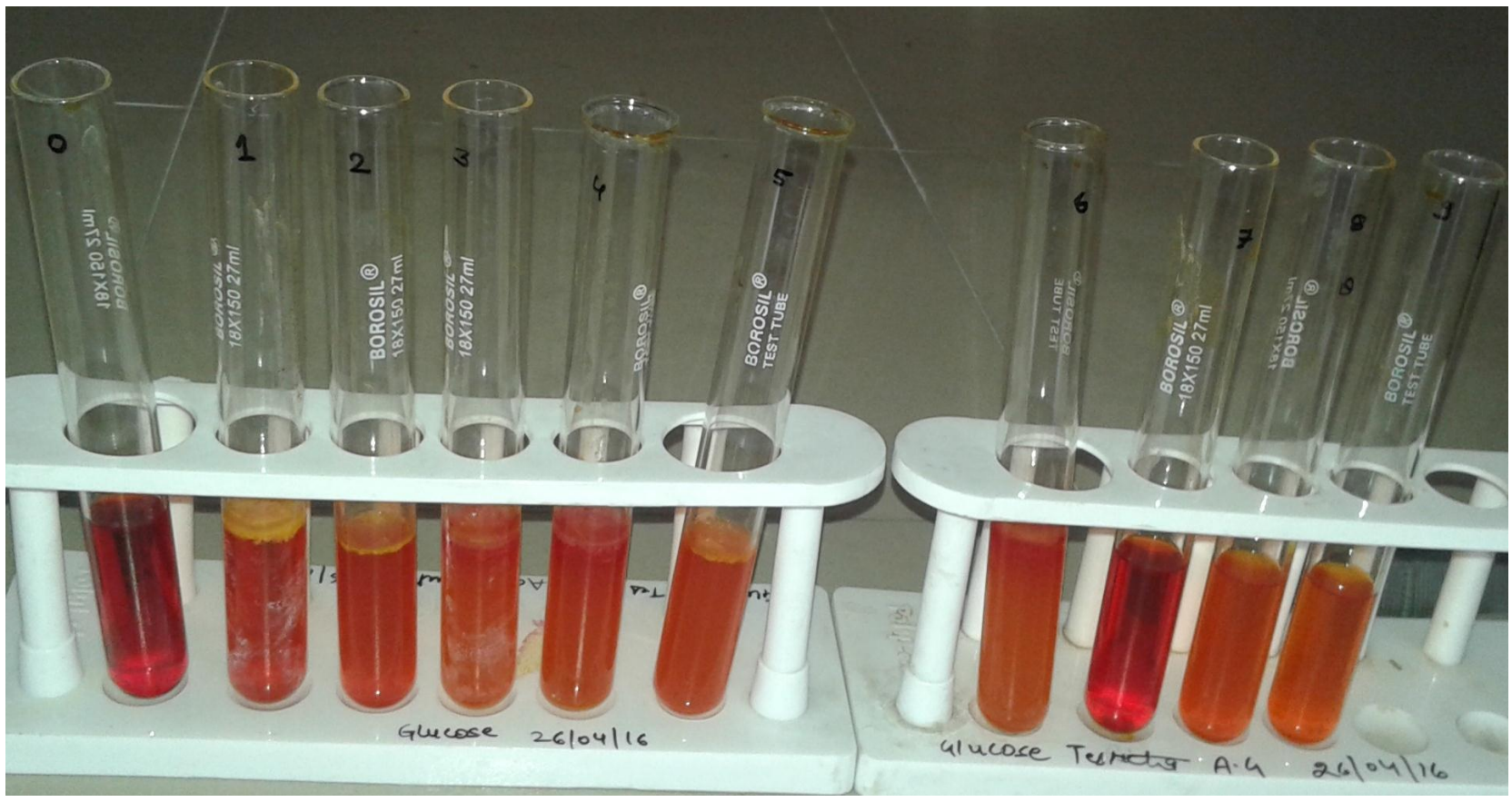


Fig 5.1: Carbohydrate fermentation Test (Sucrose)

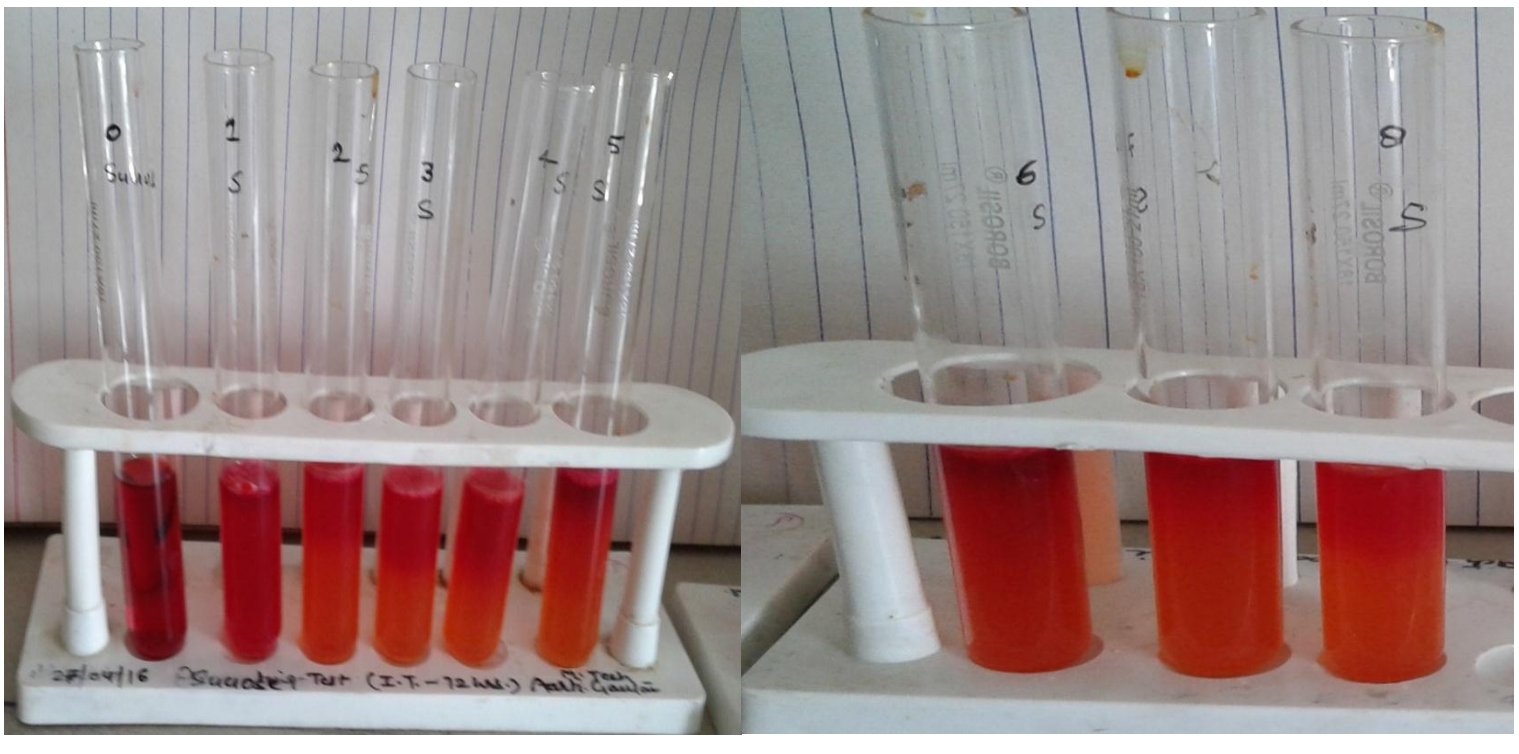


Fig 5.2: Carbohydrate Fermentation Test(Lactose)

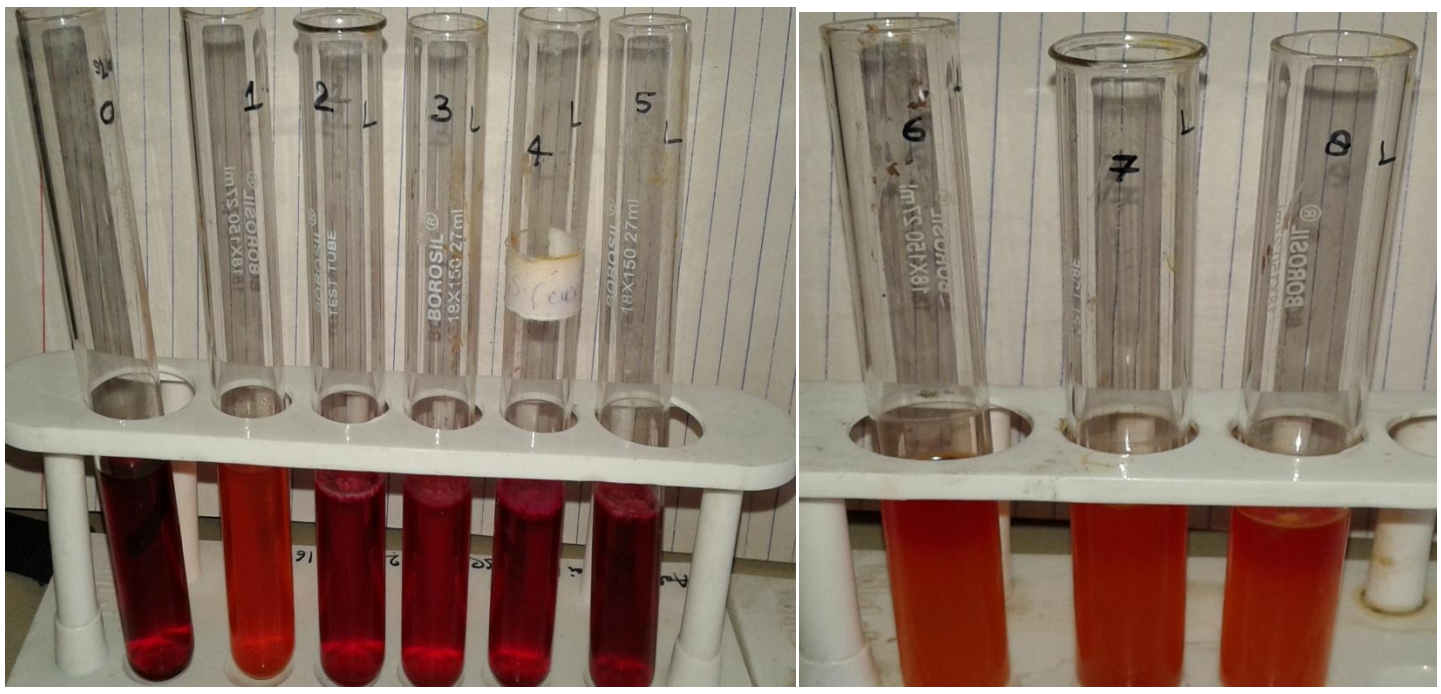


Fig 5.3: Carbohydrate Fermentation Test(Manitol)

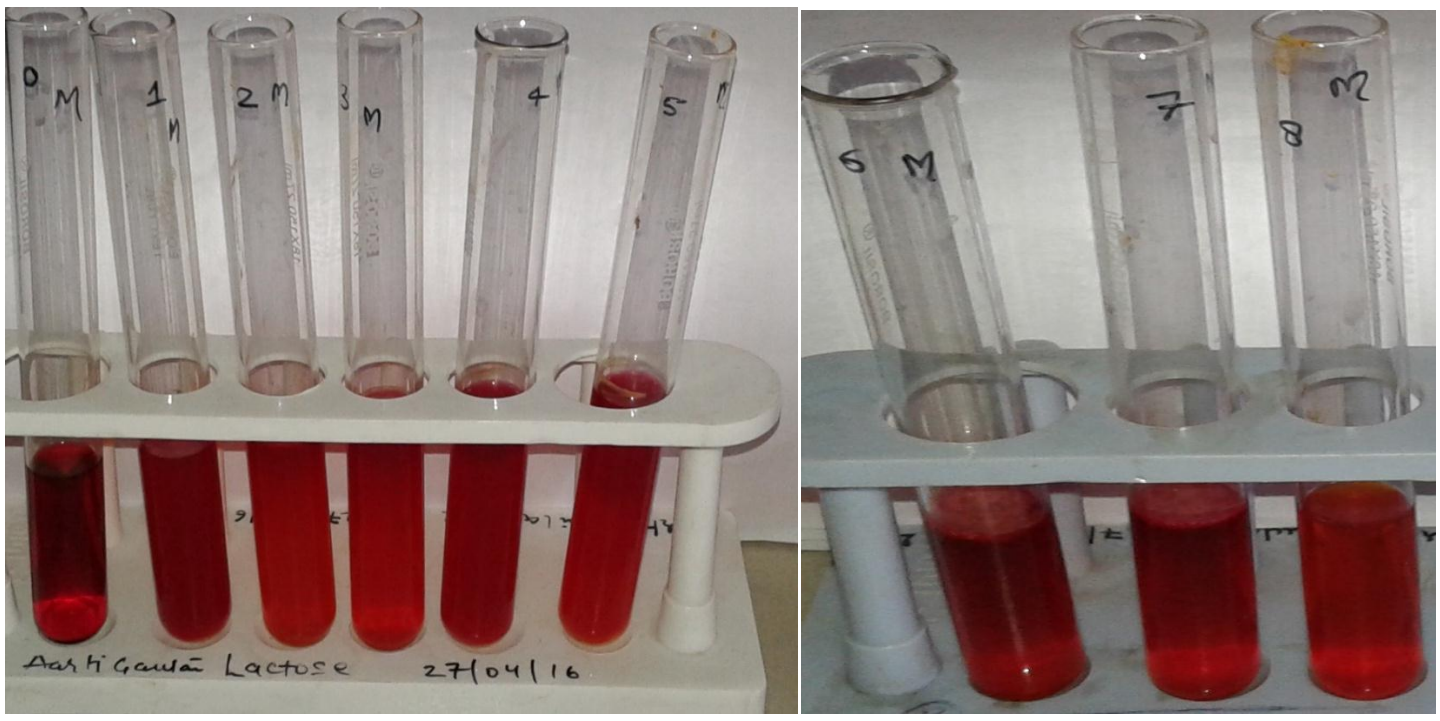


Fig 5.4: Carbohydrate Fermentation Test(Starch)

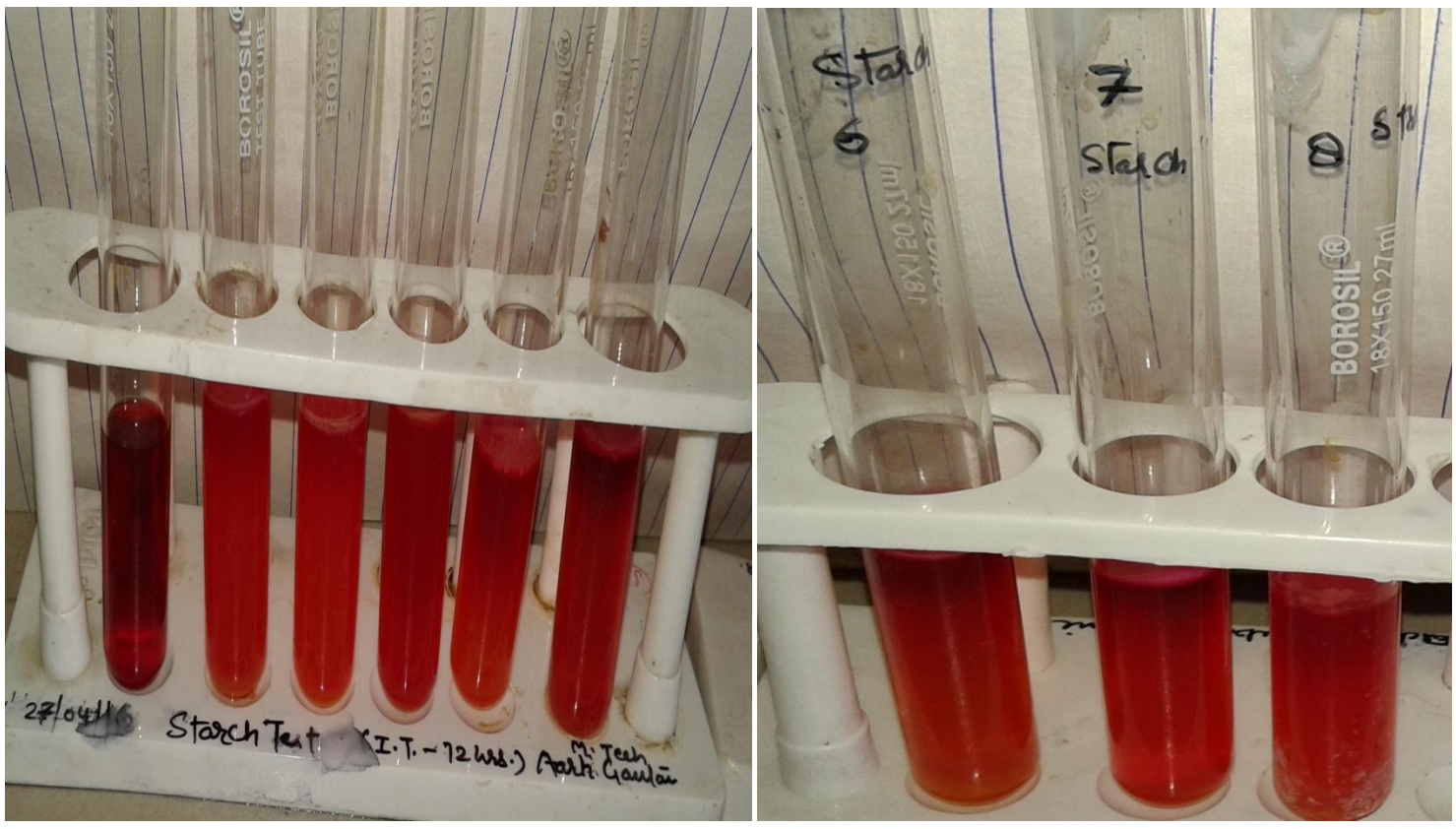
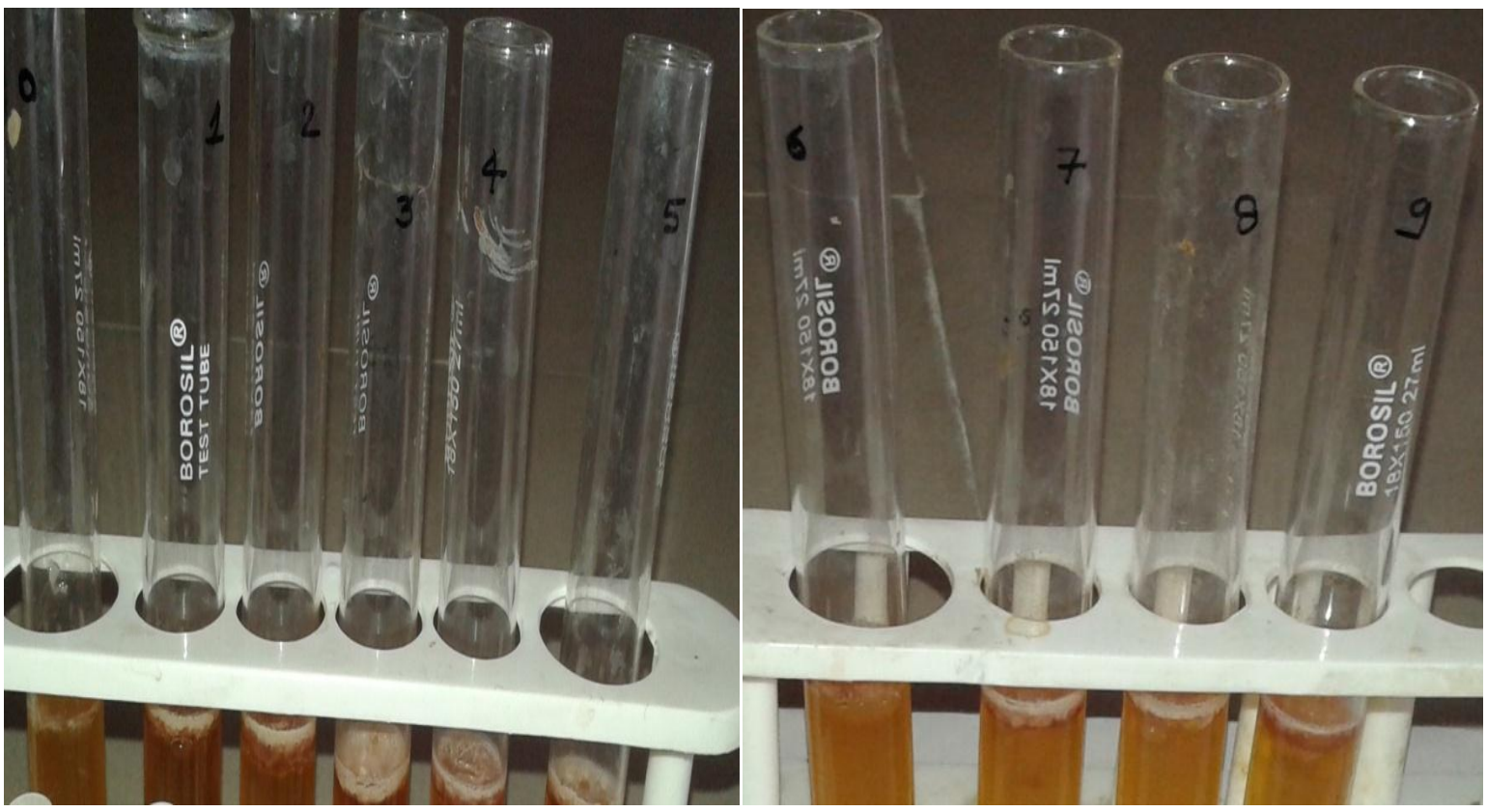
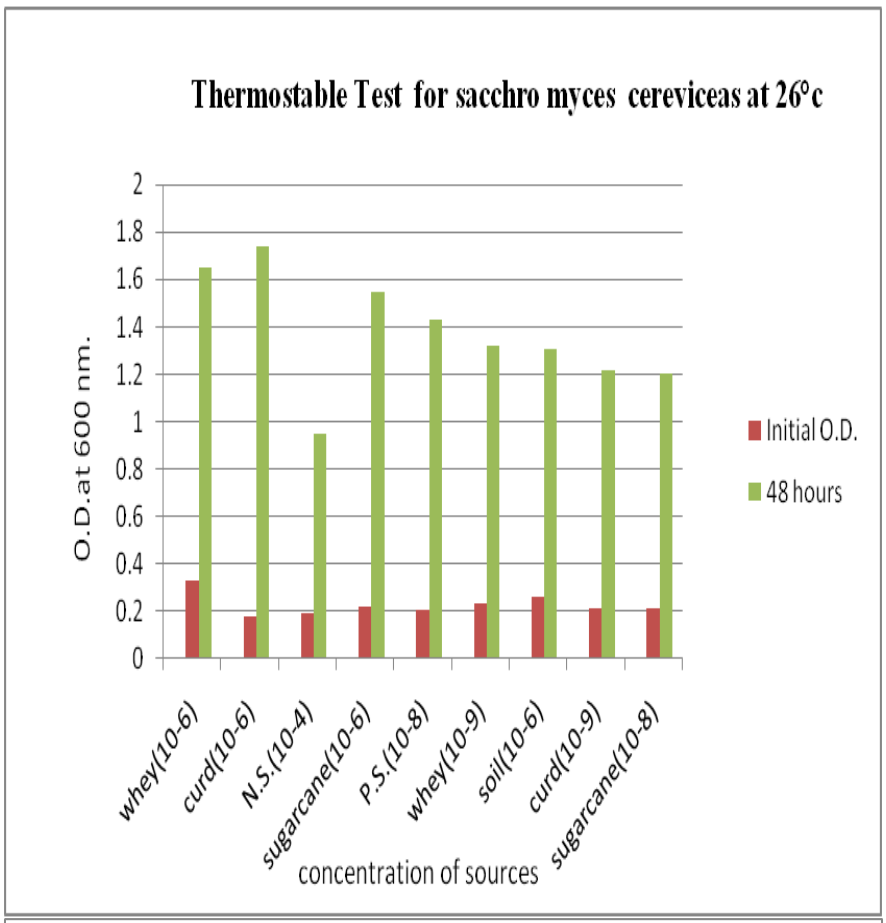
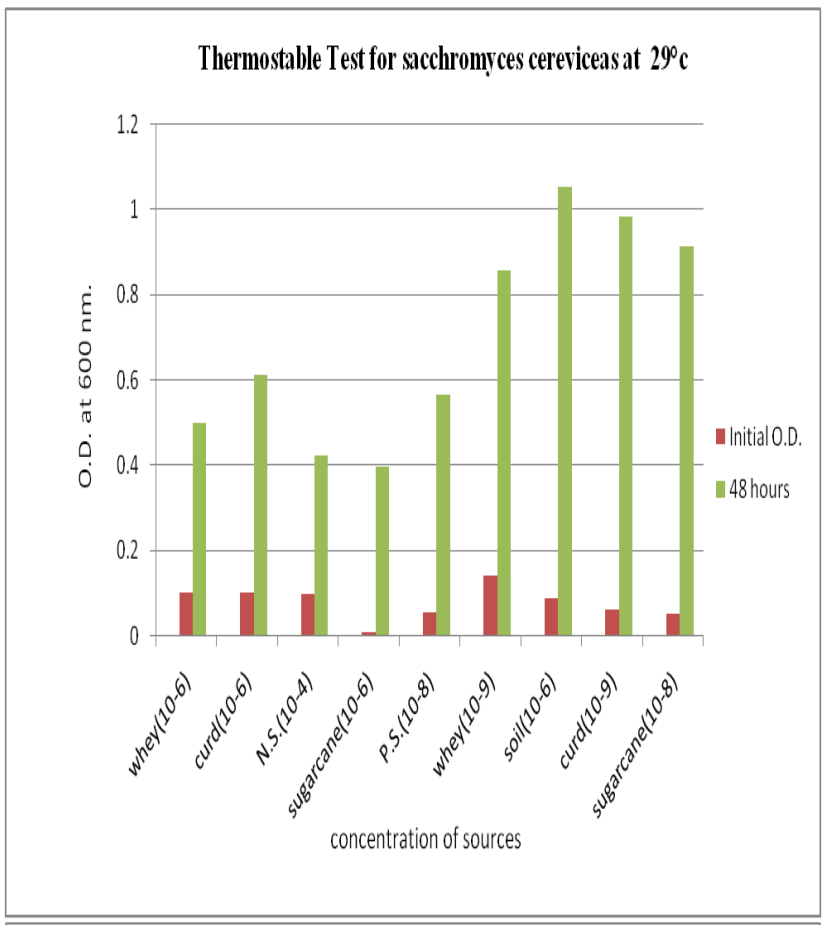


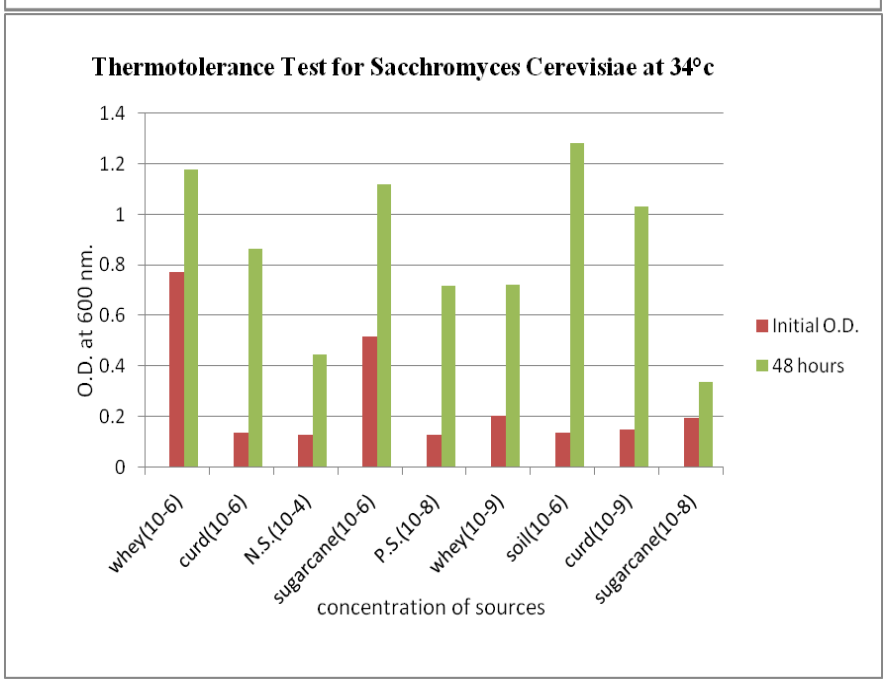
Fig 6: Flocculation Test

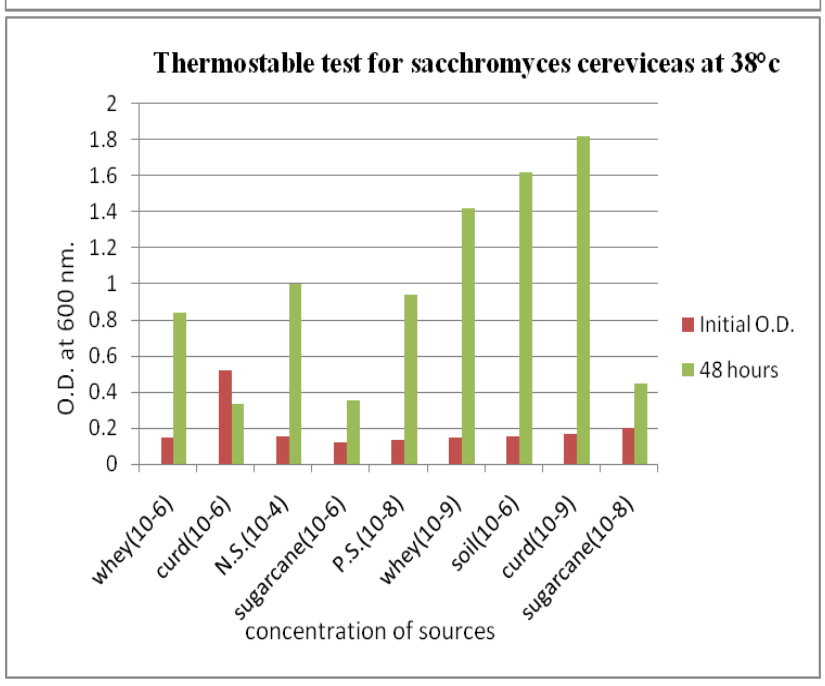


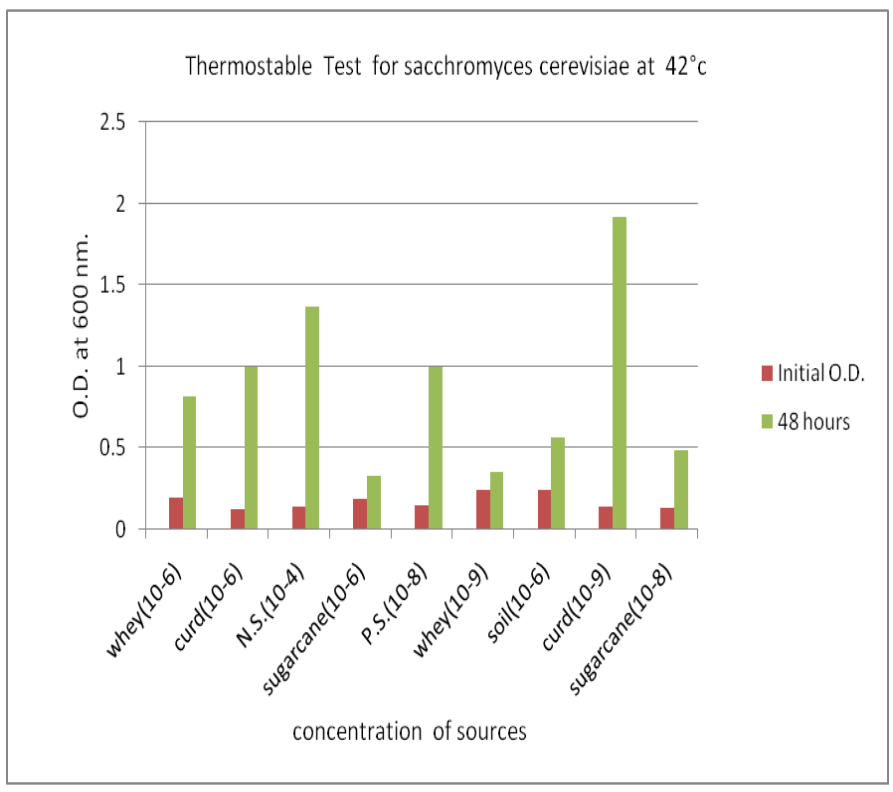
Graphs : Taxanomic identification(pH tolerance, Thermotolerance ,Ethanoltolerance and osmotolerance) of Sacchromyces Cerevisiae from different sources with different concentratio

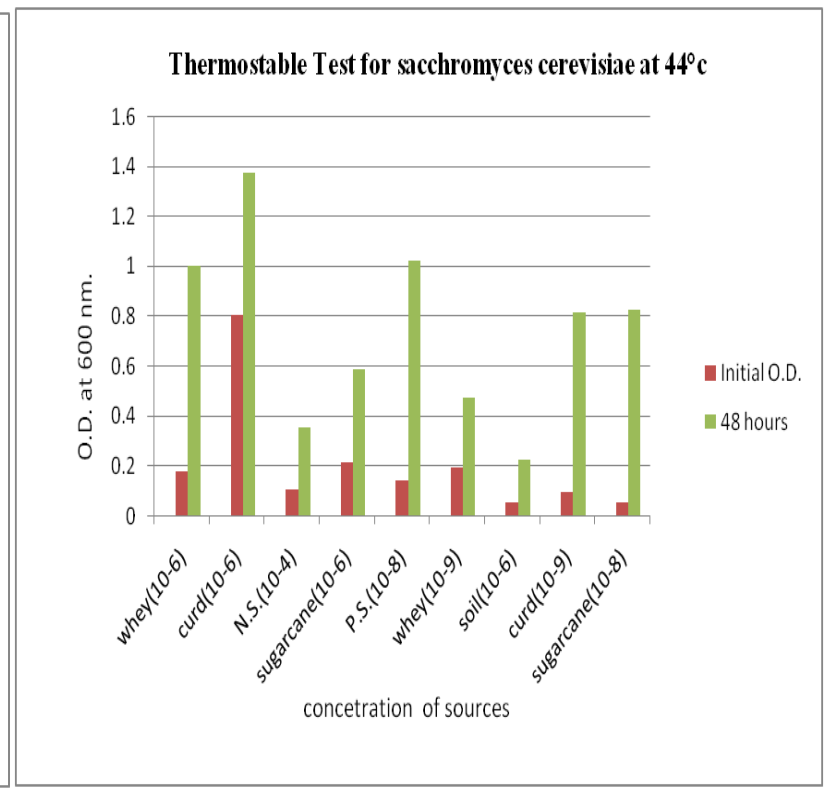


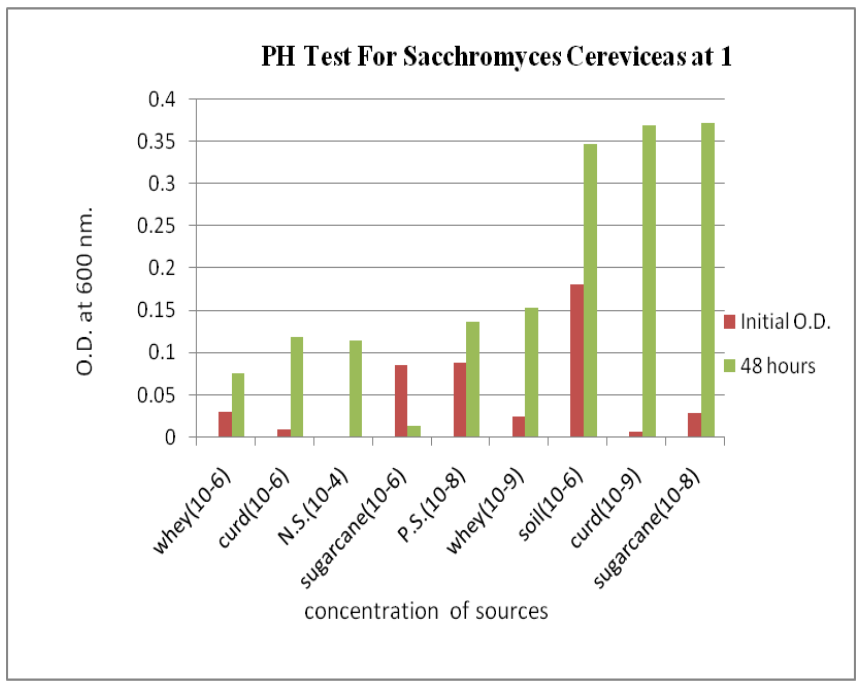


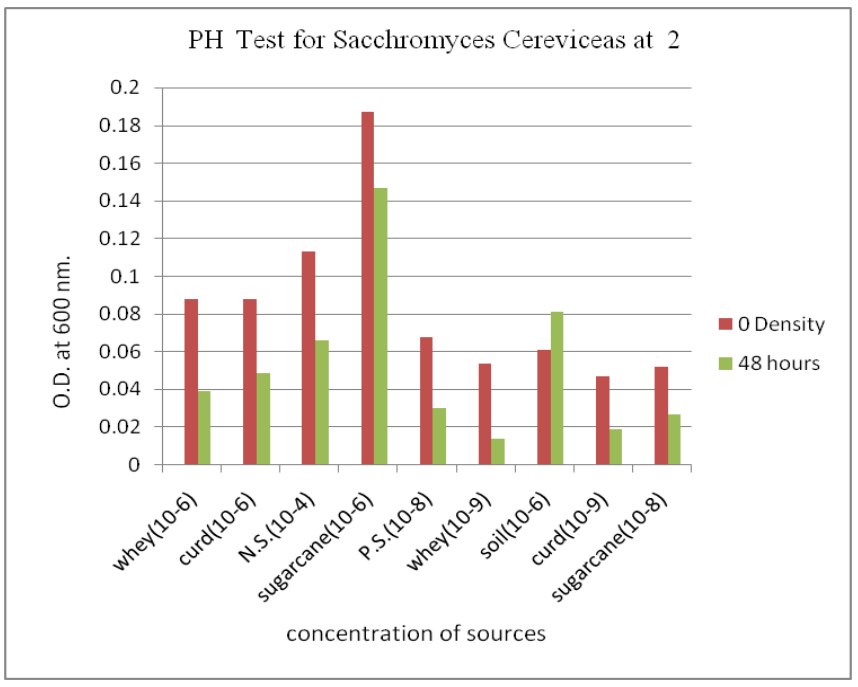


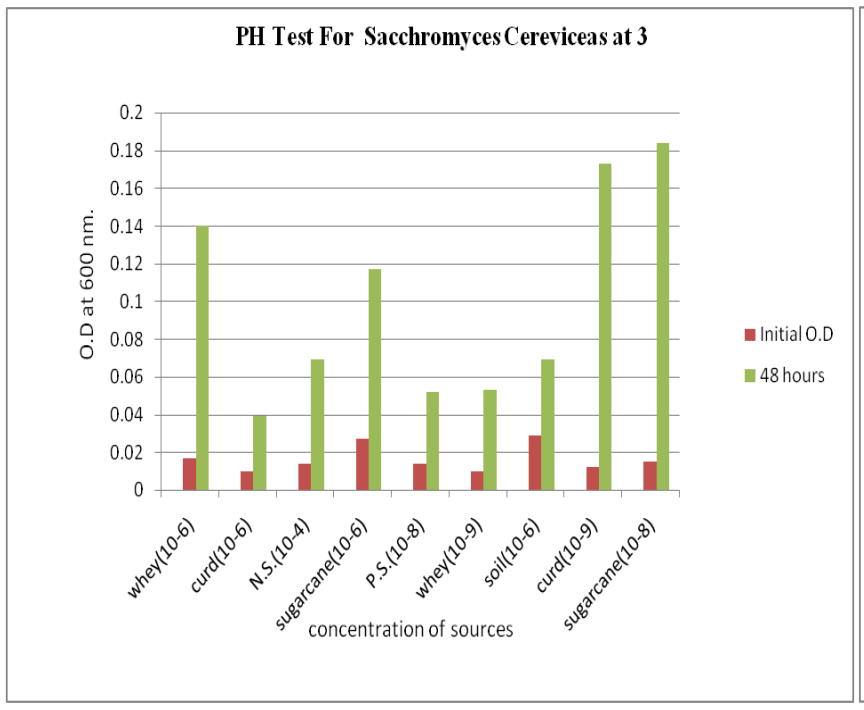


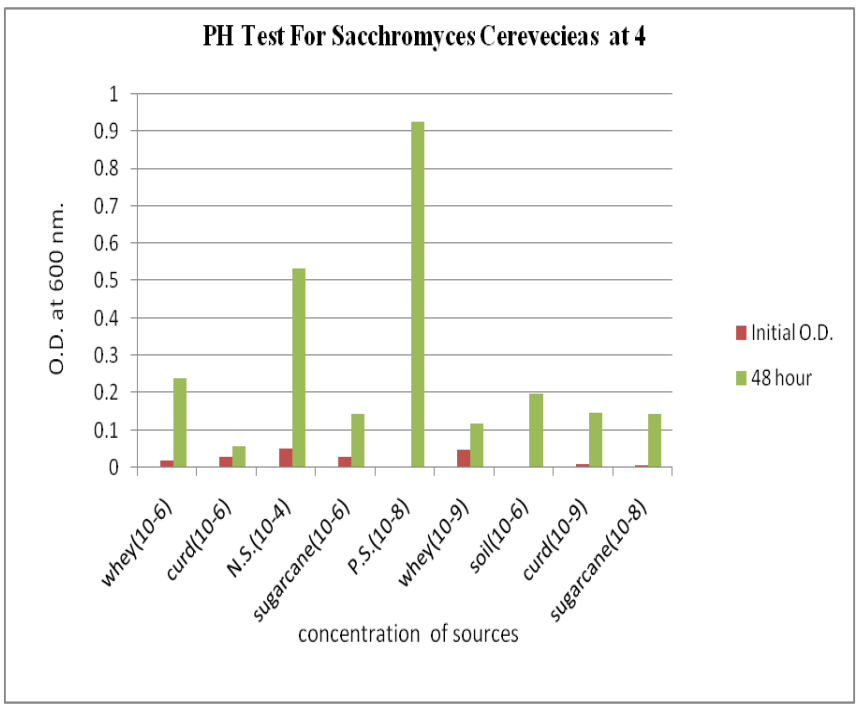


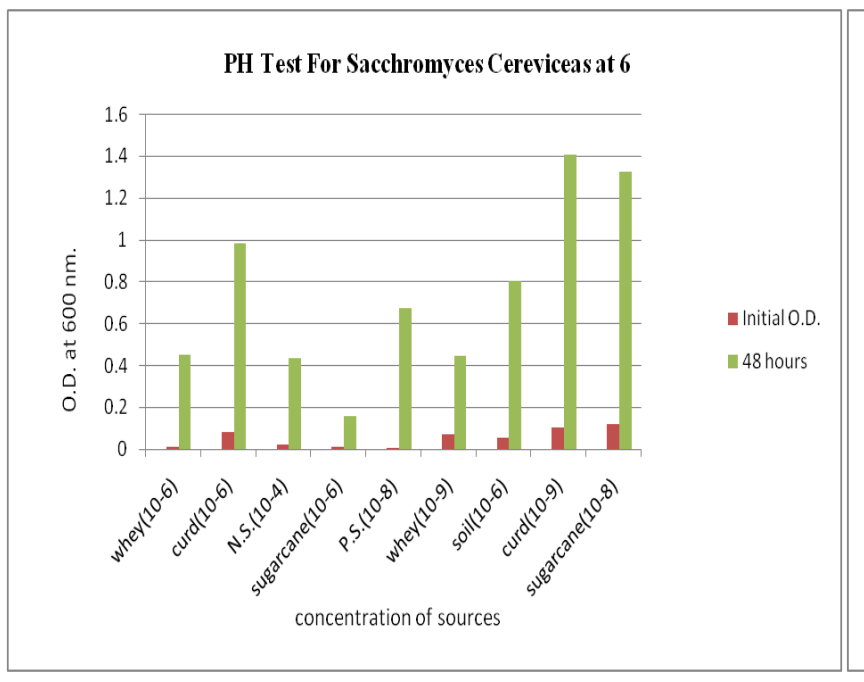


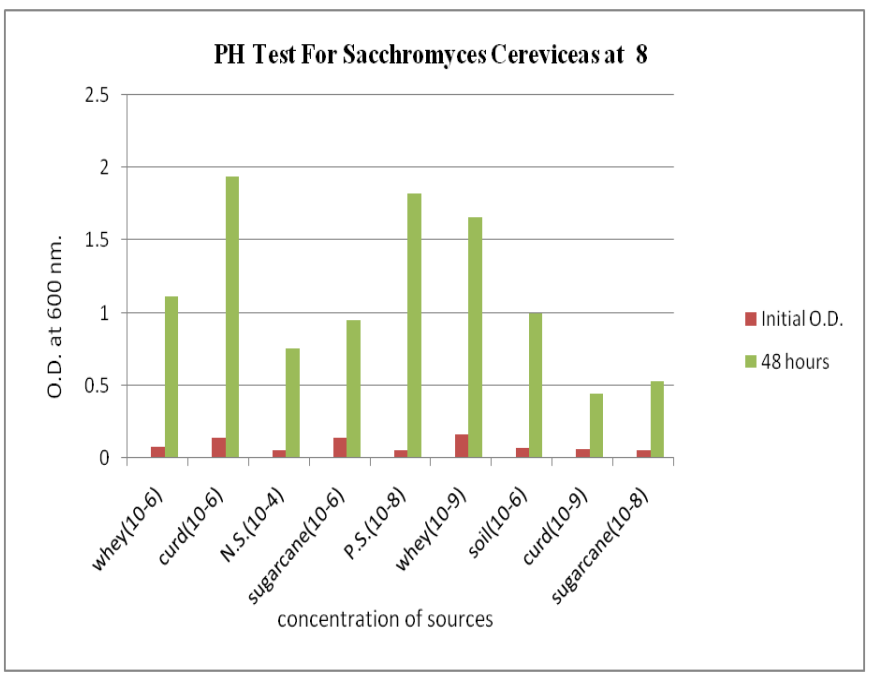

PH Test For Sacchromyces Cereviceas at 1
O.D. at 600 nm.
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Initial O.D.
48 hours
whey(10-6)
curd(10-6)
N.S.(10-4)
sugarcane(10-6)
P.S.(10-8)
whey(10-9)
soil(10-6)
curd(10-9)
sugarcane(10-8)
concentration of sources

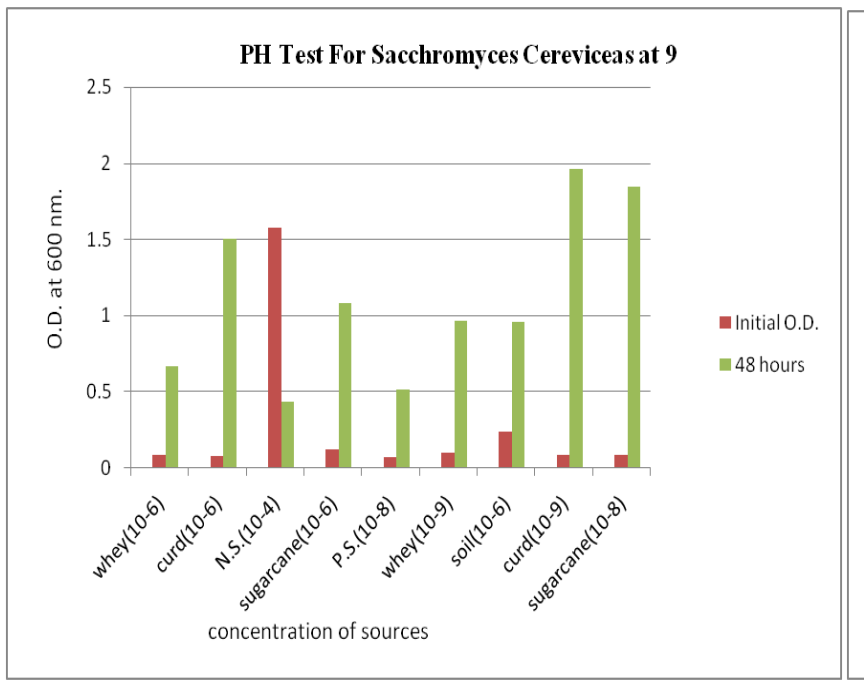

PH Test for Sacchromyces Cereviceas at 2
O.D. at 600 nm.
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0 Density
48 hours
whey(10-6)
curd(10-6)
N.S.(10-4)
sugarcane(10-6)
P.S.(10-8)
whey(10-9)
soil(10-6)
curd(10-9)
sugarcane(10-8)
concentration of sources

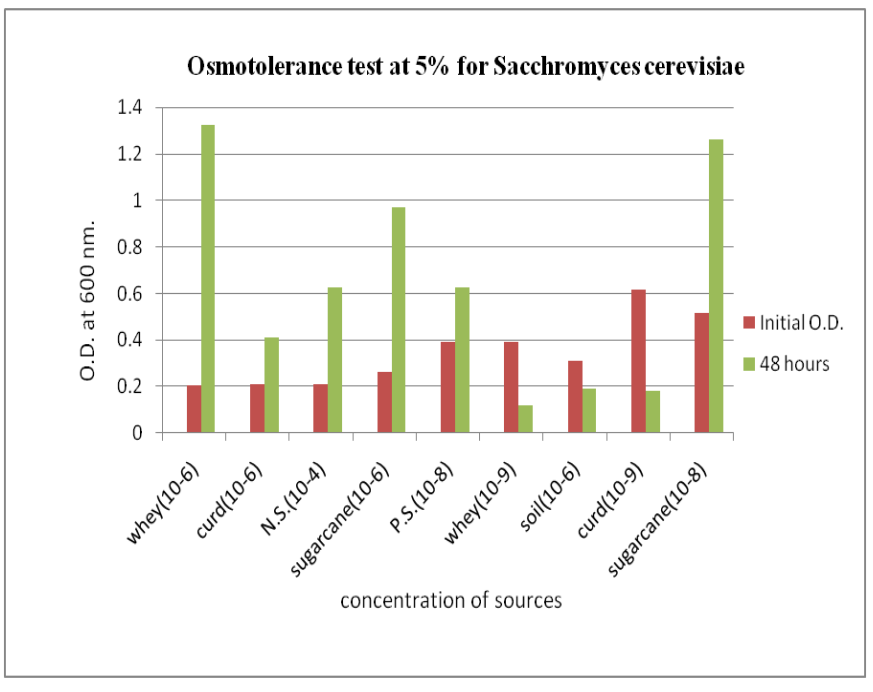

PH Test For Sacchromyces Cereviceas at 3
O.D at 600 nm.
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Initial O.D
48 hours
whey(10-6)
curd(10-6)
N.S.(10-4)
sugarcane(10-6)
P.S.(10-8)
whey(10-9)
soil(10-6)
curd(10-9)
sugarcane(10-8)
concentration of sources

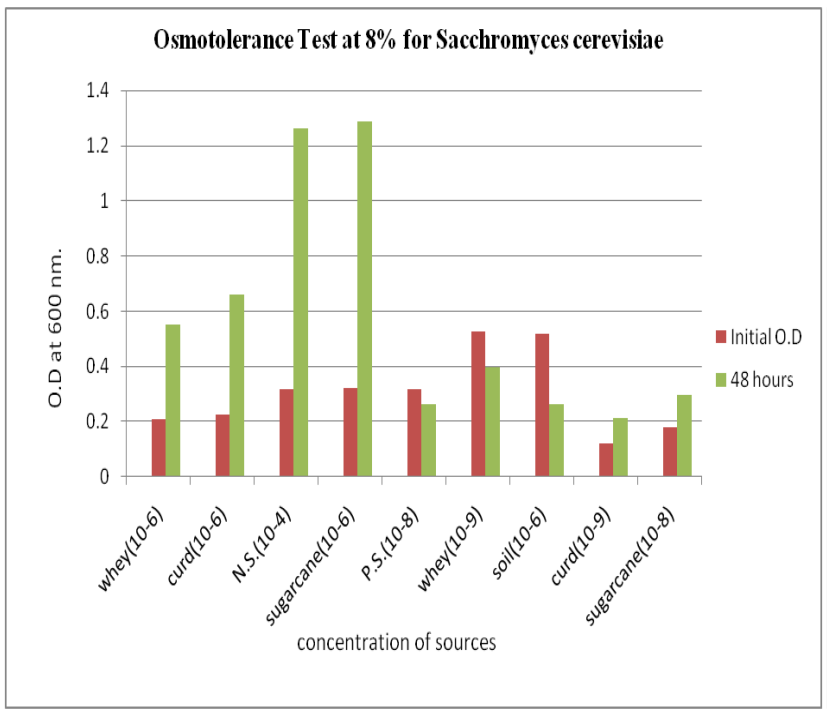

PH Test For Sacchromyces Cerevecieas at 4
O.D. at 600 nm.
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Initial O.D.
48 hour
whey(10-6)
curd(10-6)
N.S.(10-4)
sugarcane(10-6)
P.S.(10-8)
whey(10-9)
soil(10-6)
curd(10-9)
sugarcane(10-8)
concentration of sources

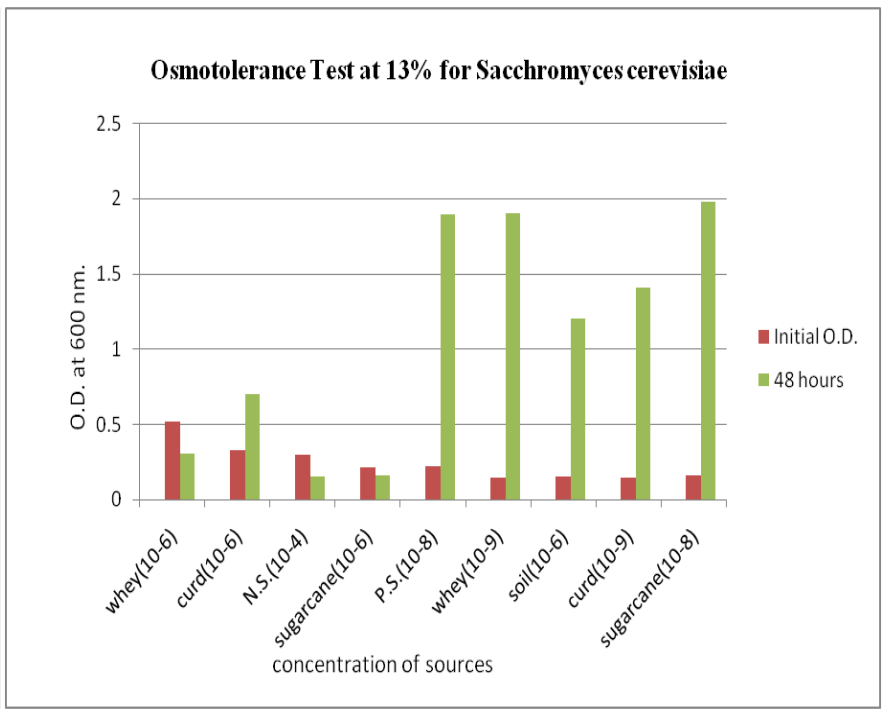

PH Test For Sacchromyces Cereviceas at 6
O.D. at 600 nm.
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Initial O.D.
48 hours
whey(10-6)
curd(10-6)
N.S.(10-4)
sugarcane(10-6)
P.S.(10-8)
whey(10-9)
soil(10-6)
curd(10-9)
sugarcane(10-8)
concentration of sources

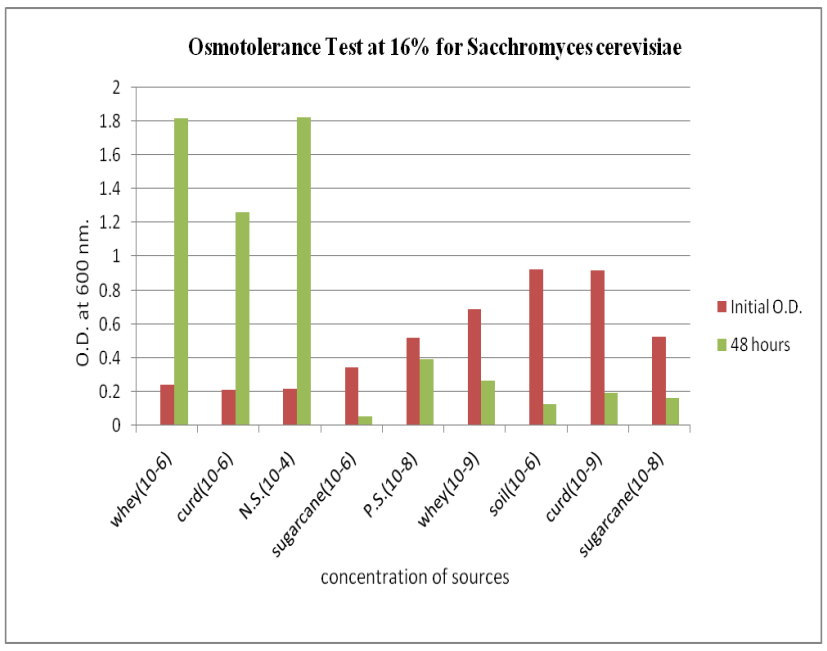

PH Test For Sacchromyces Cereviceas at 8
O.D. at 600 nm.
2.5
2
1.5
1
0.5
0
Initial O.D.
48 hours
whey(10-6)
curd(10-6)
N.S.(10-4)
sugarcane(10-6)
P.S.(10-8)
whey(10-9)
soil(10-6)
curd(10-9)
sugarcane(10-8)
concentration of sources

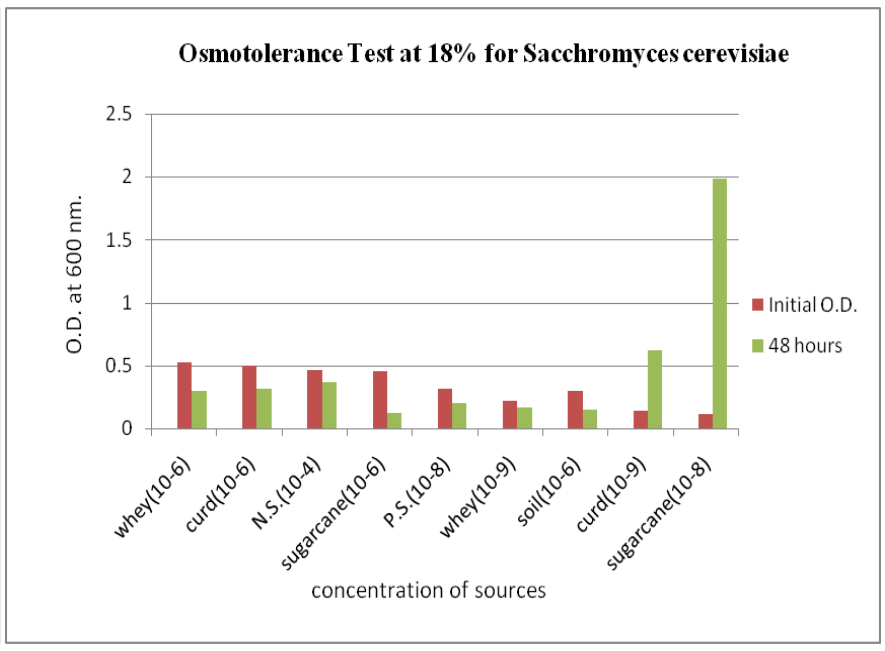

PH Test For Sacchromyces Cereviceas at 9
O.D. at 600 nm.
2.5
2
1.5
1
0.5
0
Initial O.D.
48 hours
whey(10-6)
curd(10-6)
N.S.(10-4)
sugarcane(10-6)
P.S.(10-8)
whey(10-9)
soil(10-6)
curd(10-9)
sugarcane(10-8)
concentration of sources

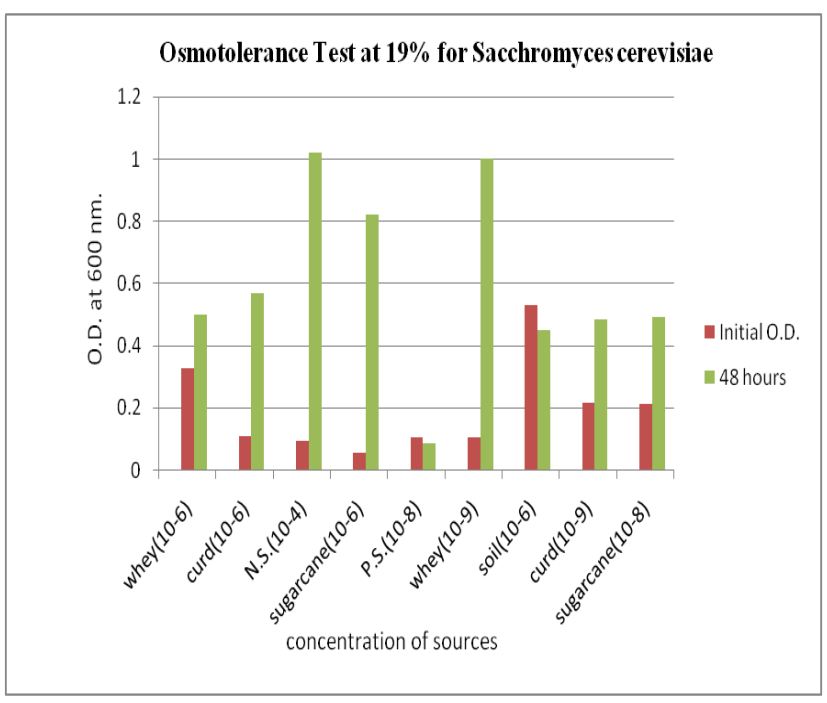

Osmotolerance test at 5% for Sacchromyces cerevisiae
O.D. at 600 nm.
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Initial O.D.
48 hours
whey(10-6)
curd(10-6)
N.S.(10-4)
sugarcane(10-6)
P.S.(10-8)
whey(10-9)
soil(10-6)
curd(10-9)
sugarcane(10-8)
concentration of sources

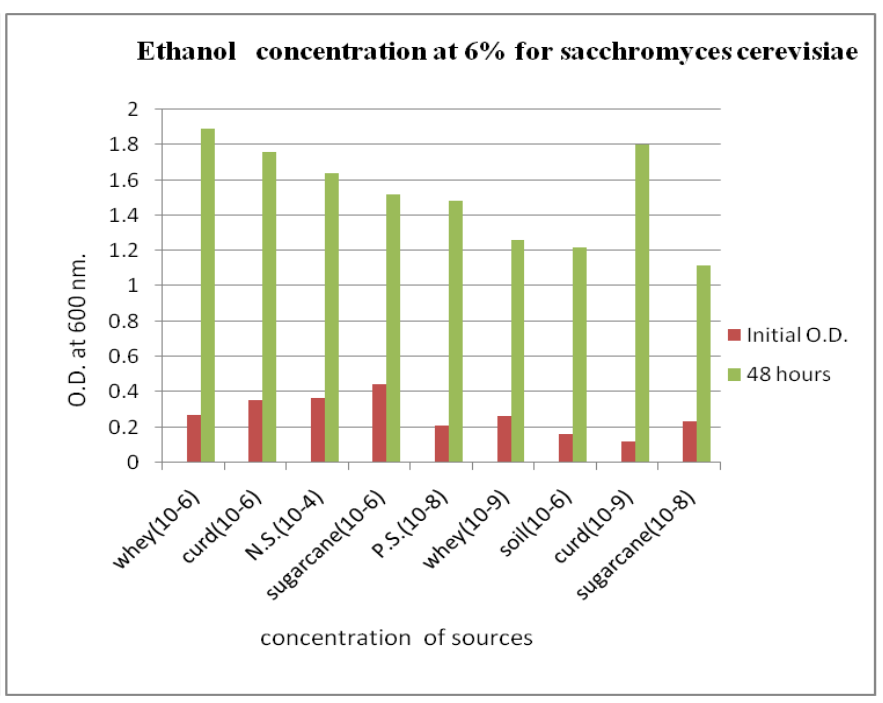

Osmotolerance Test at 8% for Sacchromyces cerevisiae
O.D at 600 nm.
1.4
1.2
1
0.8
0.6
0.4
0.2
0
whey(10-6)
curd(10-6)
N.S.(10-4)
sugarcane(10-6)
P.S.(10-8)
whey(10-9)
soil(10-6)
curd(10-9)
sugarcane(10-8)
concentration of sources
Initial O.D
48 hours

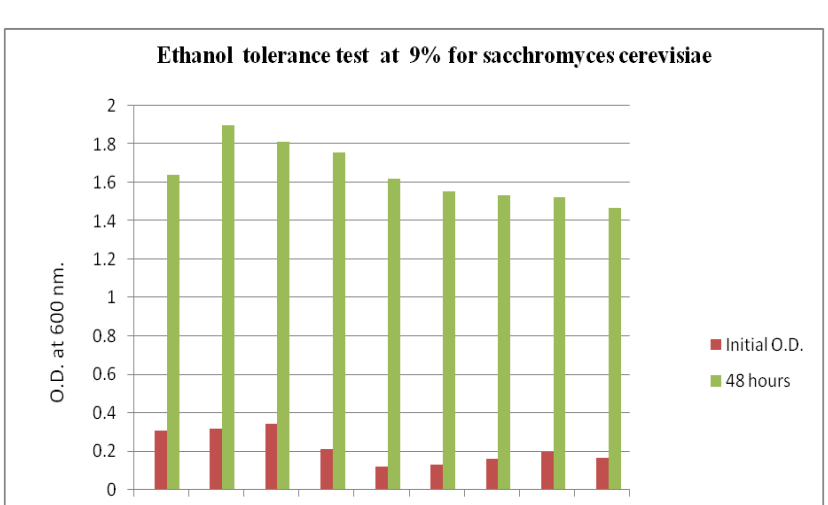

Osmotolerance Test at 13% for Sacchromyces cerevisiae
O.D. at 600 nm.
2.5
2
1.5
1
0.5
0
whey(10-6)
curd(10-6)
N.S.(10-4)
sugarcane(10-6)
P.S.(10-8)
whey(10-9)
soil(10-6)
curd(10-9)
sugarcane(10-8)
concentration of sources
Initial O.D.
48 hours

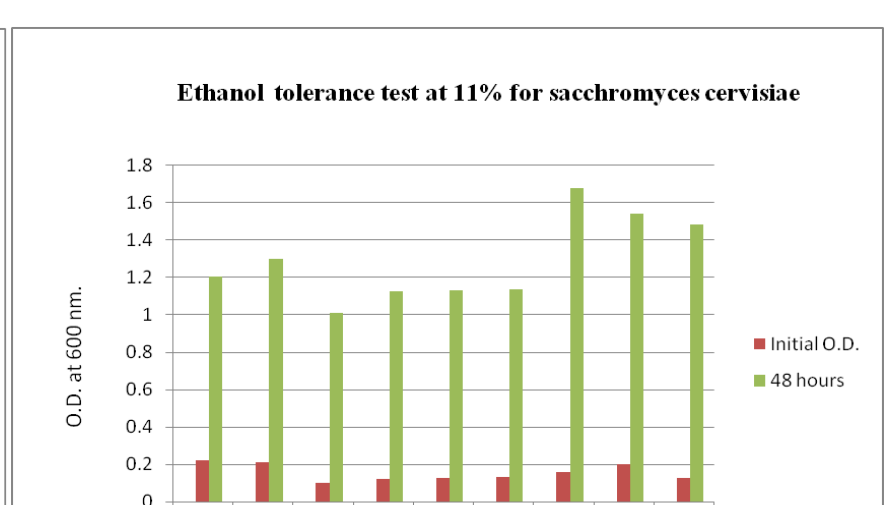

Osmotolerance Test at 16% for Sacchromyces cerevisiae
O.D. at 600 nm.
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
whey(10-6)
curd(10-6)
N.S.(10-4)
sugarcane(10-6)
P.S.(10-8)
whey(10-9)
soil(10-6)
curd(10-9)
sugarcane(10-8)
concentration of sources
Initial O.D.
48 hours

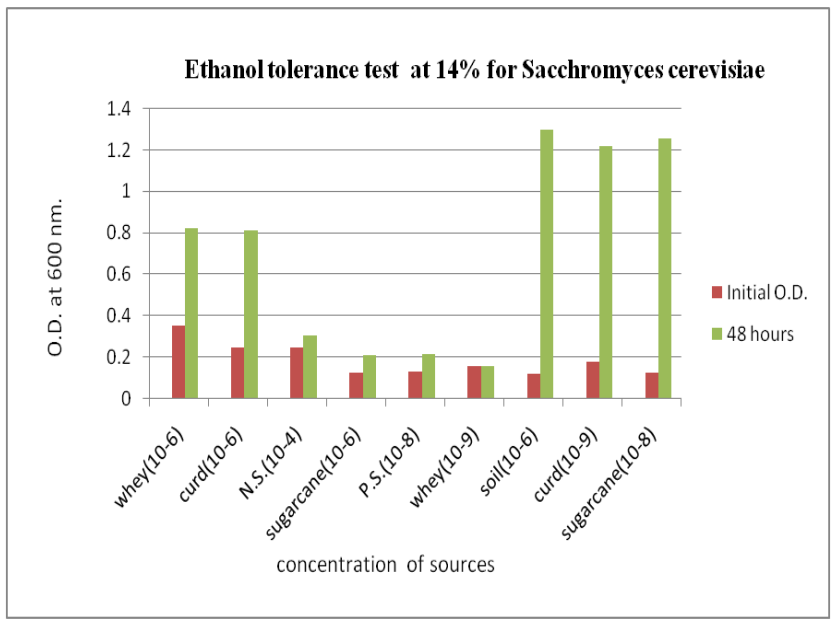

Osmotolerance Test at 18% for Sacchromyces cerevisiae
O.D. at 600 nm.
2.5
2
1.5
1
0.5
0
whey(10-6)
curd(10-6)
N.S.(10-4)
sugarcane(10-6)
P.S.(10-8)
whey(10-9)
soil(10-6)
curd(10-9)
sugarcane(10-8)
concentration of sources
Initial O.D.
48 hours

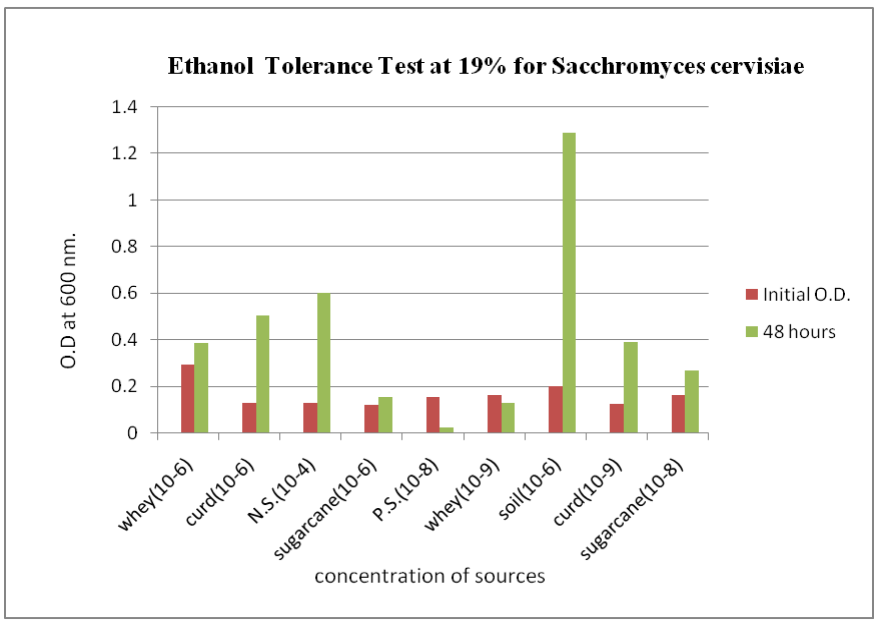

Osmotolerance Test at 19% for Sacchromyces cerevisiae
O.D. at 600 nm.
1.2
1
0.8
0.6
0.4
0.2
0
whey(10-6)
curd(10-6)
N.S.(10-4)
sugarcane(10-6)
P.S.(10-8)
whey(10-9)
soil(10-6)
curd(10-9)
sugarcane(10-8)
concentration of sources
Initial O.D.
48 hours

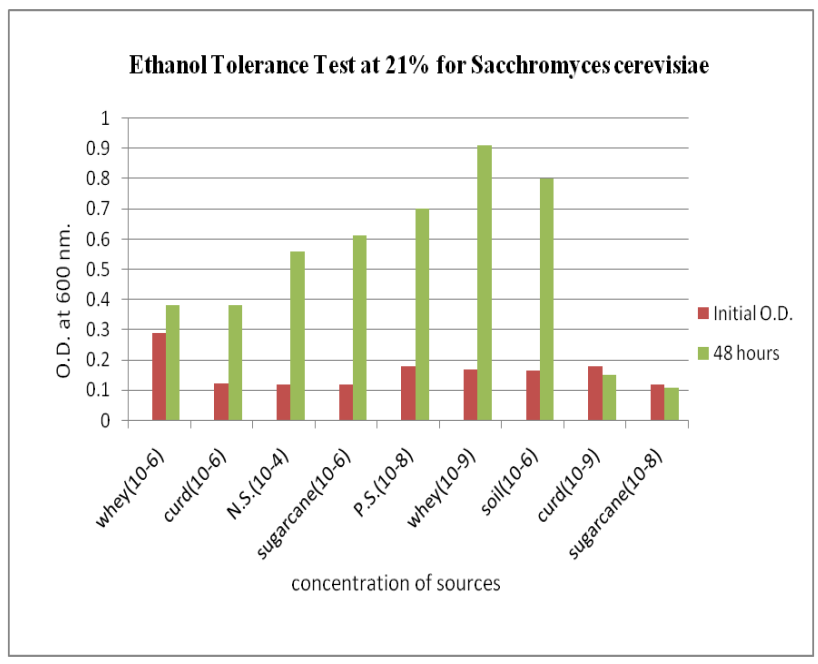

Ethanol concentration at 6% for sacchromyces cerevisiae
O.D. at 600 nm.
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
whey(10-6)
curd(10-6)
N.S.(10-4)
sugarcane(10-6)
P.S.(10-8)
whey(10-9)
soil(10-6)
curd(10-9)
sugarcane(10-8)
concentration of sources
Initial O.D.
48 hours

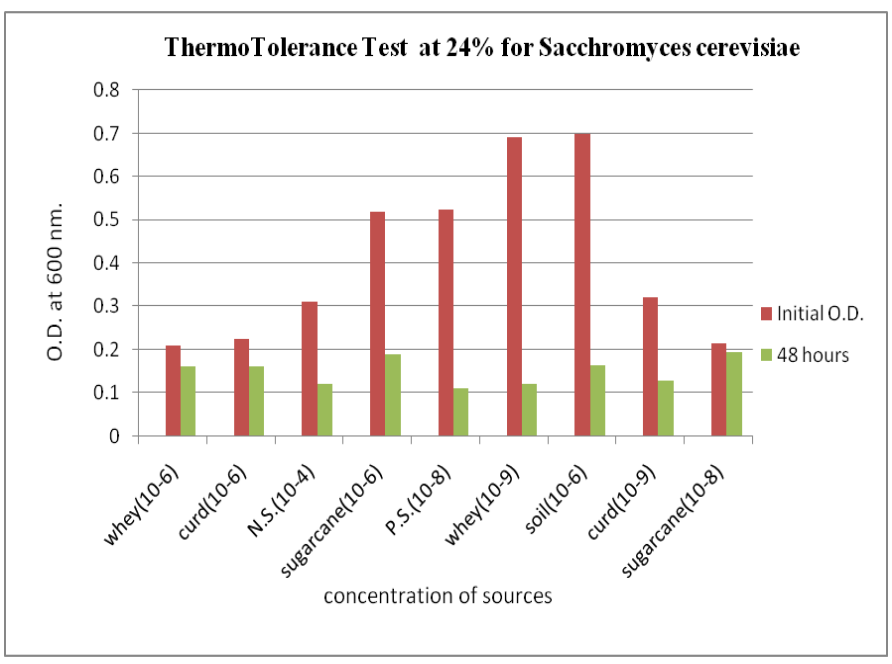

Ethanol tolerance test at 9% for sacchromyces cerevisiae
O.D. at 600 nm.
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
whey(10-6)
curd(10-6)
N.S.(10-4)
sugarcane(10-6)
P.S.(10-8)
whey(10-9)
soil(10-6)
curd(10-9)
sugarcane(10-8)
concentration of sources
Initial O.D.
48 hours


Ethanol tolerance test at 11% for sacchromyces cervisiae
O.D. at 600 nm.
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
whey(10-6)
curd(10-6)
N.S.(10-4)
sugarcane(10-6)
P.S.(10-8)
whey(10-9)
soil(10-6)
curd(10-9)
sugarcane(10-8)
concentration of sources
Initial O.D.
48 hours









Conclusion

Most of research objectives have been achieved successfully and the impacts of the other environmental conditions will be published later. However, there are some result that need to be restested and to be verified, and others to be completed. It is recommended to have more sampling points in the fermentation periods from 48 to 96 hours at some experimental conditions for measuring Sacchromyces cerevisiae ethanol productivity to verify the optimum temperatures, time, pH, and fermentation period time. This work showed that both commercial and isolated S. cerevisiae species from different sources like (sugarcane juice, curd, whey, potato soil, garden soil) investigated have a different ability to produce bioethanol under all experimental conditions mentioned. By commercial yeast, bioethanol production is high as compare to isolated strain with optimize the different parameters.

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