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Study on the development of high yielding alcohol resistant strain of *Saccharomyces cerevisiae and* the Influence of Magnetic field of *Saccharomyces cerevisiae* Inoculum for the production of Alcohol

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ABSTRACT

We have studied on the development of high yielding alcohol resistant strain of Saccharomyces cerevisiae for the production of alcohol.Overflow metabolism is an undesirable characteristic of aerobic cultures of *Saccharomyces cerevisiae* during biomassdirected processes. It results from elevated sugar consumption rates that cause a high substrate conversion to ethanol and other bi-products, severely affecting cell physiology, bioprocess performance, and biomass yields. Fed-batch culture, where sucrose consumption rates are controlled by the external addition of sugar aiming at its low concentrations in the fermentor, is the classical bioprocessing alternative to prevent sugar fermentation by yeasts. However, fed-batch fermentations present drawbacks that could be overcome by simpler batch cultures at relatively high (e.g. 20 g/l) initial sugar concentrations From the results it is clear that strain T2. which has been exposed to 15% alcohol for 18 hrs is the high yielding strain, as it gives 31.73% alcohol after distillation. We also find that as the exposure is increased, that is, with increasing exposure to 20% alcohol for 5 hrs, 18 hrs, and 20 hrs, the production of alcohol decreases. *Saccharomyces cerevisiae* yeast cells strain T2. which has been exposed to 15% alcohol for 18 hrs were exposed to a homogenous static magnetic field of 130 mT for periods of 24, 48 or 72 hours and then used as inoculum for the alcoholic fermentation. The exposure to the magnetic field improved the fermentation process kinetics. Biomass and ethanol yields of fermentations inoculated with treated inoculum were higher than those in the control fermentation, which was inoculated with an untreated inoculum.

Key Words: *Saccharomyces cereviseae* inoculum magnetic field ,ethanol fermentation, continuous process, batch fermentation,sucrose.

1. Introduction:

The environment concern over the use and depletion of fossil fuels, the search for alternative fuel is desirable [1,3,4]. Ethanol has attracted worldwide attention due to its potential use as a transportationfuel [4,5]. Ethanol is traditionally produced in the batch fermentations by yeasts, mostly *Saccharomyces cerevisiae* and their interspecies hybrids, which provide the low productivity [5,6] Fermentation processes utilize microbiology in producing chemical compounds, but have a history similar to all natural product processing. Those processes yielding simple structural chemicals *e.g.*, ethanol, butanol, or acetoneis gradually being replaced by synthetic processes with cheap and abundant raw materials. However, fermentation processes are still useful for production of complex organic chemicals such as medicines, antibiotics, and for chemicals of more complex structure, such as citric and lactic acids derived from low-cost carbohydrate sources. *Saccharomyces cereviseae* inoculum magnetic field From ancient times to the present day, in some wine regions, the influence of moon magnetism in grape must alcohol fermentation has been postulated possibly as a myth. The influence of Earth´s magnetic field on wine fermentation by *Saccharomyces cerevisiae* was observed in Roman times. It was found that variations in the magnetic field strength in different locations of the wine cellars influenced the alcoholic fermentation of the grape must.1One of the first studies of the influence of magnetic fields on the growth of yeast cells during wine fermentation was that of Kimball, published already in 1937.2 A suspension of wine yeast was exposed to a heterogeneous static magnetic field of 0.04 T for different times and the subsequent sprouting of the yeast cells was measured. Exposure for 10 to 17 minutes had no effect; while exposure for 20, 25, 30, 60, and 150 minutes inhibited sprouting. Yeast budding was only affected by heterogeneous fields; homogeneous fields produced no effect.2 Recently, there has been a resurgence of interest in the application of magnetic fields to yeasts, with various researchers applying magnetic fields stronger than that of Earth, which varies from 0.025 to 0.065 mT, depending on the location.3–6 Beyond field strength of the magnetic field, it is of high relevance whether the field is homogeneous or heterogeneous, whether the field is static or alternating, and the process temperature.4–6 The results of the influence of magnetic fields on yeast growth and metabolism are contradictory. Some studies have not shown any effect. For example, there was no statistical difference between the growth of *S. cerevisiae* when cultured within the 1.5 T magnetic field of a clinical magnetic resonance imager, and when it was cultured outside of this magnetic field. Likewise, growth of *S.cerevisiae* was not affected by exposure to a static magnetic field of 50 Hz frequency, varying between 0.35 and 2.45 mT.8,9 However, various studies have demonstrated effects. Exposure of aculture of *S. cerevisiae* to a magnetic field of 110 to 220 mT led to faster growth and higher respiration rates;9,10 a culture of *S. cerevisiae* exposed to a 3 mT homogeneous magnetic field had a more porous membrane, absorbing 50 % more copper Cu2+ ions than non-exposed control cells;11 growth of *S. cerevisiae* was reduced by exposure to an alternating 12 mT field at 50 Hz, and the surviving cells were more resistant to the ethanol production;12 magnetic field exposed cells of *S. cerevisiae* immobilized on magnetic particles also showed higher ethanol production;13 finally, cultivation of *S. cerevisiae* in a static magnetic field of 30 mT during 16 h led to a 25 % higher biomass. concentration . Unfortunately, these contradictory results make it impossible to state clearly what effect magnetic fields have on yeast growth. It has been suggested that the magnetic field influences

cell membrane permeability, active transport through the cell membrane and protein synthesis. It has also been suggested that magnetic fields can cause some essential molecules in the cell to move from their normal location, interrupting normal cell metabolism. The suggestion that the magnetic field influences the rate of chemical reactions or protoplasmic streaming is less probable The main purpose of the present research was to investigate the influence of the static magnetic field on wine yeast cells metabolism and in subsequent alcohol fermentation.

1.1. General Characteristics of a Fermentation Process:

The action of a specific microorganism on a substrate to produce the desired chemical compound is termed fermentation. The majority of processes requires oxygen and is classified as aerobic. The few processes carried out in the absence of air are classified as anaerobic. In general, the process involves addition of a specific culture of microorganisms to a sterilized liquid substrate or broth in a tank (submerged fermentation), addition of air if aerobic in a welldesigned gas-liquid contactor, and carrying out the fermentation to grow microorganisms and produce the desired chemicals. This can be done batch-wise or continuously at a given ,temperature and time, then processing the broth to remove the desired chemical. Some of the engineering problems in such processes will be discussed next with typical fermentation process examples given last.

1.2. Fermentation Industries:

The fermentation industries constitute the branch of chemical manufacture, which yield useful productsthrough the vital activities of microorganism. Fermentation, in the broad sense in which the term is now generally used, may be defined as a metabolic process in which chemical changes are brought about in an organic substrate through the activities of enzymes secreted by microorganism. It was at first thought that all fermentation processes (hydrolytic, proteolytic, oxidation, alcoholic etc.) are connected with the vital growth of the living microorganism.
With respect to oxygen supply, two types of fermentation are recognized. One fermentation, which requires free oxygen to act as a hydrogen acceptor viz. Acetic acid and citric acid fermentation, is called aerobic fermentation. Another is anaerobic fermentation in which atmospheric oxygen is no involved, but other substances, such as aldehydes or pyruvic acid, serve as hydrogen acceptor, examples alcoholic, butyl alcohol acetone fermentation etc.Large number of microorganism are available in nature which each can produce fermentative changes in the broad sense. The microbial dissimilation is the most diverse kind but the number of transformations, which have become of technical importance, is quite limited. Success of fermentaiton process on the industrial scale depends on a number of factors, notably: The ability of the selected organism to give a consistently yield of derived product in a reasonably time from a cheap, available raw material, The easy recovery of the product in pure form and the manufacture of a unique product which is in demand, but difficult to obtain by other methods. Organisms of various types are employed in the fermentation industries. These include species of strains of yeast, bacteria and molds. The microrganisms of fermentation differ in morphology, size manner of reproduction, reaction to free oxygen, growth requirements and ability to attack different substrates and in other ways. They are similar in that they grow actively and produce enzymes by which they catalyze the reactions brought about by them.The industrially important

microorganisms have at least five outstanding abilities. To grow rapidly in suitable organic substrate, To be cultivated easily in large quantities, To produce the necessary enzymes readily and profusely, in order to bring about the desiredchemical changes. To carry out the transformations under comparatively simple and workable modifications of environmental conditions. To maintain physiological constancy these conditions. Industrial operations with microorganism are obviously more complicated than laboratory work but involve in the same principles.The chemical engineering equipment must be designed carefully to meet the special conditions required in each type fermentation and must include means to
protect against contamination by other organisms. The best known chemical product obtained industrially by fermentation and one of largest volume of ethyl alcohol, but other fermentation chemicals including nbutyl alcohol, acetone, butyric acid, citric acid, vitamins, antibiotics have been also produced on commercial or pilot plan chemical manufacture by fermentation is characterized by a high degree of flexibility as to materials. In almost all cases the raw materials for fermentation process directly or indirectly by products of agriculture since firm products are the primary raw materials availability and prices of firm corps have at time been a serious handicap to the industries. However, in many processes, a wide variety of raw materials may be employed for a given fermentationand thus advantage may be taken availability and price changes. The majority of large-scale process involves carbohydrates dissimilation. In general two class of carbohydrates raw materials for fermentation process are available.

1.3. Saccharine substances and Polysaccharides.
It should be noted, however, that while most of t major fermentation of industrial importance involvestransformation of carbohydrates, in some cases, other substances are of essential substrates, for example,sugar, alcohol, proteins or lipids. In some processes, the saccharine substance used foran industrial fermentation is pure sugar such as sucrose, glucose or lactose. There are used wherespecial conditions of process, product demand or other factor make the higher cost of pure sugar permissible.In most cases saccharine substances employed arecheap by products and have other ingredients along with fermentable sugars. Black strapmolasses, a by product from the manufacture of cane sugar has been generally employed as the lowest cost raw materials for fermentation industries. Black strap molasses contain about 50% fermentable sugars mainly sucrose and inverted sucrose and also inorganic compounds nitrogenous substances and other organic compounds of diverse nature. Beet molasses is a similar by product with higher sugar content which is made avilable by acid hydrolysis and then by neutralization. In India Mohuwa Flower is used as a very good source of sugar for fermentation purposes, which is available in large quantity. The sugar content of Mohuwa is about 53%.
It is mainly available in Bihar, M.P., Assam etc.Other Saccharine by-product which have used or suggested for fermentation are whey, sulfite waste liquor, ripe apple juice, citrus fruit juice etc.
Polysaccharide raw materials that are used for fermentation process usually contain starch. Pure starch has been used for certain fermentation and large-scale use of grains including corn, wheat, rye and rice has been practiced frequently in many countries.Potatoes and sweet potatoes are other available starchy raw materials. Wood waste contain about 50% cellulose can also be employed in fermentationindustries. Utilization of cellulose wastes for fermentation process requires preliminary saccharification of the cellulose. Since cellulose is resistant to hydrolysis.

Drastic treatment is required which is proved to be somewhat costly obviously the byproduct materials such as black straw molasses have the lowest value and will sell at any figure below competitive costs of other raw materials for equivalent uses. So black strap molasses will continue to be a favored raw materials for fermentation. In India Mohuwa flower rich in sugar has been employed for fermentation. Another cheap raw is waste sulfite liquor. All fermentation industries require large volume of water, which water is not directly a substrate for fermentation, microorganisms act in aqueous mediumand any fermentation industry is basically dependent upon availability of large volume of water. From the designed data of a fermentation industry in U.S.A. it has been found that about 65 gallons of water is required per gallon of alcohol produced for preparationof fermentation mashes, for steam production for processing operation and especially for cooling.Adequate supply of water plays a vital role in a fermentation industry.

1.4. Alcoholic Fermentation:

The alcoholic fermentation is the largest sector of th fermentation industries in terms if product quantity. Number of commercial units and persons employed directly and indirectly. Large-scale production of industrial ethyl alcohol began when alcohol used for industrial purposes as denatured alcohol was made to exempt. During World War II, the demand for industrial ethyl alcohol was multiplied due to its use insynthetic rubber manufacture. Most of alcohol has been made from molasses since it the only most economic recourses of sugar for fermentation. But now a days particularly in India another raw material Mohuwa flower containing good amount of fermentable sugar have been used. The technology of the yeast fermentation of molasses is simple and well worked out. Essentially the processinvolves merely dilution of molasses, inoculation with yeast, fermentation and distillation. But in case of Mohuwa flower the procedure is same except the sugar in Mohuwa flower is first extracted by boiling with acid at pH 5. Now a days emphasis has been given a further research on alcohol fermentation industry to have Methods of obtaining higher yield Continuous fermentation Byproduct utilization and disposal.The demand for industrial alcohol is increasing in our country. The major disadvantage of the fermentation industry is the unavailability of suitable raw materials, such as molasses. Moreover in India most of alcohol fermentation industry is based upon the utilization of molasses as raw materials, which is cheap. But those industries are get to be accustomed with the use of Mohuwa flower as raw material which is rather cheap that of molasses. In our country other raw materials are different types of fruit juices and some seasonal products.Now in our country all the parts of an alcohol fermentation plant an available and there are so many engineering concerns are available which can manufacture fermenter, air filter, distillation column with high degree of accuracy required for a fermentation industry. Alcoholic fermentation, upon which the manufacture of industrial alcohol, wine and vinegar depends, is caused by yeast. On this account yeast are of very great importance in these horticultural products industries. Yeast is classified in two ways, one based upon morphology and the other upon their use in the industries. Morphologically classified into thefollowing general categories : Saccharomyces, (2) Torulospora, (3) Pichia, (4) Hansenula etc.Again from the industrial use point of view : Wine or Alcohol producing yeast (2) Baker.s yeast (3)Food yeast and Fodder yeast etc.The shape and size of yeast varies for different species.Some are predominantly round. Some are oval to long cells. Again some growth from round, oval to long cells,spares are round,

kidney form or hat shaped. They generally do not from mycellium. Their sizes vary from 5M to 9M. They multiply very rapidly. An active yeast cell in a favourable media will increase in 48 hrs to many million cells. Under conditions of temperature and moisture supply that are favourable for the process, the true yeast forms endospores. Abundant moisture, very tittle food and a temperature range of 10 to 30oF favors spore formation.Some names of industrially important yeasts are Saccharomvces cerevisiae, S. ellipsoideus, S sheresensia.

1.5. Mechanism of Fermentation:

In actual practice the fermentation of sugar to alcohol is done by a number of enzymes or iocatalysts present in yeast cells and this phenomena was established by the classic work of Buchner, reported in 1897, who demonstrated cell free fermentation and was the fore runner of much of our recent and modern research on enzyme chemistry and behaviour. He ground yeast in a mortar with sand and diatomaceous earth and then subjected it to very high pressure. He ecured yeast juice that was then filtered. If slowly transferred sugar into carbon dioxide and alcohol, although there were no living yeast cells present. Alcohol is produced through Techniques such as continuous culture; cellimmobilization and recycling of cells have been explored to achieve these objectives [7,8,22]. The continuous process can achieve substantiali improvements in the efficiency of the process and product quality, subsequently higher productivities,lower operating costs, reduced product losses and environmental advantages [9,10,11,12]. However, continuous process withfree cells has disadvantages of higher cost of cell recycling, high contamination risk, susceptibility to environmental variations and the limitations of the dilution rate due to wash-out condition [13,14,21]. Cell immobilization facilitates the larger area of contact between cells and nutrient medium, potential for high fermentation rates offered by the high cell concentrations, option of reusability of cells, protection of cells from toxic effects of lo pH,temperature,inhibitors,tolerance to high osmolalities[18,19,20].

2. Materials and methods:

Mother Culture of *S. Cerevisiae*

Culture Medium **:** A suitable agar . agar medium of the following composition made in water Yeast extract 500 mg ,Peptone 500 mg ,Glucose 1 gm ,Water 100 ml Agar 3 gm PH 5.The above materials were taken in requisite amounts in a 500ml conical flask and a 400ml solution was made. The pH was controlled at 5. The solution was then poured in 30 sterile test tubes so that each test tube contained 10ml of the solution and 100ml solution was kept aside for the preparation of the mother culture. 0.30gm agar was added in each test tube. The medium was then plugged tightly with non-absorbent cotton and autoclaved at 12oC and 15 psi for 30 mins. The test tubes were then kept in a slant position.The suspension of the mother culture made in a sterile test tube with sterile water. Baker.s yeast obtainedfrom market was suspended in autoclaved water and the mixture was shaken well. Then with the help of a sterile pipette, 1 ml of the spore suspension was diluted to 10^4 and 10^5 times with sterile water. The dilutions were made in order to get well-isolated colonies. Now aseptically 1 ml of each 10^4 and 10^5 times diluted suspensions were taken in two petridishes aseptically. 15 ml of melted culture medium at 40 . 50oC were added in the petridishes and well mixed by tilting. Then the petridishes were incubated at 37oC for 24 hrs.The yeast colonies were well separately exposed. Then with sterile

needle ascetically 10-colonies were transferred to the slant medium. These cultures were marked T1, T2, T3, T4, T5, T6, T7, T8, T9 and T10 respectively. After 24 hours of incubation at 37oC, the duplicates of these cultures were made. Production of Alcohol from the above culture A 100ml conical flask was taken and the mouth was tightened by rubber cork. The rubber cork was provided with a glass tube for the supply of sterile air.
The distillation set up.

Medium:
The above culture was then inoculated in a suitable fermenting medium. The composition of the fermenting medium is as follows: For 150ml of fermenting medium, Sucrose 10% KH2PO4 0.1% (NH4)2SO4 0.5% ,MgSO4, 7H2O, 0.05% Yeast Extract 0.1% ,pH 4.5
Organism:
Mother culture of Yeast.The above substances were weighed and dissolved in water. The pH of the medium was maintained at 4.5 by adding 1.6 hydrochloric acid solution. About 600ml of the liquid media was made following the above composition. The mouths of the conical flasks were plugged tightly with non-absorbent cotton and they were sterilized for 30 minutes at 121oC and 15 P.S.I. pressure in an autoclave. The flasks were then cooled and one flask was inoculated with the mother yeast culture. The flasks were shaken and kept for incubation for 48 hours at 28oC. After 48 hours of incubation each set was distilled at 80 to 85oC. 10 ml of distillate was collected in a 10ml specific gravity bottle. Specific gravity for the alcohol obtained was calculated. Then from the chart specific gravity verses weight percentage of alcohol in the distillate in the weight percentage of alcohol was determined.
Isolation and development of a high yielding alcohol resistant strain of S. Cerevisiae for the production of alcohol. The yeast mother culture is then sub-cultured into about 20 of the previously prepared slant solid medium. The sub-cultures are then incubated at 37oC for 48 hours. Then six test tubes are taken and alcohol of various concentrations are poured in them and exposed for various time lengths. The test tubes were labeled T1.,T2., T3., T4., T5., T6.

% Alcohol	Time(hr)
15	15
15	18
15	24
20	15
20	18
20	24

After the exposure, the cultures are then transferred into fresh slant medium and allowed to grow for 24 hrs. Then each of these cultures is inoculated in 600ml of the liquid fermenting media. The flasks are shaken and kept for incubation at 28oC for 48 hours.After 48 hours of incubation each set was distilled at 80-85C. 10 ml of distillate was collected in a 10ml specific gravity bottle. Specific gravity for the alcohol obtained from different sets was calculated . Then from the graph of specific gravity versus

weight percentage of alcohol in the distillate, the weight percentage of alcohol in different sets was determined. Magnetic field with 72 h culture of *Saccharomyces cerevisiae* yeast cells exposed to homogeneous static magnetic field of 130 mT, at 28 °C, were used in all the experiments. The magnetic fields were generated by a coil powered by a transformer. The coils were separated 1 cm and produced a homogeneous field in the vertical direction in the central area near the axis of the coils. Yeasts were located in the region within the coils where fields are homogeneous(Fig. 1).As inoculum, a yeast cell suspension in concentration $2 \cdot 10^7$ cells mL–1, previously for 24, 48 and 72 hours exposed to the static magnetic field of 130 mT, was used in all the experiments. Inoculum cells used in control experiments were grown for 24, 48 and 72 h, under identical conditions – but without the magnetic field. Substrate sugar Ethanol -growing region was used as a fermentation media in all experiments 1. Fermentation 2. 100 ml of medium was inoculated at T =28 °C and N = 100 rpm, with 1 ml yeast cell suspension previously exposed to homogeneous static magnetic field of 130 mT.3. Analytical methods Organic acids, reducing sugars, and alcohol in the ethanol and sugar were analyzed by HPLC. Fig.1, – *72 h culture of Saccharomyces cerevisiae yeast cells exposed to homogeneous static magnetic field of 300 mT, at 28 °C.* glycerol and ethanol were detected by RI detector. The peaks were quantified using external standardcalibration. The components were identified by acomparison of their retention times with those of the standards.Quantification was performed using external standards prepared from pure compounds.15Acetaldehyde, iso-amyl alcohol, 1-propanol and 2-butanol analysis were performed by gas chromatography. The components were identified by comparison of their retention times with those of the standards. Quantification was performed using internal standard. Biomass was determined gravimetrically after 5 minute centrifugation of 20 ml of fermentation broth at 4000 rpm and 24 h drying at 105 ºC. Yeast cells were prepared for ultrastructural analysis by conventional method of fixation in a mixture of 1.5 % glutaraldehyde and 2 % paraformaldehyde in 0.1 mol /l–1 phosphate buffer, postfixation in 1 % osmium tetroxide, dehydration in graded series of ethanols, and embedding in Spurr. Ultrathin sections were stained in uranyl acetate and lead citrate. Statistics All the experiments were performed in triplicate. Results *On-line* redox potential measurement was used as a monitor of yeast cell metabolic activity. It differed between fermentations inoculated with the treated and control cells. In all the experiments, the measurements started at 300 mV. For the fermentation done with the control inoculum, the aerobic phase lasted 24 h, with the potential reaching 350 mV. With the 24-h treated inoculum, the aerobic phase lasted 18 h, with the potential reaching 400 mV. The aerobic phase was very short with the other treated inocula: 15 minutes with 24 h treated inoculums, 10 minutes with the 48 h, and 6 minutes with the 72 h treated inoculum. With an increase in the magnetic field treatment, the final redox potential was lower and the time at which it was reached was shorter: The fermentations done with the control reached –120 mV at 100 h, 24 hour treated inocula – 200 mV at 96 h, 48 h –2500 mV at 72 h and at 72 hour treated inocula –350 mV at 36 h respectively (Fig. 2).The biomass determinations showed similar results (Fig. 3). Biomass levels measured at 96 h, when all cultures had reached the stationary phase, increased with increasing treatment time of the inoculums to 3.22 g/l– (24 h), 3.54 g/l (36 hr)3.98 g /l (48

h) and 4.11 g/l– (72 hour treated cells) compared to the Contro3.22g /lrespectively (Table 1).

Table 1.

	Biomass(g/l)	Fructose(g/l)	Ethanol(g/l)	Fermentation time(hr)
control	3.22	3o.40	64.33	24
magnetic field 1day	3.54	18.94	84.40	36
magnetic field 2day	3.98	11.32	92.24	48
magnetic field 3 day	4.11	6.42	98.78	72
magnetic field 4 day	4.24	3.22	99.23	90

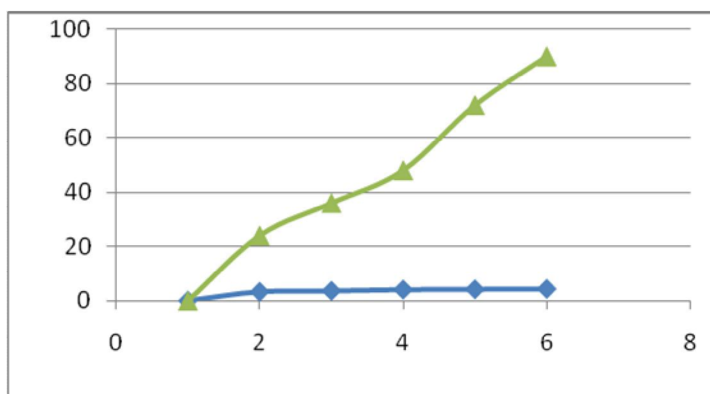

Fig 1.Concentration Biomass (g/l) vs Fermentation time(hr)

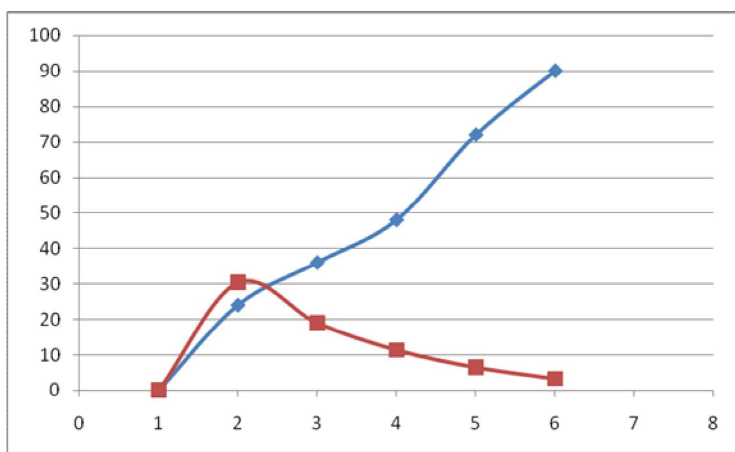

Fig 2.Concentration of fructose(gm/l) vs fermentation time(hr)

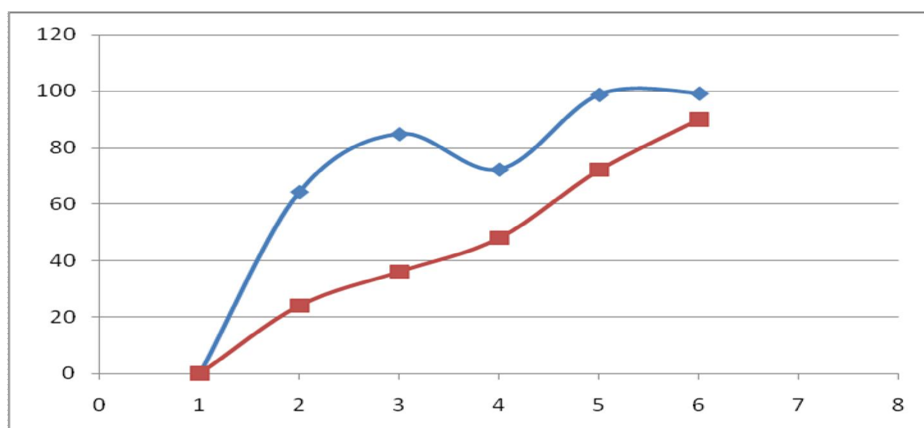

Fig 3.Ethanol concentration (g/l) vs Fermentation time (hr)

The rate of consumption of sucrose increased with increasing pretreatment of the inoculum with the magnetic field . On theother hand, fructose consumption profiles were very similar for all fermentations, irrespective of the inoculums.
Used.

3. Conclusion:

In the present research, the stimulating influence of homogeneous static magnetic field 12 mT on yeast biomass and cell metabolic activity in alcohol fermentation was found. According to the results, potentially favorable changes resulting in cellular metabolism
and fermentation process kinetics have been observed. Summarizing the results and observationsfrom the available publications (Table 1) with the results and findings of the present research, th *Saccharomyces cerevisiae* cell exposure does not cause noticeable changes in the cell structuritself, but it promotes faster growth and cell metabolism
and therefore faster process kinetics. Although no significant changes in the cell structure were found, magnetic exposure also influences cell membrane permeability and motility; therefore the synergistic effect of all these factors has to be taken into the final account. With an increase in the magnetic field, the length of the exposure resulted with lower fermentation process redox state, which resulted in a more stable and oxygen-resistant wine in a much shorter fermentation time. In the exposed samples, the highest biomass 4.24 g /l(28 % increase) was obtained in fermentation at 72 hour magnetic exposure, while at 48 h exposure 3.98 g /l (24 %), and in 24 h 3.784g /l 11 % increase) amount of biomass compared to 3.22g /lin control. Faster process kinetics was also found in the glucose consumption, while in the fructose consumptions results were quite similar for all three exposures. Although very similar results were obtained in final acetaldehyde production, it was evident that magnetic exposure influences the intensity of its biosynthesis. Similar to the 24 h, 48 h and 72 h magnetic inoculum treatments were the results of fermentations undertaken.at 24°C, 25 °C, 28 °C, respectively.

The results obtained are given in the following table 2.

culture no	Exposure to alcohol%	Time of exposure(hr)	Time of distillation (hr)	Production of alcohol
T1	15	5	48	31.73
T2	15	18	48	28,53
T3	15	20	48	27.64
T4	20	5	48	26.22
T5	20	18	48	25.78
T6	20	20	48	22.65

From the above results it is clear that strain T2. Which has been ex posed to 15% alcohol for 18 hrs is the high yielding strain, as it gives 31.73% alcohol after distillation. We also find that as the exposure is increased, that is, with increasing exposure to 20% alcohol for 5 hrs, 18 hrs, and 20 hrs, the production of alcohol decreases. Thus based on the alcohol tolerance capacity of Saccharomyces cerevisiae, the strain that yields the highest percentage of alcohol was isolated.

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5. REFERENCES:

[1]. Bakoyianis V, Koutinas AA, Agelopoulos K, Kanellaki M (1997). Comparative study of kissiris, _-alumina,and calcium alginate as supports of cells for batch and continuous wine-making at low temperatures. J. Agric. Food Chem., 45: 4884-4888.
[2].Banat IM, Nigam P, Singh D, Marchant R, McHale AP(1998). Review:Ethanol production at elevated temperatures and alcohol concentrations: Part I -Yeasts in general. W. J. Microbiol. Biotechnol., 14: 809-821.
[3]. de Vasconcelos JN, Lopes CE, de França FP (1998).Yeast immobilization on cane stalks for fermentation.Int. Sugar J., 100: 73-75.
[4]. de Vasconcelos JN, Lopes CE, de França FP (2004).Continuous ethanol production using yeast immobilized on sugar-cane stalks. Braz. J. Chem.Eng., 21: 357-65.
[5]. Göksungur Y, Zorlu N (2001). Production of ethanolfrom beet molasses by Ca-alginate immobilized yeast cells in a packed-bed bioreactor.Turk. J. Biol., 25:265-275.
[6]. Gunasekaran P, Raj KC (1999). Ethanol fermentationtechnology . Zymomonas mobilis. Curr. Sci., 77: 56-68.
[7]. Kesava SS, Panda T (1996). Ethanol production byimmobilized whole cells of *Zymomonas mobilis* in a continuous flow expanded bed bioreactor and acontinuous flow stirred tank bioreactor. J. Ind. Microbiol., 17: 11-14.
[8]. Valach M, Navrátil M, Horváthová V, Zigová J,.turdík E, Hrabárová E, Gemeiner P (2006). Efficiency of a fixed-bed and a gas-lift threecolumn reactor for continuous production of ethanol by pectate- and alginate-immobilized *Saccharomyces cerevisiae* cells. Chem. Pap., 60: 154-159.
[9]. Verbelen PJ, Schutter DPD, Delvaux F, Verstrepen KJ, Delvaux FR (2006). Immobilized yeast cell

systems for continuous fermentation applications. Biotechnol. Lett., 28: 1515-1525.
[10]. Yu J, Zhang X, Tan T (2007). An novel immobilization method of *Saccharomyces cerevisiae* to sorghum
bagasse for ethanol production. J. Biotechnol., 129: 415-420.
[11]. Kocher GS, Kalra KL, Phutela RP (2006). Comparative production of sugarcane vinegar by different immobilization techniques. J. Inst. Brew., 112: 264-266.
[12]. Kourkoutas Y, Psarianos C, Koutinas AA, Kanellaki M, Banat IM, Marchant R (2002). Continuous wheyfermentation using kefir yeast immobilized on delignified cellulosic material. J. Agric. Food Chem., 50: 2543-2547.
[13]. Krishnan MS, Blanco M, Shattuck CK, Nghiem NP,Davison BH (2000).Ethanol production from glucoseand xylose by immobilized *Zymomonas mobilis*CP4(pZB5). Appl. Biochem. Biotechnol., 84-86: 525-541.
[14]. Kumar S, Singh SP, Mishra IM, Adhikari DK (2009a).Recent advances in production of bioethanol fromlignocellulosic biomass. Chem. Eng. Technol., 32: 517-726.
[15]. Kumar S, Singh SP, Mishra IM, Adhikari DK (2009b).Ethanol production from glucose and xylose at highemperature by *Kluyveromyces* sp. IIPE453. J. Ind.Microbiol. Biotechnol., 36: 1483-1489.
[16]. Kumar S, Singh SP, Mishra IM, Adhikari DK (2010).Feasibility of ethanol production with enhanced sugarconcentration in bagasse hydrolysate at high temperature using *Kluyveromyces* sp. IIPE453.Biofuels, 1: 697-704.
[17]. Liang L, Zhang Y, Zhang L, Zhu M, Liang S, Huang Y (2008). Study of sugarcane pieces as yeast supportsfor ethanol production from sugarcane juice and molasses. J. Ind. Microbiol. Biotechnol., 35: 1605-1613.
[18]. Love G, Gough S, Brady D, Barron N, Nigam P, Singh D, Marchant R, McHale AP (1998). Continuousethanol fermentation at 45°C using *Kluyveromyces marxianus* IMB3 immobilized in calcium alginate andkissiris. Bioproc. Eng., 18: 187-189.
[19]. Nigam P, Banat IM, Singh D, McHale AP, Marchant R (1997). Continuous ethanol production by thermotolerant *Kluyveromyces marxianus* IMB3 immobilized on mineral kissiris at 45°C. W. J.Microbiol. Biotechnol., 13: 283-288.
[20]. Ozmihci S, Kargi F (2008a). Ethanol production from cheese whey powder solution in a packed columnbioreactor at different hydraulic residence times. Biochem. Eng. J., 42: 180-185.
[21]. Ozmihci S, Kargi F (2008b) Fermentation of cheese whey powder solution to ethanol in a packed-columnbioreactor: effects of feed sugar concentration. J. Chem. Technol. Biotecnol., 84: 106-111. Rebro.
[22].. *Funk, R. H., Monsees, T., Ozkuour, N.,* Prog. Histochem.Cytochem. **43** (2009) 177.
[23]. *Kimball, G. C.,* J. Bacteriol. **35** (1937) 109.
[24]. *Fojt, L., Klapetek, P., Strašak, L., Vetterl, V.,* Micron. **40**(2009) 918. 4. *Egami, S., Naruse, Y., Watarai, H.,* Bioelectromagnetics **31**(2010) 622.
[25]. *Valentine, R. L., Pedro, J. J.,* Crit. Rev. Env. Sci. Technol.**27** (1997) 319.
[26]. *Otabe, E. S., Kuroki, S., Nikawa, J., Matsumoto, Y., Ooba,T., Kiso, K., Hayashi, H.,* Journal of Physics – Conferenceeries **156** (2009) 12016.
[27]. *Ruiz-Gomez, M. J., Prieto-Barciab, M. I., Ristori-Bogajoc,E., Martinez-Morillo, M.,* Bioelectrochemistry **64** (2004)151.
[28]. *Malko, J. A., Constantinidis, I., Dillehay, D., Fajman, W. A.,*Bioelectromagnetics **15** (1994) 495.
[29]. *Motta, M. A.,* Biotechnol. Progr. **17** (2001) 970.
[30]. *Motta, M. A.,* Biotechnol. Progr. **20** (2004) 393.
[31]. *Gorobets, S. V., Gorobets, O. Y., Goiko, I. Y., Kasatkina, T. P.,* Biophysics **51** (2006) 452.
[32]. *Stašak, L., Vetterl, V., Šmarda, J.,* Bioelectrochemistry **55**(2002) 161.
[33]. *Liu, C. Z., Wang, F., Ou-Yang, F.,* Bioresour. Technology**100** (2009) 878.

[34]. *Santos, L. O., Alegre, R. M., Garcia-Diego, C., Cuellar, J.,*Process Biochem. **45** (2010) 1362.
[35]. BIO-RAD Guide to Aminex HPLC Columns for Food and Beverage Biotechnology, and Bio-Organic Analysis, Bio- Rad Laboratories, pp 1–58, USA, 1997
[36]. Hofmann, G. A. 1985. Deactivation of microorganisms by an oscillating magnetic field. U.S. Patent 4,524,079.
[37]. Frankel, R. B., Liburdy, R. P., Biological effects of static magnetic fields. In: Handbook of Biological Effects of Electromagnetic Fields. (Polk C. and Postow E. eds.) 2nd
Ed. CRC Press, pp 64–78., Boca Raton, 1995
[38]. *Berovic, M., Mavri, J., Wondra, M., Kosmerl, T., Bavcar,D.,* Food Technol. Biotechnol. **41** (2003) 353.