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Deviation in Production of Bioethanol Applying Stress Tolerant Yeast: A Current Approach

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BACKGROUND: *Biofuel is a form of green energy which is being produced using living organisms. It produces less carbon as compared to the fossil fuels so it can be a promising alternative to the commercial fuel available. Fungal strains are commonly used agents to convert the carbohydrate content of the substrates into the ethanol following glycolysis pathway. Each and every micro-organism needs a specific condition for growth and produce higher yield of ethanol. By altering the parameters whether increasing or decreasing it might be possible that the organism adapt the changes and work in an efficient manner metabolically or vice versa. Physical and chemical conditions are the important conditions that are to be focused on but at present scenario, genetic engineering do plays an important role for altering the metabolism of the organism. Carbon source that is to be used is the major requirement of the organism to produce ethanol as it will provide nutrition to the organism. Considering the incubation time, inoculum size and the agitation rate, one can say that these work interdependently.*

RESULT AND CONCLUSIONS: *All these factors are the part and parcel of cell's activity aiding in the production of bioethanol for the future world to focus on green energy so as to save environment from greenhouse gases (GHG). The sole purpose of this review is to let the organism to adapt in stressed out conditions and review the prime parameters whether increased or decreased that might affect the growth and production of the desired product.*

KEYWORDS: *Biofuel, fossil, glycolysis, parameters, incubation, greenhouse.*

I. INTRODUCTION

Bioethanol is being widely explored as a renewable fuel source because in many considerations it is superior to gasoline fuel. Ethanol provides energy that is inexhaustible and less carbon intensive than oil [1, 2]. Ethanol is an essential industrial chemical with emerging potential as a biofuel to replace fossil fuels [3]. Production of ethanol from inexhaustible carbohydrate materials has been attracting worldwide interest and research has been directed to the production of ethanol by immobilized microorganisms using continuous culture [4]. It is an important organic compound used as solvent in laboratories, industries, and households. Freshly, the use of ethanol as a substitute fuel has received much attention as a solution for many problems caused by insufficient gasoline fuel obtained from depleting petroleum stocks. It is produced from various substrates such as saccharide, starchy and cellulose materials. Collection of suitable and economically substrate is an important cost component for industrial ethanol production [5-7]. Amongst these biofuels, ethanol has great demand as it is widely accepted and it is clean burning. In many countries, ethanol is used as either an alternate fuel or blended with petrol or gasoline. Many investigators have studied on the production of ethanol using various raw materials. The fruit wastes like papaya, mangoes [8], banana peels [9], pineapple [10] and grapes [11, 12] were used in the production of ethanol [13].Technically, ethanol can be produced from a wide variety of renewable feedstock, which can be roughly classified into three main groups: (1) those containing considerable amounts of readily fermentable sugars (sugar cane, sugar beets, and sweet sorghum), (2) starches and fructosan's (corn, potatoes, rice, wheat, and agave) and (3) cellulosics (Stover, grasses, corn cobs, wood, sugar cane bagasse). Sugar cane, beet and sweet sorghum maintain the simple sugars, as sucrose, glucose and fructose that can be readily fermented by yeasts [14, 15].Yeast, fungus, etc. are very commonly used for production of ethanol. Commonly *Saccharomyces cerevisiae* strains are being used for the production of ethanol. The yeast's capacity to produce ethanol is dependent on the tolerance of a strain to ethanol, temperature and other properties [16].Thus the review focuses on increasing the productivity of bioethanol with the help of

stress tolerant factors along with current approach of RNA interference application for providing a better alternative to the future needs.

II. PHYSICAL STRESS TOLERENCE

TEMPERATURE

Temperature is one of the most important factors that affect the ethanol production by yeast using molasses as a carbon sources. The fermentation process is always accompanied with evolution of heat that raises the temperature of the fermenter [1]. Ethanol fermentation at enhanced temperature is a main necessity for effective ethanol production in tropical regions where normal day-time temperatures are typically high all over the year. The rewards of rapid fermentation at enhanced temperature not only shrink the risk of contamination nonetheless also reduces the cooling costs. To attain enhanced temperature fermentation it is essential to use an competent yeast strain that can tolerate high temperature [17].For bioethanol production at higher temperatures, yeast cells die resulting in a cutback in alcohol yield when the substrate is concentrated, while excellent temperature for maximum productivity occurs at 32°C for maximum strains. It is therefore, necessary to select the optimum temperature at which yeast strains can ferment the sugars [18, 19].Taking into considerations the strains for temperature variations to be carried out there is a requirement of thermo-tolerant strains that can withstand high temperatures during the experimentations. For example, *K. marxianus* DMKU3- 1042 is considered to have an optimal temperature of 40 º C at which it can grow and ferment but if temperature is reduced to 28 º C then also it shows growth at a better level as compared to the increase in temperature of the same species till 48 º C at which minimum fermentation might be possible (Fig. 1). Another example states that *S. cerevisiae* showed the best performance at 35°C when cultured [20]. But since at 45°C no growth was observed and no fermentation was observed then it was concluded that *S. cerevisiae* have a range from 30–35°C range which is known to be optimal for fermentation [21, 22].Hence, temperature plays most important operating variables is temperature. The dependency of the maximum ethanol production rate on the temperature was explained by the superposition of activation energy for ethanol production [23, 24]. Therefore by standardizing the temperature or any particular temperature yeast gets stressed out for higher ethanol production.

pH (Hydrogen ion concentration)

The hydrogen ion concentration is an important environmental factor affecting cell growth and metabolite production. Therefore several studies investigating the influence of pH on microorganisms have been published. For the managed operation of bioreactors using immobilized yeast cells it is very important to know the precise effect of pH on the fermentation media on cell growth and ethanol production [25]. With increase in pH yeast produces acid rather than alcohol. Molasses has generally alkaline pH and must be acidified prior to fermentation [26, 27]. Taking the example of *S.cerevisiae* K-7 strain grew in the medium containing barley without salt at pH 2.0 and thus did not produce fuel bioethanol. In comparison to this strain, increased amount of bioethanol was produced by independently culturing all the strains of *I. orientalis* in the same medium. No ethanol production was observed using K-7 strain in the medium containing 50 g/L sodium sulphate (Na_2SO_4) at pH 3;however, 10 of the 13 strains so taken in the experiments of *I. orientalis* produced bioethanol between 0.5% and 5.3% (v/v)under the same condition of 50 g/L sodium sulphate at pH 3. Considering another experimental setup only 0.5% (v/v) ethanol was produced upon culturing theK-7 strain containing 25 g/L Na_2SO_4 at pH 2.5. However, all of the *I. orientalis* strains, produced increased concentrations of ethanol, between 4.9 and 5.3% (v/v), not including the NBRC 0011 strain, under the same condition. Thus, almost *I. orientalis* strain produced increased quantities of bioethanol compared to the K-7 strain of *S. cerevisiae* after incubation in acidic media (indicating the pH stress tolerance of the respective strains) containing high concentrations of Na_2SO_4. In the similar manner, observation of larger differences were observed in ethanol production amongMF-121 strain as 5.3% (v/v) and the NBRC 10737 strain as 3.3% (v/v) at 50 g/l of Na_2SO_4 at pH 3.0. At 50 g/l of Na_2SO_4 at pH 2.0, which was the most critical condition, the MF-121 strain fermented glucose to ethanol at 1.3% (v/v) value of concentration. However, little or no ethanol was observed using other strains of *I. orientalis* under 50 g/l of Na_2SO_4 at pH 2.0 (Table. 1)[28].Thus, the MF-121 strain produced the maximum amount of bioethanol under the combined stress of decreased pH between 2.0 and 3.0 pH units and a high salt concentration of 50 g/l sodium sulphate [29]. Concluding, looking over the above example, control of pH during ethanol fermentation is important for two reasons:

1) The growth of other harmful microorganisms is retarded by acidic solution.
2) Yeast grows well in acidic conditions.

AGITATION

Agitation plays a vital role for uniform mixing of the medium components within the fermenter (dispersion of cells and nutrients) as well as mass transfer phenomena (e.g., oxygen transfer rates). The effect of

agitation on ethanol production is vital for the efficacious progress of the fermentation method. Agitation is important for 3 major reasons:
[1] Sufficient mixing
[2] Mass transfer
[3] Heat transfer

It not only assists mass transmission amid the diverse phases present in the culture, but also upholds consistent chemical and physical environments in the culture by unbroken mixing. Agitation creates shear forces, which influence microorganisms in several ways, causing morphological changes, alteration in their progression and product formation and also destroying the cell structure [30, 31]. An aeration is considered to be most important factor affecting xylose fermentation by yeasts, since it regulates the partitioning of the carbon flow substrate between growth and product formation [32]. The agitation helps in the aeration in the ethanol production.In one of the study it was observed that an increment in the rate of agitation from 100 to 200 rpm, did not influenced the consumption of sugar and production efficiencies for ethanol fermentation[28]. Agitation rate in combination with the aeration rate and aeration timing influenced the *S. cerevisiae* NP 01 to utilize substrate in the form of sugar readily, directing to the higher yield of ethanol production. After the experimentation the results so recorded were supported by Lin whose work suggested that the presence of a particular amount of dissolved oxygen made the yeast adjustable and physiologically healthy to become productive and fruitful [33].According to the range for specifically agitation, the order of influence was agitation rate > aeration timing >aeration rate. Both the transition intensity of nutrients from the fermentation broth to the yeast cells and permeation that of ethanol so produced from the yeast cells to the fermentation broth could be enriched by increasing the agitation rate (from 100 to 200 rpm). Thus the processes would improve the sugar exploitation and deteriorate the spontaneity of the ethanol to the yeast cells [34, 35]. However, the increased agitation rate at extreme would not exert for upgrading ethanol concentration because of constraints in the yeast metabolism [28].Taking another example of *S. cerevisiae* and its co-culture, it can be clearly seen that agitation rate do plays a significant role in the ethanol production but up to a certain limit due the restriction of microorganism's metabolism. As a result, agitation fashions forces, which encourages microorganisms in several ways, causing physiological fluctuations, in progression and in product formation (Table 2).
Aeration is considered to be a vital factor since in fermentation by yeasts, it standardises the apportioning of the carbon flow from substrate specifically mass transfer and heat transfer between the cells and vicinity for growth and product formation in the form of biofuel and secondary metabolites.

CARBON SOURCE

The carbon source is very important in yeast to produce ethanol. Because the yeast takes the carbon source from external and use in the metabolic pathway. If the substrate is better and contain higher carbon source than the yeast is in stress and produces maximum ethanol. The carbon source is in form of monosaccharide, polysaccharides, and disaccharides. The complex carbon source is breakdown into a simple monosaccharaides so that the molecule so broken down can be used easily by the microorganisms for the production of bioethanol. When glucose was used as the carbon source, it allowed higher growth rate compared to other substrates [36]. The oxidation state of the carbon source significantly affected the yield of metabolites, at the time of ethanol production. The NADH/NAD+ cofactor pair, in particular, is very important in microbial catabolism, where a carbon source, like a glucose, is oxidized through a series of reactions utilizing NAD+ as a cofactor and produces reducing equivalents in the form of NADH. In one of the study, glucose (oxidation state = 0), sorbitol (oxidation state = –1), and gluconate (oxidation state = +1) were used as carbon sources [37] as a substrate for the microbe to utilise it. When reducing sugar content was focussed as one of the parameter, it was described in one of study that, in the media at the end of fermentation the concentration of reducing sugar can be seen in the graph plotted, that explained the amount of sugar which is left by the microbe and is not converted into bioethanol (Fig. 2).Consequently, these results indicated that higher the amount of starter (%), reducing sugar content in the fermentation medium is decreasing. This means that sugar as a source of carbon is converted into ethanol by *S. cerevisiae* microorganism, indicating that higher the amount of starter higher microbial population will be there in the medium which will lead to increased amount of sugar utilization by microbes for growth and for production of fuel, so that by the end of the fermentation of sugar is left fewer and is not consumed by the agents of fermentation.
According to the Moat and Foster (1979), sugar is a source of carbon and energy used for microbial growth and metabolite production [8, 38].

INOCULUM SIZE

Microorganisms are the major agents aiding in the field of fermentation, these microbes utilize the sugar content of the substrate and thus produces the desired product. These microbes when taken in a varying

quantity (%) play a vital role in the process. Taking the example of *S. cerevisiae*, average concentration of microbe is 5% starter, 7.5% and 10% respectively 8.35 x 106; 1.07 x 108; and 8.27 x 106 or in log form is 6.9222, 7.0295, and 6.9173.

This type of microbe *S. cerevisiae* has a growth pattern as follows, progressively increment in the amount of starter used, the number of microbes progresses towards the declination. This decrement in the number of microbes was expected, because of the increasing number of microorganisms in the fermentation medium that will increase in the competition in getting the nutrients. Both the graphs (Fig.3 & Fig. 4) indicate the microbial concentration at different starter concentration and at different incubation time as these two plays an important role when the inoculum size is taken into consideration this phenomenon supports the interdependency of the parameters onto each other for the interactions to be considered.The longer the fermentation the average number of microbes will be increasing, but the subsequent decline will be seen as the nutrients concentration decreases [38]. Accordingly, the results showed that the duration of fermentation will affect the growth of microbes. From these data it became clear that the number of microbes increased until fermentation for 4 days, further decrease the number of microbes was observed. No increase in the quantity of microbes, because the nutrients in the medium decreases at a particular concentration of microbes, therefore caused inhibition of growth by the presence of ethanol so produced after the process of fermentation.

INCUBATION TIME

The cells increased exponentially at the beginning of incubation, then entered a stationary phase after several days incubation. It is a very important for the growth of the yeast so as to promote the production of ethanol (Fig. 5). Considering the work of Trisasiwi et al., [38] on *Saccharomyces cerevisiae* incubation time is being focused thatlengthier(stressed) the incubation duration consumption of reducing will increase indicating the levels of reducing sugar in the medium is gradually and progressively decreasing. This means that the longer the duration of fermentation, increased amount of sugar will be used by microbes to grow and produce primary and secondary metabolites, etc. Standardisation of incubation time will positively help in the growth and metabolism of the organism but coin having its two sides the incubation should not exceed a proper limit since after the stationary phase the growth curve move towards the decline phase where the cells number starts decreasing indicating the completion of the nutrient media. It is a very important at the industrial level to produce ethanol at low cost and minimum time; this is the reason for which incubation time is being focussed for the growth and production of bioethanol as an alternative for the automobile energy.

LIGHT

Light is a very vital signal for every single living cell, and since exceptional adaptation to both the valuable and damaging effects of light knowingly improves the fitness of microorganism; it can be measured as crucial for effective competition and existence in nature. In contrast to plants, yeast use light as a source of information but not as a source of drives i.e. energy. During periods of studies on yeast, at least 100 fungal species, on behalf of all phyla, had been instituted to react to light. Light connected signalling phenomena have mainly been studied in *Neurospora crassa*, influences on expanded metabolic procedures and regulators have been illuminated in other yeast. Established on the genes indulged in the signalling cascade of light and their impact on metabolic production pathways in several yeast [39, 40] can be easily perceived. As a consequence this suggest that if light is being provided to the organism it will carry out its metabolic activities properly with proper passage of the signals so required during the process to be carried out for the production of bioethanol.

METALLIC STRESS TOLERANCE

Metals play a vital role in carrying out the reaction no matter humans or organisms they are useful in each and every aspect. These are very important for increase the ethanol production. There are several types of metals that are being focussed for the production of ethanol. These are Co^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, Mo^{6+}, Ni^{2+}, Zn^{2+}, SeO_4^- and WO_4^-[41]. The metals so used are first analysed and standardised for the maximum production of ethanol and since these are not toxic so there is no requirement of any pre-treatment or hydrolysis as the metal ions concentration is non-toxic to yeast[42]. Fundamental studies of ethanol reactions on metals and oxides, examining the relation between surface properties (structure, surface defects, etc.) and the reaction yields, have helped to more our understanding of the various processes[43]. Trace metals always act as cofactors or coenzymes in many cellular processes deficiency of which will affect the fermentation of lignocellulosic hydrolysate [35].Hence, it was found that there are specific metals for specific yeast and once these are analysed they can be used in the process for providing the assistance to the yeast for the production of ethanol.

GENETIC ENGINEERING

Genetic engineering technology presents undeniable potential for future agriculture and biofuel production. It has been focussed will be on the various possibilities that genetic engineering can offer to increase bioethanol production at large level. One strategy is to modify the characteristics and properties of yeast so that they can be converted more readily to the desired products. Enhanced stress tolerance in plants has been achieved mainly through the manipulation of effector genes [44]. To categorize genes that could be exciting targets for succeeding genetic engineering directing to obtain more robust and vigorous industrial yeast strains. The practice of genetic engineering tactics to upsurge the appearance of the nominated genes in industrial strains will be the next logical and reasonable step, to find out even if these influences may lead to the generation of supplementary robust industrial strains, which will be capable to deal with the most important fermentation tensions and, thus, to boost ethanol production rate and final ethanol titers [45].Combining different types of enzymes, and genetically engineering new enzymes, that work together to release both hemi-cellulosic sugars and cellulosic sugars, can be termed "bioprocessing." Bioprocessing is a key area of research in the efficient production of ethanol for biofuels. Genetic engineering is playing a major part in getting the best possible conversion rate in thermo tolerant yeasts. Biotechnological advances, especially in genetic engineering of commercially useful enzyme producing strains, are playing an important role in solving some of the bottlenecks that occur in production, and will be vital in securing cellulosic ethanol as a competitive liquid fuel for the future [46]. In the coming years, genetic modification of crops, yeast for biofuel production will be done by modifying quantity or quality of biomass. RNAi technology has emerged as an attractive tool to study the gene functions in plants and yeast through genetic engineering [47-49]. Genetic engineering has already played a key enabling role in the development of cellulosic biomass conversion technologies by dramatically reducing the cost of cellulase production from about $5.50 per gallon of ethanol to $0.10-15 per gallon of ethanol.

Preliminary results indicate that biomass yield increases of >300 % in some grass species can be achieved via genetic engineering, making the goal of a 15 ton per acre feedstock well within the realm of feasibility [50]. Recent advances in genetic engineering of yeast are expected to facilitate the introduction of more efficient as well as novel uses of yeast in industrial procedure. In addition, because they are eukaryotic cells with a relatively short generation time and small haploid genomes, effective genetic systems have been developed for many fungal species. This has resulted in their use as excellent model systems for basic metabolic processes [51].
There are two ways how the production of these unwanted by-products can be reduced by genetic engineering. Either the genes of the pathways that produce the by-products can be knocked out or the genes from the ethanol producing pathway can be overexpressed. Knock out is thought to be more feasible in this case because it is irreversible and it is generally easier to manage for organisms with a poorly developed genetic system. Genetic engineering, however, has not been very fruitful in developing stress resistance, but there seem to be promising results from evolutionary engineering, which could produce multiple-stress resistant yeast strains [52, 53].

III. CONCLUSION

Ethanol is an important organic compound used as solvent in laboratories, industries, and alcoholic beverages. Recently, the use of ethanol as an alternative fuel has received very importance and will achieve heights in near future. Utilization of the waste material will provide a benefit for the cost-effectiveness during the production and will thus proves itself a better alternative for the food feedstock's used till date. Ethanol so produced from the waste will be a boon for the industrial growth and will consequently help in the environment remediation. For maximization of the production standardization of different parameters like temperature, pH, agitation, incubation time, carbon source and metallic quantity is very much required.

IV. FUTURE PROSPECT

Genetic engineering will be valuable in future as it can alter the microbial activity for the production of bioethanol. One such technique that will be focussed in near future will be gene silencing. It will provide a hidden potential benefit of the microorganism that might focus on the inhibition of one or more pathways so as to subject the carbon source on one of the desired pathway to retrieve ethanol at a comparatively higher amount. In future the ethanol will be very important as a replaceable material to fuel as the fossils are not renewable and will get over in few years. Not only alcohol production but also pigments, flavours, etc. will get retrieved in a greater amount with the aid of genetic engineering. Further, with the help of genetic engineering microorganisms can be forced to work on to the stressed out conditions that might save time and once cultured monetary saving might also be possible.

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Table 1: Ethanol production under combined stress condition of low pH and Na_2SO_4 [29]

Medium		Ethanol (%, v/v) [d]												
Na_2SO_4 (g/l)	pH	*I. orientalis*												*S. cerevisiae*
		MF-121	0011	0012	0013	0155	0201	0584	0841	1162	1279	1395	1664	10737	K-7
0	3.0	4.9[a]	5.1[a]	5.0[a]	5.3[a]	5.2[a]	5.1[a]	5.2[a]	5.1[a]	5.1[a]	5.1[a]	5.3[a]	5.4[a]	5.5[a]	5.4[a]
0	2.5	5.4[a]	4.7[a]	5.1[a]	5.3[a]	5.4[a]	5.2[a]	5.3[a]	5.3[a]	5.2[a]	5.3[a]	5.2[a]	5.3[a]	5.4[a]	5.3[a]
0	2.0	5.7[a]	5.1[a]	5.0[a]	5.3[a]	5.3[a]	5.3[a]	5.2[a]	5.0[a]	5.4[a]	5.7[a]	5.4[a]	5.7[a]	5.4[a]	0.0[c]
10	3.0	4.9[a]	5.0[a]	5.0[a]	5.1[a]	5.1[a]	5.1[a]	5.0[a]	5.0[a]	5.1[a]	5.0[a]	5.3[a]	5.2[a]	5.2[a]	5.3[a]
10	2.5	5.1[a]	4.8[a]	5.1[b]	5.2[a]	5.2[a]	4.8[a]	5.2[a]	4.9[a]	5.2[a]	5.2[a]	5.4[a]	5.3[a]	5.3[a]	5.1[a]
10	2.0	5.5[a]	4.4[a]	5.1[a]	5.1[a]	3.0[a]	5.2[a]	5.3[a]	5.2[a]	5.3[a]	5.5[a]	5.2[b]	5.5[a]	5.4[a]	0.0[c]
25	3.0	4.9[a]	0.0[c]	5.0[a]	5.0[a]	5.1[a]	5.1[a]	4.8[a]	4.6[a]	5.0[a]	4.9[a]	5.3[a]	5.3[a]	5.1[a]	3.5[a]
25	2.5	5.1[a]	0.0[c]	5.0[a]	5.1[a]	5.1[a]	5.0[a]	4.9[a]	4.9[a]	5.3[b]	5.1[a]	4.9[a]	5.1[a]	5.0[a]	0.5[c]
25	2.0	5.2[a]	0.0[c]	2.4[b]	4.6[a]	0.0[c]	5.2[a]	2.8[a]	4.9[b]	2.3[a]	5.3[a]	5.2[b]	5.3[a]	3.3[a]	0.0[c]
50	3.0	5.3[a]	0.0[c]	0.7[b]	3.1[a]	0.0[c]	2.6[b]	0.0[c]	3.0[b]	2.5[b]	1.9[b]	1.7[b]	0.5[b]	3.3[b]	0.0[c]
50	2.5	3.7[a]	0.0[c]	0.0[c]	0.1[b]	0.0[c]	1.5[b]	0.0[c]	1.1[b]	0.3[b]	1.0[b]	0.6[b]	0.1[c]	0.1[b]	0.0[c]
50	2.0	1.3[b]	0.0[c]	0.0[c]	0.0[c]	0.0[c]	0.0[c]	0.0[c]	0.0[c]	0.0[c]	0.1[b]	0.0[c]	0.0[c]	0.0[c]	0.0[c]

[a] Final $OD_{600} \geq 5$
[b] $2 \leq$ Final $OD_{600} < 5$
[c] $0 \leq$ Final $OD_{600} < 2$
[d] The theoretical maximum of ethanol production from 100 g/l glucose is 6.4% (v/v).

Table 2: Fermentation kinetic parameters of experiments with using glucose and xylose as substrates for co-culture in agitated flask condition [38].

Parameters	0 rpm	50 rpm	100 rpm	150 rpm	200 rpm
Initial substrate concentration (g/l)	20.00	20.00	20.00	20.00	20.00
Substrate consumed (%)	98.51	95.32	95.24	95.17	97.62
specific glucose consumption rate (g/l h)	3.21	4.16	3.27	3.32	4.26
specific xylose consumption rate (g/l h)	0.71	0.62	0.53	0.52	0.54
Maximum ethanol concentration (g/l)	7.05	8.02	7.16	6.52	6.47
Maximum cell concentration (g/l)	2.52	2.84	2.47	2.71	2.94
Specific growth rate (ln (X/X_0)/h)	0.14	0.09	0.11	0.32	0.14
Ethanol productivity (Q_P, g /l h)	0.51	1.02	0.82	0.84	0.85
Ethanol yield ($Y_{P/S}$, g /g)	0.35	0.40	0.36	0.33	0.32
Cell yield ($Y_{X/S}$, g/g)	0.12	0.14	0.12	0.14	0.15
Ethanol yield ($Y_{P/X}$ in g P/g X)	2.81	2.82	2.90	2.41	2.20

P, product (ethanol); X, cell mass.

Fig. 1 Growth of *Kluyveromyces marxianus* strains at 28°C and 48°C.

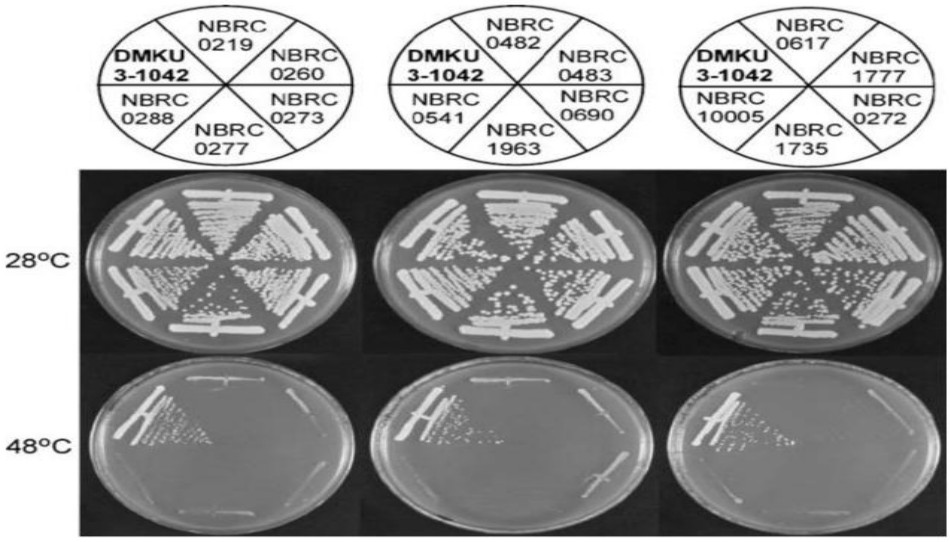

Fig. 2 The average value of reducing sugar content in various concentration of starter.

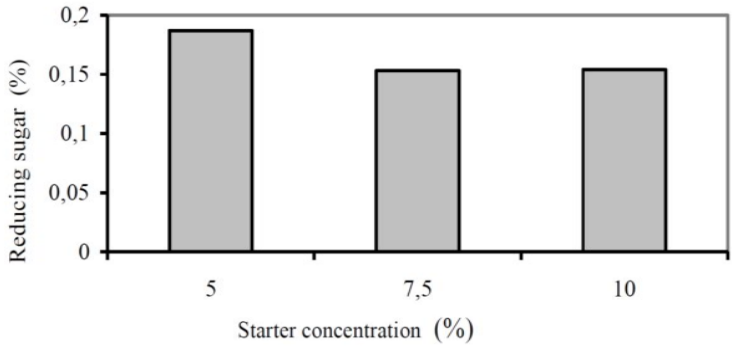

Fig. 3 Average total microbial at vary of starter concentration.

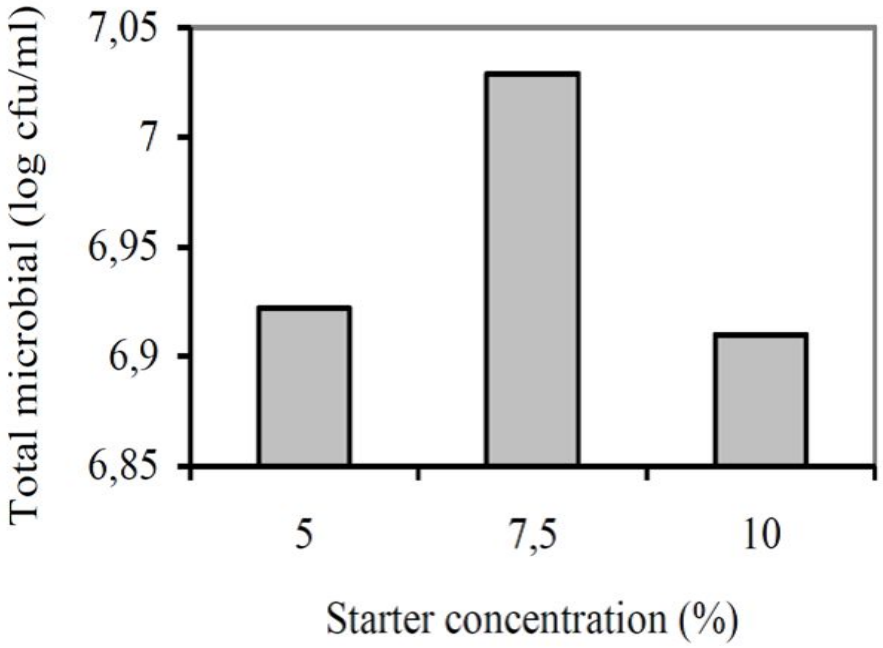

Fig. 4 The average number of microbes in various incubation times.

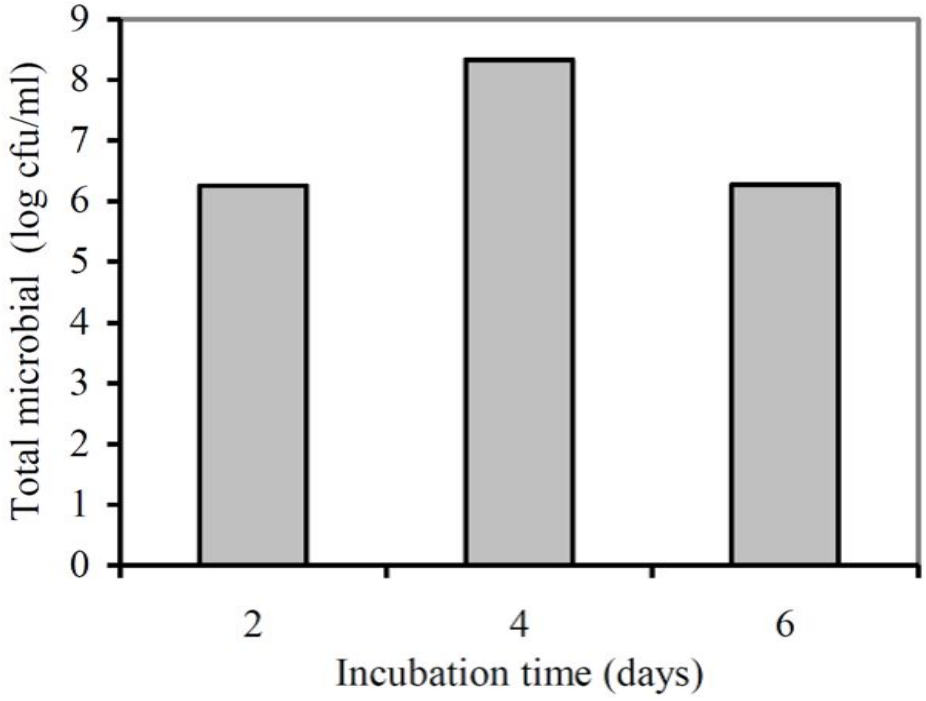

Fig. 5 The average value of reducing sugar content in various incubation times.