

Production of Alcohol by *Saccharomyces* sp. using Natural Carbohydrate Sources

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Abstract

A more general definition of fermentation is the chemical conversion of carbohydrate into alcohol or acids. The yeast species *Saccharomyces cerevisiae* has been used in baking as well as in brewing industry for production of alcoholic beverages for thousands of years. It is extremely important as a model organism in modern cell biology research for alcohol production and is one of the most thoroughly researched eukaryotic organisms. Ethanol produced by yeast cells can be used as a fuel in gasohol, as an antiseptic & antidote as well as in perfumes and tinctures. It is a base for other organic compounds like ethyl halide, diethyl ether etc. So it is important to find out economical and feasible method to enhance the production of alcohol above 7% (v/v). The present work involves use of different readily available cheaper carbon sources for production of alcohol within short duration; as well as study regarding the effect of co-factors on alcohol production by *Saccharomyces* species. The carbon sources used involves traditional sweetening agents like honey and molasses along with different fruits. Efficiency of fermentation was examined by formation of reducing sugars by yeast during the fermentation process. Growth media supplemented with compound sugars along with proteinous source affects carbohydrate utilization & ethanol production by yeast. Higher concentration of protein adversely affects alcohol production. Yeast cells exhibit higher ethanol production when grown in media with higher percentage of carbohydrate. But higher sugar concentrations above 8% (w/v) have inhibitory effect on ethanol yield. Addition of antioxidants rather than over ripened fruits to molasses media enhances ethanol production.

Key Words: yeast, ethanol fermentation, *Saccharomyces*, carbohydrate, glycogen

Introduction

Ethanol Fermentation is a biological process in which sugars are converted to alcohol using yeast under anaerobic conditions. A more general definition of fermentation is the chemical conversion of carbohydrate into alcohols or acids. The process of ethanol fermentation can be used on commercial scale for the production of alcoholic beverages and gasohol. Yeast is a unicellular eukaryote classified in the kingdom 'Fungi' with about 1,500 species currently known. The yeast species *Saccharomyces cerevisiae* has been used in baking industry for rising of bread dough as well as in brewing industry for production of alcoholic beverages for thousands of years. Day by day the fuel prices are rising along with increase in the demand by population in the world. So other energy crises like biofuel, agro based industrial product, solar energy are used as an alternate energy sources. European Union has given priority to produce bioethanol from agricultural residues as well as to replace up to 20% of petroleum fuel by ethanol within next 15 years (Hahn-Hagerdal M *et al.*, 2006). To save the petrol, Government of India is trying to add 10% ethyl alcohol for the full utilization and combustion of petrol & diesel. Bioethanol used for production of gasoline can reduce vehicle carbon dioxide emissions by 90% (Ward and Singh, 2002). Currently, approx. 80% of total world ethanol production is obtained

from the fermentation of simple sugars by yeast (Lin Y. and Tanaka S., 2006). Ethanol, a by product of sugar industries is produced by fermentation of molasses. Higher concentrations of sugar and the accumulation of ethanol in the cell can inhibit the growth of yeast (Ribereau-Gayon, 1985). So, yeast cells cannot produce ethanol higher than 7% (v/v) which ultimately inhibits the cell growth by producing osmotic stress across the cell wall (Dake M.S., 2002). For higher ethanol production various modifications have been carried out like mutation of yeast strain, changes in nutrient composition of the medium, prefermentation etc. Sugar tolerant yeast strains were isolated from sugar fermented vegetable extract (OK T & Hashinaga, 1997; Bertolini MC *et al.*, 1991). The yield of ethanol depends upon the type of yeast strain, physiology of yeast, media composition and other environmental parameters like pH, temperature etc. (Arvindekar A.U., 1995). Thus it is important to emphasize the importance of yeast genetics and physiology since yeast activities during fermentation impacts directly on yeast growth, alcohol productivity, stress-tolerance, sugar utilization, production of volatile compound and off-flavor development (Attfield PV & Bell P.J.L, 2003; Lambrechts MG & Pretorius IS, 2000; Piskur J & Langkjaer RB, 2004). Addition of assimilable nitrogen source can increase the rate of fermentation by reducing the time required for completion of fermentation (Bely *et al.*, 1990). So the present study

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involves use of various cheap & easily available carbon and nitrogen sources to investigate their effect on the ethanol production. Effect of these natural sugar sources is estimated in terms of the amount of both sugars and proteins utilized by yeast cells during fermentation process. Apart from the sugar source, use of other additives like vitamins & plant products was implemented to visualize their effect on ethanol production. Similarly efficiency of fermentation was also measured in terms of the concentrations of ethanol produced by yeast cells.

Materials and Methods

Microorganisms and Culture conditions: Bottom flocculating strain of *Saccharomyces italicus* NCIM - 3300 was obtained from National Chemical Laboratory, Pune and is routinely maintained on YEPD agar at 4°C. Peptone, yeast extract and malt extract were from Difco Laboratory, Detroit, Mich. All other chemicals were from AR grade. Sugar concentration was measured by DNSA method. Proteins were analysed by Folin Lowry method (Lowry O H *et al.*, 1951). Ethanol concentration was measured by ceric ammonium nitrate method.

Media Composition: YEPD- Variation in P concentration; variation in YE concentration; variation in G concentration. YE- 0.3% yeast extract, P- 0.5% peptone, D-varaible percentage carbohydrates- (Sugarane juice, Honey, Molasses, Grape juice, Glucose, Fructose, Maltose, Sucrose, and Lactose)

Results & Discussion

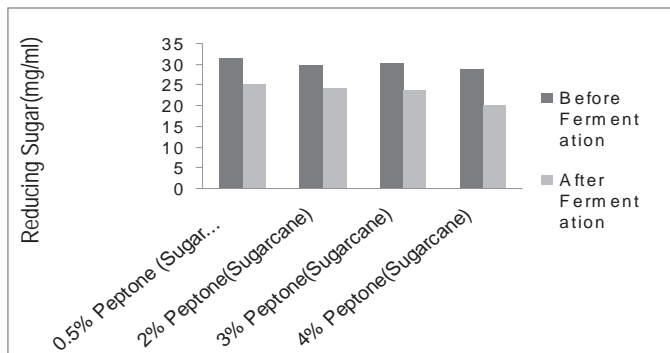


Figure 1. Variation in Peptone (P): Amount of reducing sugars before & after fermentation for sugarcane juice media with variable % of peptone (0.5 % - 4 %).

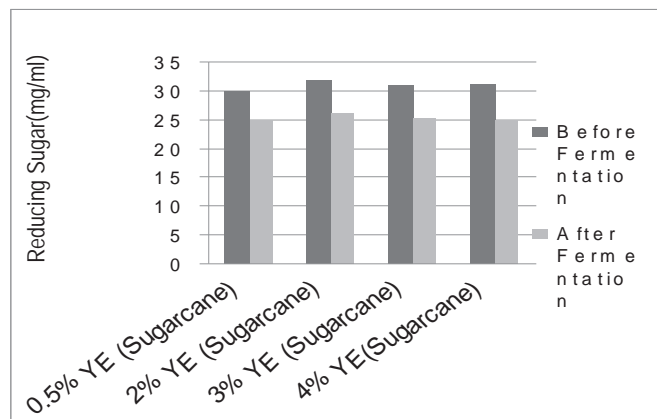


Figure 2. Variation in yeast extract (YE): Amount of reducing sugars before & after fermentation for sugarcane juice media with variable % of yeast extract ((0.5 % - 4 %).

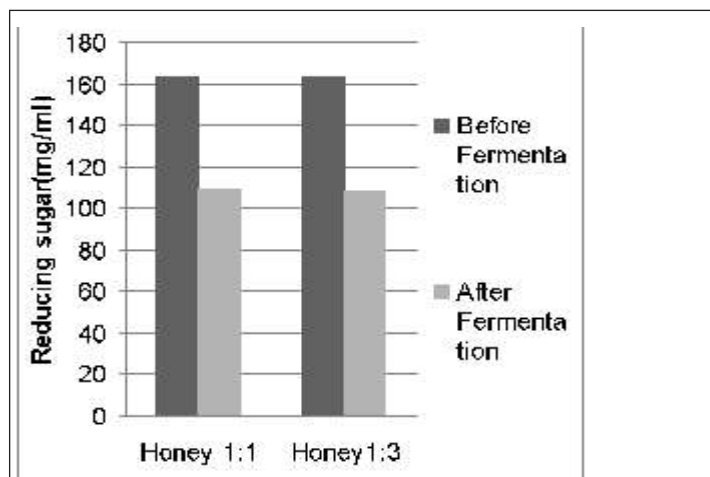


Figure 3. Variation in carbohydrate (Honey): Amount of reducing sugars before & after fermentation for media containing Honey (1: 1 & 1:3 diluted) as a carbon source.

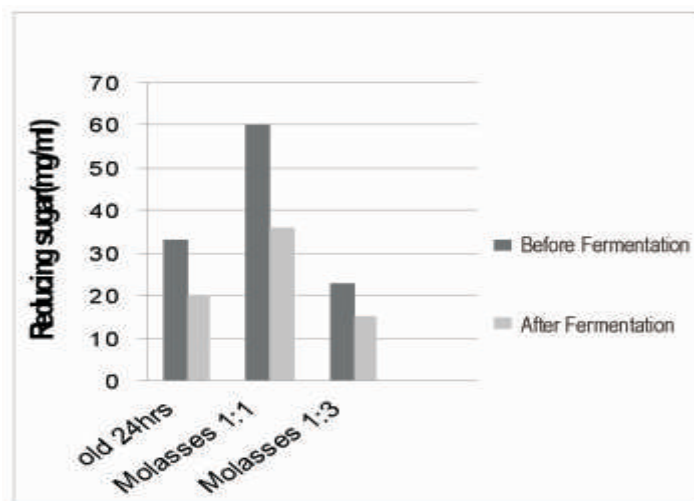


Figure 4. Variation carbohydrate (Molasses): Amount of reducing sugars before & after fermentation for media containing Molasses (1:3 dilution) as a carbon source using Traditional Method (24 hrs. fermentation) & Molasses (1:1 & 1:3 dilution) as a carbon source using Non-Traditional Method (48 hrs. fermentation).

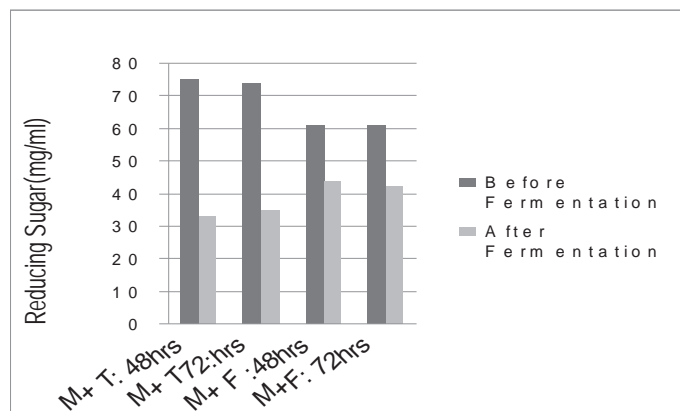


Figure 5. Amount of reducing sugars before & after fermentation for two different Molasses media-one containing antioxidant (Tamarind) & the other with fruits (Banana, Chiku, Mosambi). For both media fermentation was carried out for 48 hrs & 72 hrs duration.

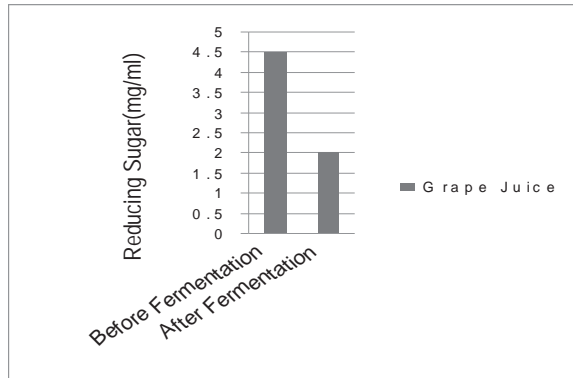


Figure 6. Carbohydrate (Grape juice): Amount of reducing sugars before & after fermentation for media containing grape juice as a carbon source.

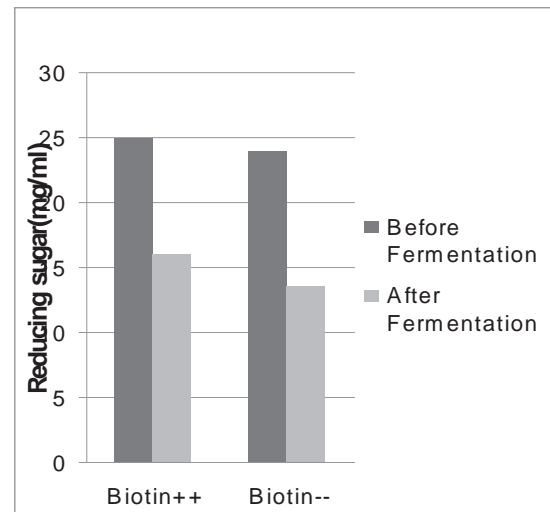


Figure 8. Carbohydrate (Sugarcane juice) with & without Biotin: Amount of reducing sugars before & after fermentation for Molasses media with & without Biotin.

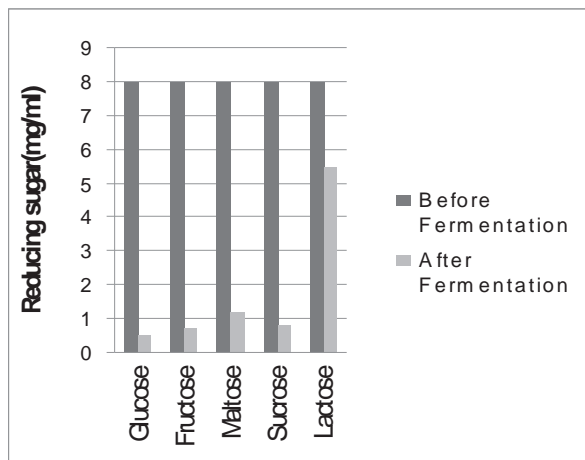


Figure 7. Variation in a carbon source: Amount of reducing sugars before & after fermentation for media containing variable sugars (8 % of Glucose, Fructose, Maltose, Sucrose & Lactose) as a carbon source.

Figures 1 - 8 indicate the amount of reducing sugars utilized by yeast cells during the process of fermentation. Sugar concentration is measured before & after fermentation. Fig. 9 indicates ethanol yield. When yeast cells were grown in sugarcane juice media with variable concentrations of peptone and yeast extract, the amount of reducing sugars utilized by cells gets increased during fermentation. But the yield of ethanol decreased in the presence of such higher concentration of nitrogenous source. When growth media of yeast was supplemented with natural carbon source – honey; utilization of reducing sugars and subsequent ethanol production, was found to be increased. Honey, used as a carbon source exhibited comparatively higher concentration of ethanol than sugarcane juice. Amount of sugar utilized during fermentation and ethanol production was found to be higher in case of traditional method

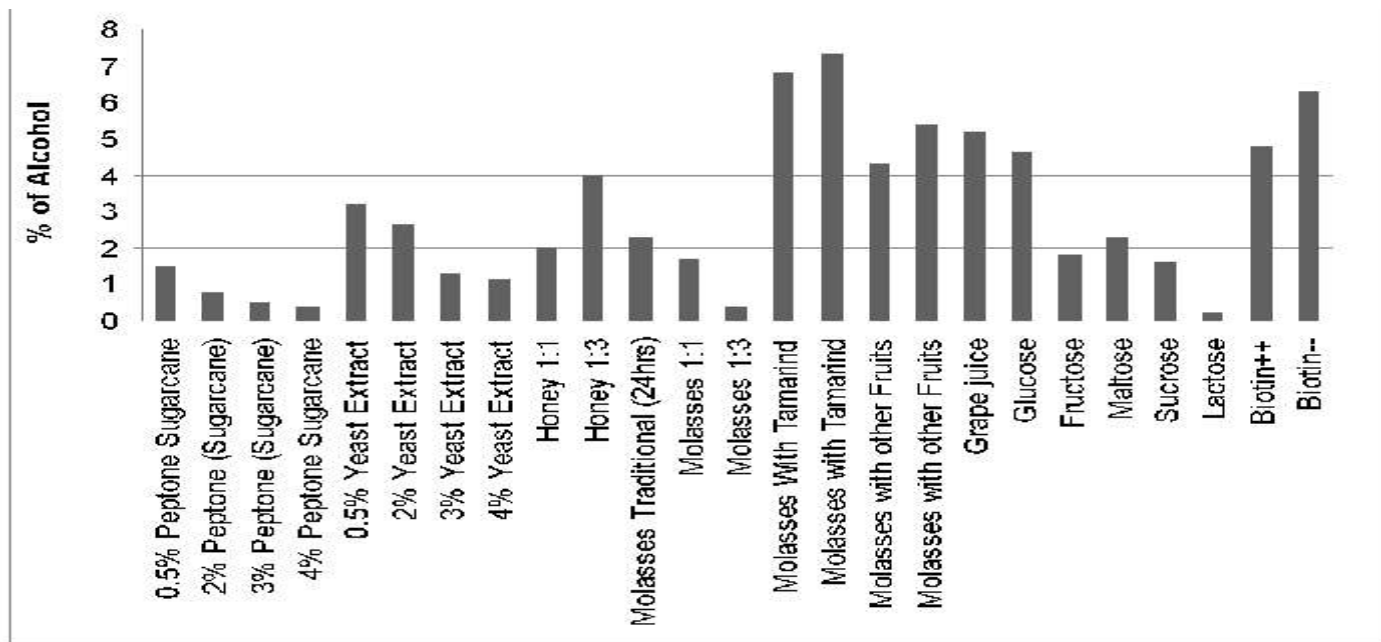


Figure 9. Effect of media with variable percentage of carbon & nitrogen sources on ethanol production: (Category I - Sugarcane Juice with variable % of peptone (0.5% - 4%); Category II -Sugarcane Juice with variable % of yeast extract (0.5% - 4%) ; Category III – Honey; Category IV- Molasses (Traditional Method -24 hrs. fermentation) & Molasses (Non Traditional Method - 48 hrs. Fermentation); Category V - Molasses + antioxidant & Molasses + fruits ; Category VI - Grape Juice; Category VII - Sugars (Glucose, Fructose, Maltose, Sucrose, Lactose); Category VIII- Sugarcane juice with & without Biotin.

where molasses was used as a carbon source. Production of ethanol was enhanced when yeast cells were grown in grape juice media rather than honey or sugarcane juice media. Addition of antioxidants to molasses media displayed highest ethanol yield which gets lowered by addition of fruits instead of tamarind. A higher concentration of ethanol was obtained for The Fermentation period of 72 hrs is optimum for better ethanol production rather than 48 hrs. Yeast cells fermented all mono & disaccharides like glucose, fructose, maltose & sucrose used as a carbon source in YEPD media. The least fermented sugar was found to be lactose. The highest ethanol production was observed for glucose followed by maltose as a fermentable carbon source. But yeast cells grown in presence of biotin lowered the ethanol yield.

Figures 10 - 17 indicates utilization of protein by yeast cells before & after fermentation. Yeast cells grown in the presence of higher concentrations peptone and yeast extract showed decrease in ethanol production. Natural carbon sources like honey, grape juice, molasses & sweet fruits increased protein utilization as well as ethanol production by yeast.

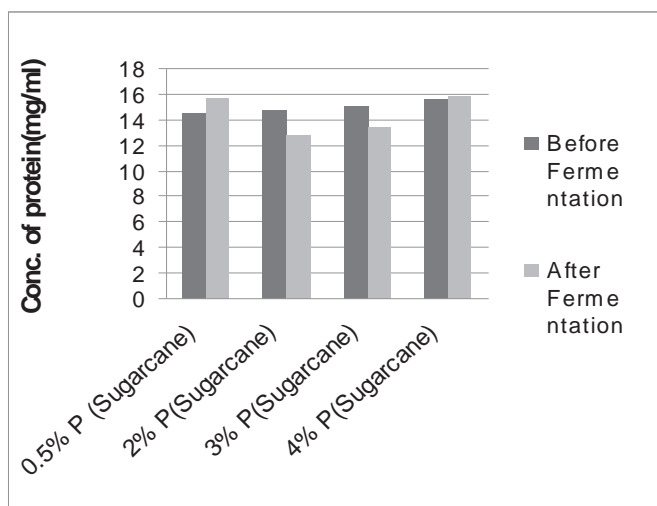


Figure 10. Variation in Peptone (P): Amount of protein before & after fermentation for sugarcane juice media with variable % of peptone (0.5% - 4%).

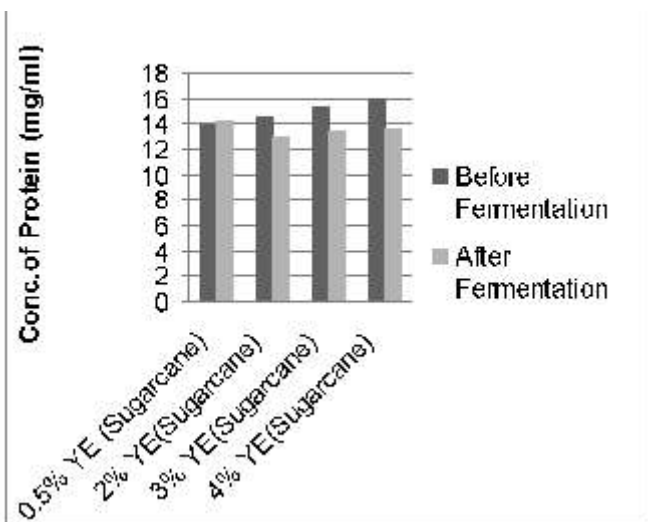


Figure 11. Variation in yeast extract (YE): Amount of protein before & after fermentation for sugarcane juice media with variable % of yeast extract (0.5% - 4%).

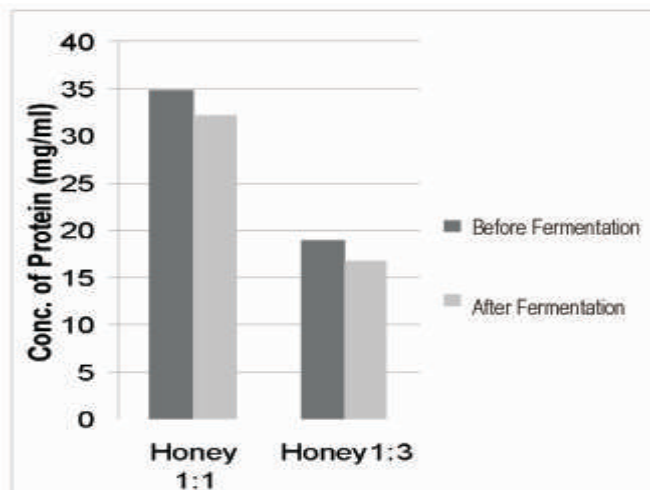


Figure 12. Variation in carbohydrate (Honey): Amount of protein before & after fermentation for media containing honey (1:1 & 1:3 diluted) as a carbon source

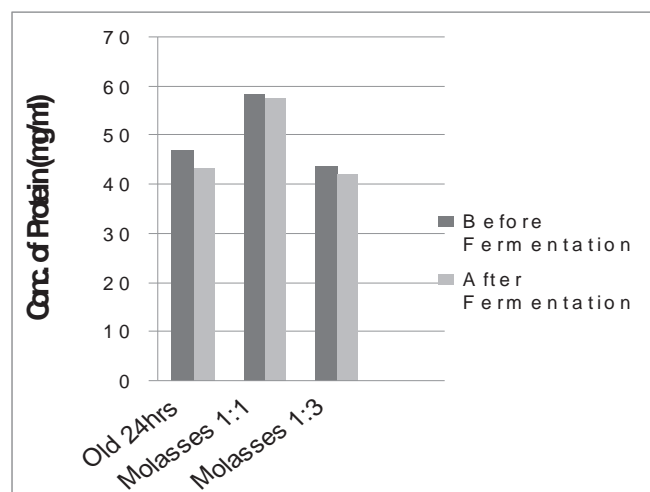


Figure 13. Variation in carbohydrate (Molasses): Amount of protein before & after fermentation for media containing Molasses (1:3 dilution) as a carbon source using Traditional Method (24 hrs. fermentation) & Molasses (1:1 & 1:3 dilution) as a carbon source using Non-Traditional Method (48 hrs. fermentation).

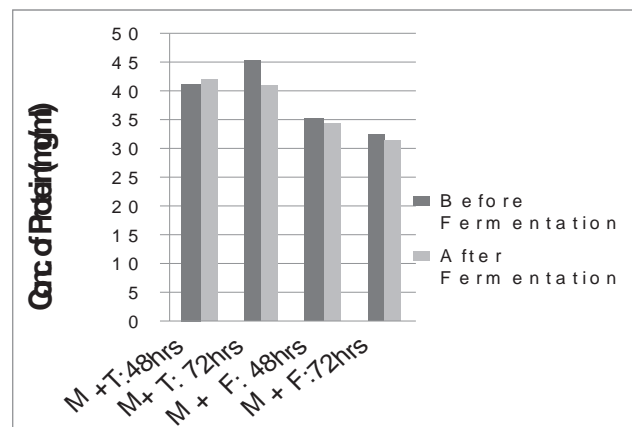


Figure 14. Variation in carbohydrate along with antioxidants: Amount of protein before & after fermentation for two different Molasses media – one containing antioxidant (Tamarind) & the other with fruits (Banana, Chiku, Mosambi). For both media fermentation was carried out for 48 hrs & 72 hrs duration.

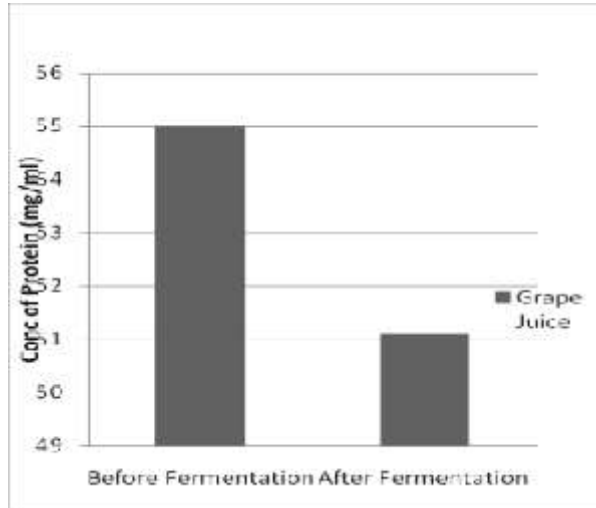


Figure 15. Carbohydrate (Grape juice): Amount of protein before & after fermentation for media used as a containing grape juice as a carbon source

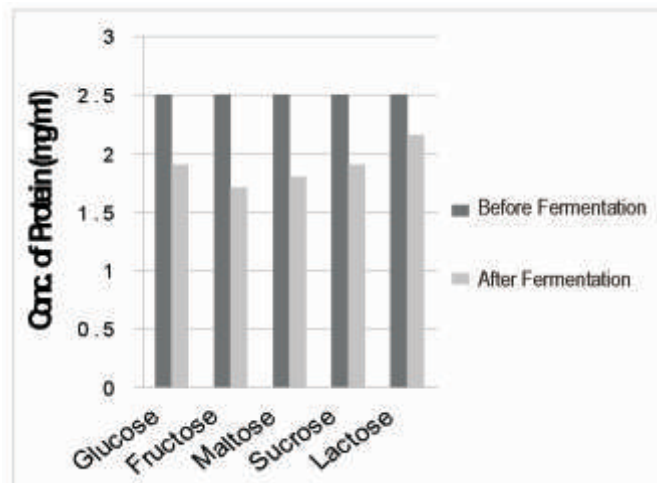


Figure 16. Variation in carbon source: Amount of protein before & after fermentation for media containing variable sugars (8 % of Glucose, Fructose, Maltose, Sucrose & Lactose) as a carbon source

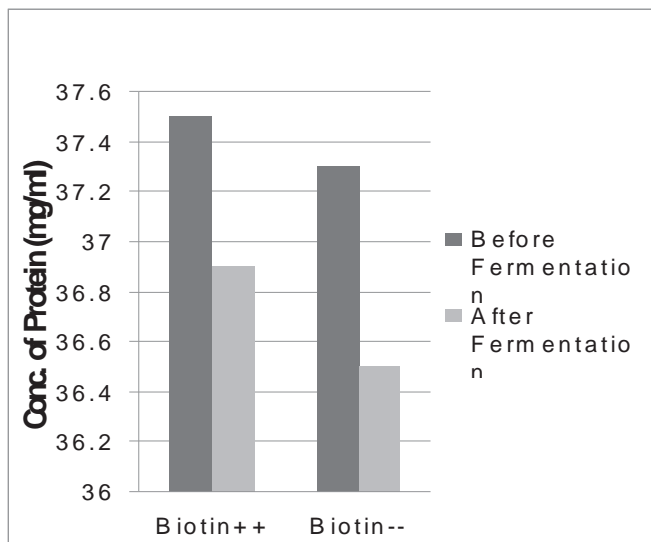


Figure 17. Carbohydrate (Sugarcane juice) with & without Biotin: Amount of protein before & after fermentation for Molasses Media with & without Biotin)

Conclusion

Decrease in ethanol production in the presence of higher concentration of nitrogenous source indicates that the amount of sugar utilized by yeast cells is diverted towards production & storage of carbohydrate in the form of glycogen rather than ethanol production. So, utilization of sugars by yeast cells gets increased with increase in the concentration of nitrogen source. Higher concentration of sugars in case of sugarcane juice media causes metabolic repression of yeast which results reduced yield of ethanol. Amount of sugar utilization and ethanol production varied in the presence of different sugars like mono & disaccharides. This is because monosaccharides such as glucose is transported across cell membrane by a simple facilitated transport mechanism whereas disaccharides like sucrose is converted to glucose & fructose at the cell surface using invertase which is present in yeast cell wall (Johannes, P., et al., 1994). In this way variation in ethanol content shown by yeast cells as a result of variable sugars used as a carbon source is due to their different pathways of metabolism. It is observed that higher utilization of sugars corresponds to higher protein utilization by yeast cells, and ultimately higher ethanol yield. Addition of antioxidants also increases protein utilization & subsequent ethanol production. Presence of biotin decreases ethanol yield.

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