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Variations of Two Pools of Glycogen and Ethanol in *Saccharomyces carlsbergenesis*

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Saccharomyces carlsbergenesis maintains two pools of glycogen, one soluble and the other insoluble and also topographically separated and metabolize them separately. Glycogen gets accumulated in the cells and its concentration greatly varies. The variations of these two pools of glycogen as a function of fermentation time, different sugar concentrations, different peptone concentrations and in different media when studied shows that sucrose is a better sugar than glucose. Since the yield of ethanol and glycogen is higher, optimum insoluble glycogen and ethanol are found to be at 36 h of fermentation. Though glycogen may serve as a substrate under anaerobic conditions for ethanol production, the availability of co-enzymes, co-factors and the necessary enzymes for the conversion are equally important.

Key Words: *Saccharomyces carlsbergenesis*, **Yeast, Glycogen, Ethanol, Two pools of glycogen.**

INTRODUCTION

The cell wall of yeast is a complex structure and composed of α-glucan, β-glucan, mannan and chitin. The β-glucan represents one of the major components of the yeast cell walls. In *Saccharomyces* the cell wall glucan has been found to be composed of two components: a high molecular weight β (1→3) linked glucan containing a small proportion of β (1→6) inter linkages and the minor fraction is a polymer consisting predominantly of β (1→6) linked glucosyl units containing about 20% of β (1→3) inter chain linkages[1, 2]. Mannan is in the form of mannoproteins and is attached to fibrillar inner layer of β-glucan[3] and chitin is a major constituent of bud scars[4].

α-Glucan (glycogen), a homopolymer of glucose, is highly soluble in water and thus can serve as energy reservoir and is synthesized or degraded on demand to balance glucose supply. The presence of glycogen in an organism is a guarantee of its survival during starvation condition. Glycogen has been isolated and characterized from a variety of micro-organisms including yeasts, *Mycobacteria*[5], *E Coli*[6] and *Aerobacter aerogenes*[7]. Further more precise information about the structure of yeast glycogen was given by Northcote[8].

A report showing that out of the total glycogen present only a minor fraction was water soluble referred as soluble pool of glycogen and the remainder was

water insoluble which was associated with alkali insoluble mass[9]. These observations suggest that two pools of glycogen are present in yeast; one is the soluble pool and the other insoluble pool. These two pools of glycogen differ in their location within the cell.

The attachment of cell wall glycogen to β-glucan in *Saccharomyces cerevisiae*[10] and in *Saccharomyces carlsbergenesis*[11] was established in our laboratory through the use of lyticase, a specific β (1→3) lysing enzyme for solubilization of cell wall linked glycogen. A general observation was that increased cell wall glycogen content is related to increased ethanol. The level of glycogen depends on the status of cells used for isolation, the way the cells are stored, pH, temperature and medium composition in which the cells are grown. The position, content and functions of other cell wall components of yeast are well established except that of glycogen. This prompted us to study their relation in *Saccharomyces carlsbergenesis*.

EXPERIMENTAL

Saccharomyces carlsbergenesis 3312 was purchased from National Chemical Laboratory, Pune and is routinely maintained on agar slants having composition 1% glucose, 0.5% peptone, 0.3% yeast extract and 2.5% agar (w/v).

In all the experiments 500 mL of nutrient medium was inoculated with equal quantity of inoculum (10% v/v). The fermentation was carried out at room temperature (25°C). The cells were harvested under cold conditions from each flask after 48 h of fermentation except in studies with fermentation time. These cells were further digested with alkali and the amount of soluble and insoluble glycogen was estimated. Glycogen isolation and measurement was carried out as stated by Arvindekar *et al.*[10]. The supernatant was tested for ethanol content. For measurement of ethanol, the medium was distilled and ethanol content determined by ceric ammonium nitrate method. Very similar quantities of soluble, insoluble glycogen and ethanol were obtained in several repeated experiments.

Different medium compositions were used for study of variations of two pools of glycogen and ethanol in *Saccharomyces carlsbergenesis*

Increasing sugar concentration

The concentration of glucose and sucrose were chosen as 2, 4, 6, 8 and 10% with 0.5% peptone and 0.3% yeast extract in a set of five flasks each.

Change in medium composition

- Sugarcane juice was diluted so that it contains 10% sugar concentration.
- Sugarcane juice (10% sugar content) and 0.3% yeast extract.
- Sugarcane juice (10% sugar content), 0.3% yeast extract and 0.5% peptone.

Concentration of sugar was determined using phenol sulphuric acid method.

Fermentation time

Sugarcane juice was diluted so as to obtain 10% sugar concentration, 0.5% peptone and 0.3% yeast extract. After completion of the required time interval, the cells were harvested at 0, 3, 6, 9, 12, 24, 36, 48, 60 and 72 h under cold conditions.

Increasing peptone concentration

The medium contained 10% sucrose, 0.3% yeast extract and 0.5, 1.0, 1.5 and 2.0% peptone in a set of four flasks.

RESULTS AND DISCUSSION

It is observed that variation in ethanol content and that of insoluble glycogen have a high degree of correlation. The level of glycogen storage varies with time, fermentation parameters and type of sugar utilized. In view of this a detailed study of these factors is essential and was attempted in the present study. The work carried out in the present study was found to be useful to assess the effect of different sugars on glycogen content, effect of nitrogen and growth factors on glycogen content and variation of insoluble glycogen content with fermentation time in relation to alcohol and cell weight. The intention of this study was to optimize the conditions that would favour optimum storage of glycogen in healthy and viable yeast cells. This means that when these conditions are met, optimum yield of ethanol can be obtained.

Increasing sugar concentration

When accumulation of glycogen in different sugar medium with different sugar concentration was studied, it was noted that the level of soluble and insoluble glycogen increases exponentially up to 10% of glucose and sucrose concentration. In other words, the nature of variation in case of soluble and insoluble glycogen for both the sugars is same but the cells, which were grown in sucrose medium, show excess deposition of insoluble as well as of soluble glycogen than the cells, which were grown in glucose medium. The variation found in the content of soluble and insoluble glycogen are shown in Figs. 1 and 2, respectively. When we tried to correlate the insoluble glycogen with ethanol it was observed that ethanol content increases with increasing glucose concentration (Fig. 3). Similar observation emerges out in case of sucrose medium (Fig. 4). This shows that the presence of excess carbohydrate in the medium leads to deposition of storage polysaccharide but the cells grown in glucose show comparatively lower values of both glycogen and ethanol than those grown in sucrose medium. This also means that the yeast cells grow in glucose and sucrose medium in different manner and sucrose is better sugar than glucose since the yield of ethanol is higher.

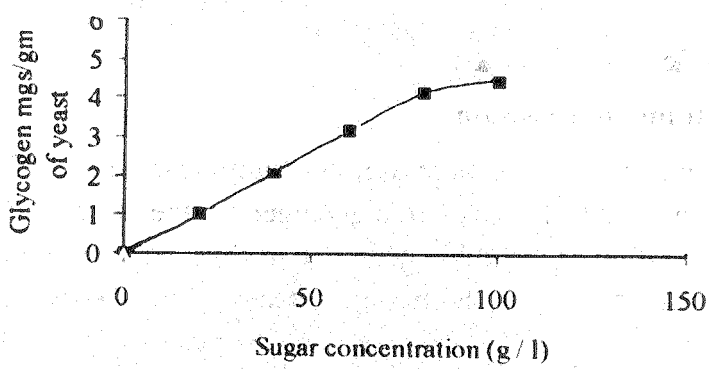

Fig. 1. Variation in soluble glycogen with increasing sugar concentration [glucose (■) and sucrose (▲)]

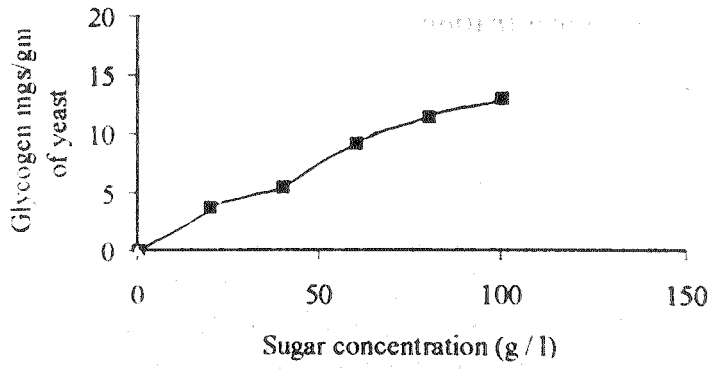

Fig. 2. Variation in insoluble glycogen with increasing sugar concentration [glucose (■) and sucrose (▲)]

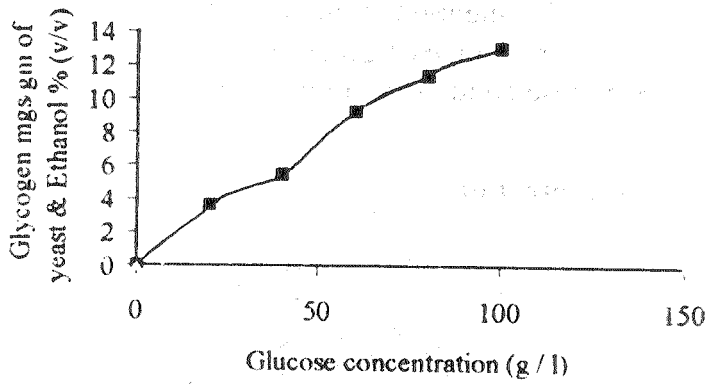

Fig. 3. Variation in insoluble glycogen and ethanol with increasing glucose concentration [glycogen (■) and ethanol (▲)]

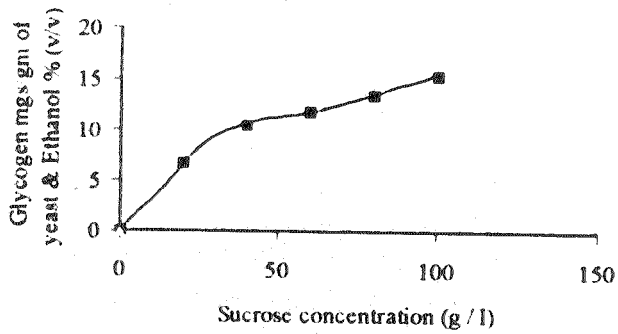

Fig. 4. Variation in insoluble glycogen and ethanol with increasing sucrose concentration [glycogen (■) and ethanol (▲)]

Change in medium composition

Three medium compositions were used for studying the effect of medium. The variation found in soluble and insoluble glycogen for the three media was shown in Fig. 5. Concentration of insoluble glycogen in cells, which were grown in only sugarcane medium, shows very high values than in other two media while cells grown in medium containing peptone and yeast extract store minimum or normal amount of glycogen. The reason is cells in medium containing peptone and yeast extract may not be subjected to nutritional stress and hence they show normal

deposition of glycogen, but cells in only sugarcane medium are under nitrogen limitation, suffer from nutritional stress and hence deposit excess glycogen. The effect in terms of concentration of ethanol in these three media was shown in Fig. 6. Somewhat unexpected results were obtained for the cells in only sugarcane medium where they show excess deposition of glycogen, the ethanol concentration is much less than in yeast extract and peptone. Thus one can note that though there is a correlation between insoluble glycogen and ethanol if cells are starved of nitrogen or growth factors, the relationship does not hold always true. In other words, if cells are grown in pure sugarcane juice, then sugarcane juice with necessary growth factors and in sugarcane juice with growth factors and essential nitrogen, it is observed that yeast cells store abnormally high levels of insoluble glycogen in cells which are starved of growth factors and nitrogen. Ethanol content on the other hand was found to be highest in cells provided with all the three. This indicates that though glycogen may serve as a substrate under anaerobic conditions for ethanol production, the availability of co-enzymes, co-factors and necessary enzymes for the conversion are equally important.

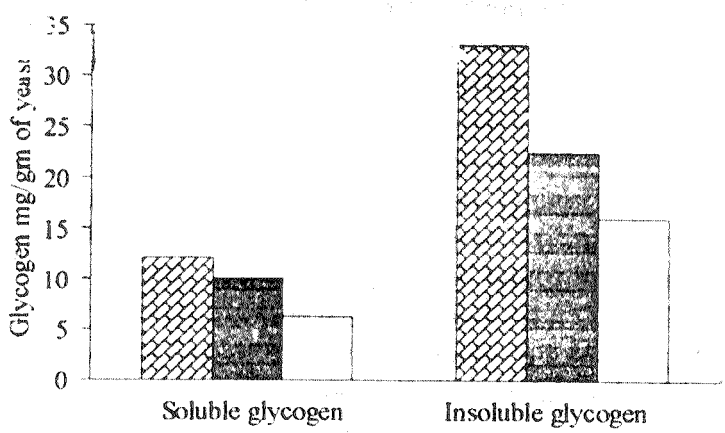

Fig. 5. Variation in the soluble and insoluble glycogen in three medium composition [sugarcane medium (▨), sugarcane and yeast extract (■) and sugarcane, yeast extract and peptone (□)]

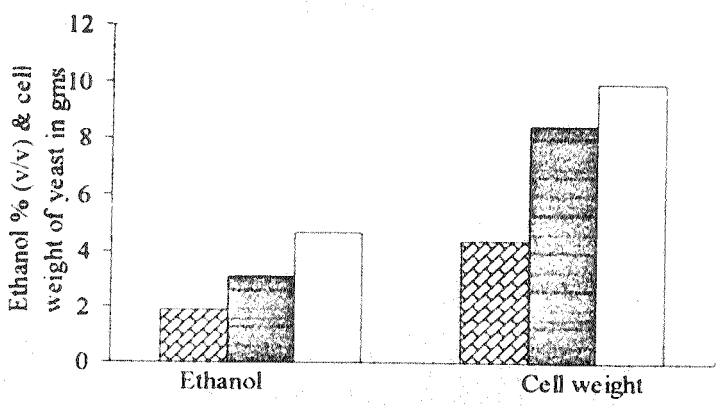

Fig. 6. Variation in ethanol content and cell weight in three medium composition [sugarcane medium (▨), sugarcane and yeast extract (■) and sugarcane, yeast extract and peptone (□)]

Fermentation time

The literature survey reveals that large number of variations are reported in case of glycogen with amounts as low as 1% to as high as 23% of total dry weight[12]. This may be due to the conditions of cells, its nutritional status and lastly time of harvest. In the present study an attempt is being made to find out whether insoluble glycogen levels vary with fermentation time. This may also help to decide what should be the optimum time for harvesting cells, so as to yield high glycogen and thereby optimum ethanol, under the conditions used in this study, namely 10% sucrose, 0.5% peptone and 0.3% yeast extracts.

Yeast cells are unable to utilize the wort sugars for the first 3–6 h after pitching in fresh wort[13]. The data in Table-1 show sugar remaining in the medium and relative increase in wet weight of the cell with fermentation time. It is observed that there is no uptake of sugar for the first 3 h, while over 40% of the sugar is utilized within 12 h and almost the entire sugar is taken up by 36 h of fermentation. The maximal increase in the cell weight is between 12 h and 36 h, a period in which most of the medium sugar is consumed by yeast. The variations in insoluble glycogen and ethanol are depicted in Table-2. The values of this fraction are seen to decrease steeply in the first 3 h of fermentation from 5.2–1.7 mg/g of wet weight; it increases in an almost exponential manner from 12 h, reaches a maximum at 36 h and then slowly declines thereafter. The observed sharp dip in its value in first 3 h is significant. It is to be noted that the oxygen uptake from the medium is over within this period[14]. Yeast can synthesize lipid only in presence of oxygen, which is available in the first 3 h of pitching. It is obvious that it is the insoluble glycogen fraction, which provides energy and carbon skeleton for synthesis of lipids as it is seen that yeast does not utilize media sugar within this period. Optimum insoluble glycogen and ethanol are found at 36 h. Normally in breweries fermentation is carried out up to 48 and 72 h to obtain optimal ethanol. A reduction in fermentation time would scale up ethanol production severalfolds and would be desirable from the point of view of industrial economics.

TABLE-1
CELL WEIGHT AND CONCENTRATION OF SUGAR IN MEDIUM WITH FERMENTATION TIME

Fermentation time (h)	Cell weight	Concentration of sugar in medium
0	0.4	10.0
3	0.4	10.0
6	1.3	9.2
9	2.0	8.8
12	2.6	7.2
24	5.9	2.0
36	10.2	0.5
48	11.0	0.4
72	10.0	0.0

Cells harvested after required time interval. Wet weight of cells g/500 mL of medium. Sugar concentration g/100 mL.

TEBLE-2
VARIATION OF INSOLUBLE GLYCOGEN AND ETHANOL WITH FERMENTATION TIME

Fermentation time (h)	Isoluble glycogen	Ethanol % (v/v)
0	5.20	0
3	1.70	0
6	1.90	0.44
9	2.10	0.92
12	4.00	1.30
24	10.36	2.80
36	16.50	4.70
48	15.60	4.60
60	14.20	4.30
72	12.00	4.00

Insoluble glycogen is calculated in mg glycogen/g yeast (wet weight).

Increasing peptone concentration

Yeast requires nitrogen to meet the needs of protein biosynthesis and nucleic acid biosynthesis. In our earlier observations it is shown that in nitrogen limitations even if glycogen accumulation is more, there is a lower production of ethanol. The nitrogen source is required for the synthesis of enzyme system that is required for production of ethanol. By considering all these observations we made further experiments using different peptone concentrations and keeping all other parameters constant. Fig. 7 shows the observed variations of soluble, insoluble glycogen and Fig. 8 shows variations found for ethanol and cell weight with increasing peptone concentration. The cells which were grown in 0.5% peptone concentration show maximum deposition of insoluble glycogen but ethanol content is less while in 2% peptone concentration even if glycogen deposition is less, ethanol concentration found is more. This proves that availability of nitrogen promotes cell growth and may be providing essential nutrients required for ethanol production.

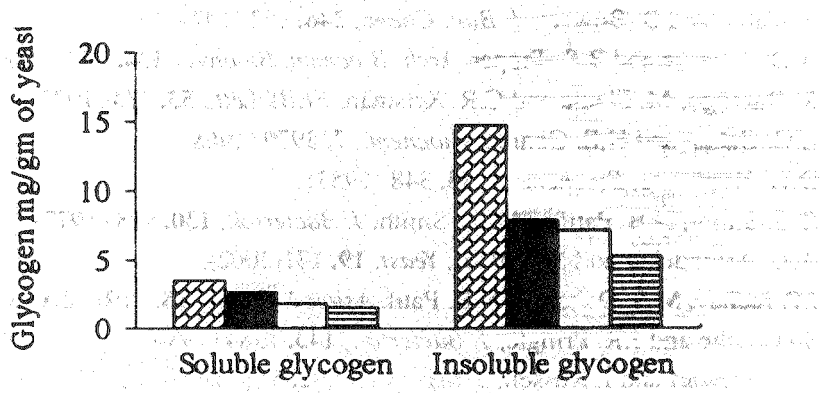

Fig. 7. Variation in soluble and insoluble glycogen with increasing peptone concentration [0.5% peptone (▨), 1% peptone (■), 1.5% peptone (□) and 2% peptone (▭)]

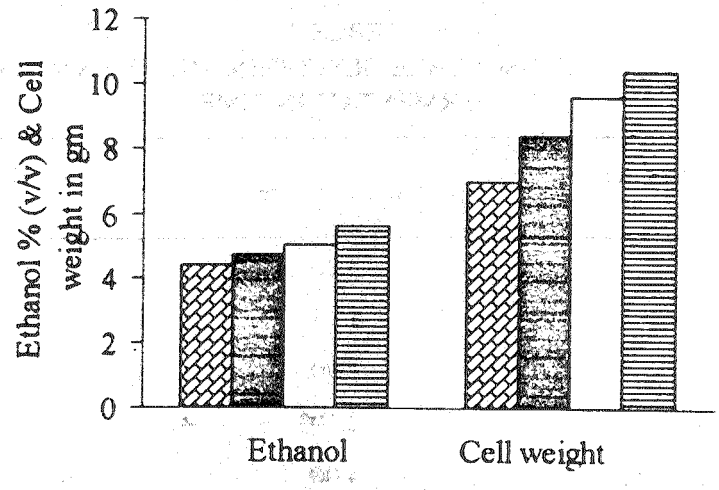

Fig. 8. Variation in ethanol content and cell weight with increasing peptone concentration [0.5% Peptone (▨), 1% Peptone (■), 1.5% Peptone (□) and 2% Peptone (▭)]

Conclusion

Saccharomyces carlsbergenesis maintains two pools of glycogen, one soluble and the other insoluble linked to cell wall. The storage of this fraction depends on various factors like type of sugar used, conditions of nitrogen limitations, ethanol concentration and fermentation time, etc. The increase in the level of insolubie glycogen occurs when the cells are subjected to nutritional stress or increased ethanol concentration, which may lead to osmostress, is a natural response of the cells as a means of protective measures to store energy which reserve to be used in crisis. Not only its use would be to combat stress, but under normal situations, it plays the vital role of providing energy and carbon skeleton for lipid synthesis, when cells are pitched in fresh medium and are unable to utilize wort sugars when oxygen supply is available and lipid synthesis is essential for its propagation.

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