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Optimization of culture conditions for baker's yeast cell mass production – a preliminary study

K. Inparuban*, S. Vasantharuba*, S. Balakumar** and V. Arasaratnam**@

*Department of Agricultural Chemistry, Faculty of Agriculture, University of Jaffna, Sri Lanka.
**Department of Biochemistry, Faculty of Medicine, University of Jaffna, Sri Lanka.

Abstract

The aim of this study was to find out suitable culture conditions to improve the cell mass production of Saccharomyces cerevisiae. S. cerevisiae was grown in a medium (30°C, pH 5.0) with sucrose (Table sugar) from 10 to 50gL^{-1}, along with (gL^{-1}) yeast extract (2.5), bacteriological peptone (1.15), $(NH_4)HPO_4$ (0.25) and $MgSO_4.7H_2O$ (0.025). Highest cell mass of 4.43gL^{-1} was obtained in the medium with 50gL^{-1} of sucrose. When the oxygen supply and diffusion were improved by either, mixing in a shaker (100rpm), by an impeller (100rpm) or aeration (100bubbles/min), highest cell mass (4.52gL^{-1}) was obtained with aeration. Increase in aeration rate to 200bubbles/min increased the yeast cell mass to 5.41gL^{-1}. For volumetric scaling up, medium to flask volume ratio was maintained as 1:2, in 1, 2, 3, and 5L flasks, and the highest cell mass (5.53gL^{-1}) was produced in 2L flask. By optimizing the culture conditions the yeast cell mass production was increased by 1.25 times.

Key words: *Baker's yeast, Cell mass production, Aeration, Reactor volume ratio*

INTRODUCTION

Yeast constitutes an interesting group from a technical and industrial standpoint in the microbial world. Strains of *Saccharomyces cerevisiae*, among known genera and species of yeast, is used commercially for baking, alcohol beverage production, food stuffs, animal feeds and

@Corresponding Author
e-mail: arva26arva@yahoo.com

the production of biochemicals [1]. The *Saccharomyces cerevisiae* biomass, mainly in the form of baker's yeast, represents the largest bulk production of any single-cell microorganism in the world. Several million tons of fresh baker's yeast cells are produced for human food use [2]. The most important requisites in the commercial production of baker's yeast are rapid growth and high biomass yield, coupled with good dough-leavening property. These are achieved by the use of a well-established fed-batch fermentation method, with sequential stages differing in fermenter size and in the aeration and feeding conditions [3].

When considering the culturing of baker's yeast there are several culture conditions such as concentrations of sugar and nutrients in the medium, oxygen supply, pH, temperature, reactor volume etc. that determine the final outcome. This study was carried out in order to determine the possibility of baker's yeast cell mass production in Jaffna. Jaffna is situated in the north of the Island of Sri Lanka. The demand for baked items among the consumers is noticeably high in Jaffna. But the baker's yeast required for the fermentation procedure in baking is totally imported into the country at a cost. In addition, the need in Jaffna for baker's yeast is met only by transporting it into Jaffna from the Southern part of the country. Due to the conflict circumstances prevailing in the country the availability of baker's yeast is rare in this region. At times the bakers face critical situations due to unavailability. Thus it becomes essential to focus on the possible ways that bakers could follow to produce the needed baker's yeast easily and locally.

MATERIALS AND METHODS

Materials

The materials used in the experiments were purchased from standard sources. Yeast extract and peptone were purchased from Oxoid, U.K. Table sugar was purchased in the local market.

Microorganism

The baker's yeast was the commercial strain of *Saccharomyces cerevisiae* of Fermipan from Gist-Brocades, Waterings-1, Delft-Holla, The Netherlands.

Analytical methods

Reducing sugar [4], total sugar [5], ethanol [6], yeast growth (measuring OD at 610nm) and dry weight (gravimetric method) of the yeast cells were measured using standard methods. The commercial table sugar was analyzed for total sugar concentration and the amounts taken were equivalent to $50gL^{-1}$ sucrose.

Cultivation of baker's yeast

Activation of yeast cells

The pre-cultivation of the *Saccharomyces cerevisiae* cells was done in 20mL of sterile sucrose solution ($50gL^{-1}$) by inoculating yeast grains (1g) and incubating in a reciprocal shaker water bath at 100rpm for 18 hours at 30°C.

Growth of yeast cells in fermentation medium

The basic growth medium contained (gL^{-1}); sucrose, 50; yeast extract, 2.5; bacteriological peptone, 1.15; $(NH_4)_2HPO_4$, 0.25 and $MgSO_4.7H_2O$, 0.025 at pH 5.0. To the fermentation medium (250mL), 10mL of the activated yeast cells were inoculated and incubated at 30°C on a reciprocal shaker (at 100rpm). Samples were analyzed for yeast cell mass dry weight, ethanol production and the residual sugar.

Effect of different concentrations of sucrose

Yeast extract, Peptone and Sucrose (YPS) medium (250mL) was prepared in 1000mL conical flasks, with varying concentrations of sucrose, (10, 20, 30, 40 and $50gL^{-1}$) keeping the amount of the other constituents constant and the experiment was carried out as described above.

Effect of different types of mixing

The YPS medium (250mL) with the sugar concentration which recorded the highest cell mass production was incubated either in a reciprocal shaker (100rpm), or by mixing, using a four - bladed impeller (at 100rpm) or by aeration (100 bubbles/min).

Effect of different aeration rates

The YPS medium (250mL) with the optimum sugar concentration in 1000mL conical flasks and inoculated with activated yeast were subjected to different aeration rates (100, 150 and 200bubbles/min) to select the optimum condition.

Effect of medium volume to reactor volume ratios

The YPS medium with the optimum sugar concentration was taken in 1, 2, 3 and 5L conical flasks inoculated with activated yeast cells and incubated under optimized conditions while maintaining the medium to reactor volume as 1:2.

RESULTS AND DISCUSSION

Effect of different concentrations of sucrose on yeast cell mass production

Baker's yeast can be grown in glucose, fructose, maltose or sucrose as the principal carbon source whereas nitrogen and other minor nutrient requirements are satisfied by inorganic salts [7]. Since the aim of the study was to scale up the baker's yeast production to commercial level, table sugar available in the local market was selected as the carbon source. When the yeast growth medium contained different amounts of sucrose, with increasing sugar concentrations, the cell mass production also increased and the highest cell mass was obtained at 30 hours of incubation in all media and further increase in incubation time reduced the cell mass (Figure 1). Comparatively, the highest yield (8.86%) of cell mass was obtained in the medium with $50gL^{-1}$ sucrose. In the medium with 40 and $50gL^{-1}$ of sugars almost the same amount of alcohol was produced (Figure 2).

Sugar can be metabolized via two different energy producing pathways, fermentation or oxidation, depending on the sugar concentration in the medium [8]. The sugar limitation is utilized to avoid extensive overflow metabolism, which otherwise would result in too high ethanol production and an accompanying reduction of biomass yield [9]. Di Serio *et al.*, (2001) stated that, at a high sugar concentration, oxidation is suppressed and only fermentation takes place (Crabtree effect). Oxidation predominates when sugar is below 50-100 $mgdm^{-3}$. At a low sugar concentration, ethanol is produced, too (Pasteur Effect) [8]. In an investigation done by Olson and Johnson using Glucose ($10gL^{-1}$), NH_4H_2PO4 ($6gL^{-1}$), KH_2PO_4 ($0.2gL^{-1}$) and $MgSO_4.7H_2O$ a baker's yeast cell mass yield of 23g per 100 glucose was obtained [10]. No residual sugar was found in all the media at 30 hours (Figure 2). Hence, the absence of sugar in the media could have been the reason for the cell mass reduction from this point onwards. Since the sucrose was also used for ethanol production (Figure 2), the medium might not be getting sufficient oxygen for the aerobic oxidation of the carbon source. Thus it is important to supply sufficient oxygen to the medium.

Effect of different types of mixing of the medium

In order to determine the best agitation type for the media for yeast growth, this experiment was carried out by three ways viz on a reciprocal shaker (GALLENKAMP), agitated with an impeller (IKA® dual speed mixer) and aeration (Airmec – Air compressor). In the aerated medium (100bubbles/min) highest biomass of $4.52(\pm 0.02)gL^{-1}$ was obtained where as the medium incubated on a reciprocal shaker and with mixing $4.16(\pm 0.225)$ and $4.04(\pm 0.225)gL^{-1}$ of dry cell mass were obtained respectively at 30 hours (Figure 3). Thus, the cell mass yield of the medium incubated on a shaker, with mixing and aeration were 8.32, 8.08 and 11.04% (g dry weight of cell mass/ g sugar utilized × 100) respectively. The yield of ethanol production (g alcohol produced/g glucose utilized × 100) in the medium which was

incubated on a shaker, with mixing and aeration were 10.40, 12.13 and 8.74% respectively, whereas, the efficiency of the alcohol production (g alcohol produced/ theoretical maximum × 100) was 19.34, 22.5 and 16.25% respectively. The initial reducing sugar concentration in all media was maintained as 50.0(± 0.030) gL^{-1}. During incubation, the amount of residual sugar in all media declined and reached zero at 30 hours. The substrate uptake was 100%. Thus aeration was selected as a suitable condition, and further studies were conducted to evaluate the best aeration rate for yeast growth.

In the aeration setup, the only agitation of the fermenter liquid is carried out by the action of the air bubbles, which has no detrimental effects on yeast cells. For small scale culture, e.g. less than 1L, it is possible to supply adequate amounts of oxygen by growing the microbe in an Erlenmeyer flask which is agitated constantly [11]. Ejiofer *et al.*,(1996) conducted a study in which *S. cerevisiae* production was tested on hydrolyzed cassava starch and here the yeast inoculum flasks were incubated on an orbital shaker at 30°C and 150rpm for 22 hours [12] and Sweere *et al.* (1987) carried out regime analysis for yeast cultivation with a $120m^{-3}$ bubble column reactor [13].

In this experiment, in mechanical mixing using impeller, the reduction in the cell mass yield may be due to the action of the rotating blade. However, agitation facilitates the transfer of oxygen from the gas phase in the flask to the liquid phase. Provided that the volume of growth medium does not exceed 10 – 20% of the flask volume it is possible to achieve cell densities of 1 – 2g dry weight per liter before oxygen becomes limiting. Reciprocal shaking of the medium was comparatively efficient than mixing. However for large scale fermentation process it is possible to combine aeration with agitation using impeller. Since with increasing aeration rate the cell mass production increased, from the subsequent experiment the best aeration rate was determined.

Effect of different aeration rates

For large scale culture and/or high cell densities, the oxygen demand of the culture can be met only by aeration [7]. This present study was intended on investigating the influence of aeration on yeast growth. When the media were aerated at different aeration rates (100, 150 and 200bubbles/min) it gave a positive effect on cell mass production (Figure 5). The maximum cell mass (dry weight) productions obtained were 4.01(±0.078), 5.14(±0.103) and 5.41(±0.078) gL^{-1} respectively with cell mass yields of 8.02, 10.28 and 10.82% respectively (Figure 5). The results indicated that increased aeration had a positive effect on yeast cell mass production. To maximize yeast yields, it is important to supply enough oxygen to keep the dissolved oxygen content in the medium for yeast cells at an optimal level [14]. Increasing aeration rates showed an affirmative influence on yeast culture. The ethanol production in the medium decreased with increasing aeration rates (Figure 6). The media

contained an initial sugar concentration of 50 (±0.836) gL^{-1}. The residual sugar decreased with the fermentation period and it ranged between 1.0 - 5.0gL^{-1} after 24 hours (Figure 6).

In an experiment to evaluate the effect of aeration rate on yeast cell mass production Fadel and Foda (2001) placed yeast cultures on rotary shakers adjusted at 50, 100 and 200rpm during the fermentation period. In this case promising results were obtained using high level aeration (200rpm) and it is stated that the cell counts were markedly increased (12%) and at the same time the alcohol yields were decreased [15]. A rapidly growing culture has a very high demand for dissolved oxygen. In practice, this is achieved by blowing air through the culture. The gas must be dissolved in the growth medium so that it can interact with the membrane-bound electron transport system to affect the oxidation of reduced pyridine nucleotide cofactors [7]. As the prime objective of this research work was to produce high cell mass yields, aeration rate of 200bubbles/min was selected as a suitable condition.

Effect of medium volume to reactor volume ratios on yeast cell mass production

Primrose states that with scale-up many of the physical and chemical parameters which influence the behavior of a microorganism are changed as the scale of operation is changed. Therefore, the scale-up criterion should be regulated at a proper degree without causing detrimental effects to the culture maintained [11]. This experiment was carried out with the aim of finding out a suitable media to reactor volume ratio for yeast growth. Reactors with different volumes were utilized to maintain the yeast growth medium. The cell mass production showed a varying pattern (Figure 7). Here, 1L medium in 2L flask recorded the highest cell mass dry weight of 5.53(±0.02) g and the least alcohol production compared to the others (Figure 8). All the media initially contained 50(±0.384) gL^{-1} of sugars, which declined to zero after 28 hours. The sugar consumption was 100%. Since medium (1L) maintained in a 2L flask gave the highest cell mass production, on laboratory scale this medium to reactor ratio was selected as an optimized condition. As the results indicate, the reactor volume plays a significant role in the cell mass production.

CONCLUSION

Baker's yeast growth was evaluated under different conditions, and the optimum was selected for further studies. Different sucrose concentrations were substituted in the medium and tested for yeast growth and 50gL^{-1} of sucrose concentration was found to be the best concentration with comparatively high cell mass. It was evident that aeration was more suitable for yeast growth than shaking and mixing, which led for more cell mass yield. Among the aeration rates tested, 200bubbles/min increased the cell mass production compared to others. Medium (1L) in 2L flask was indeed more suitable for yeast growth and it was

selected for further studies. Hence it could be concluded that, at laboratory scale the proposed medium could give higher baker's yeast cell mass with $50gL^{-1}$ of sucrose concentration along with aeration of 200bubbles/min. In addition a medium to reactor volume ratio of 1:2 was also found out to be efficient.

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The financial support and assistance of the University of Jaffna is greatly acknowledged.

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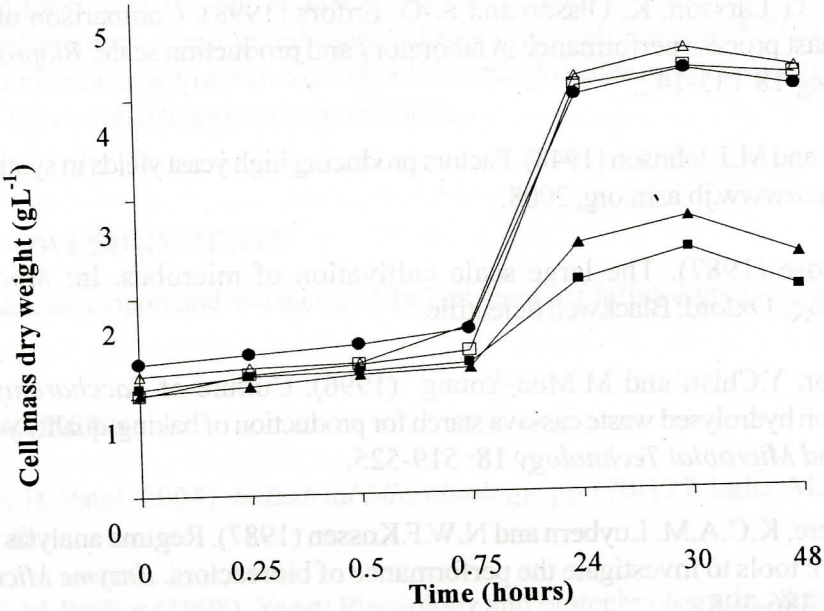


Figure 1: Growth of *S. cerevisiae* in fermentation medium containing different concentrations of sucrose (—■—), 10; (—▲—), 20; (—●—), 30; (—□—), 40 and (—△—), 50gL^{-1} at 30°C and pH 5.0.

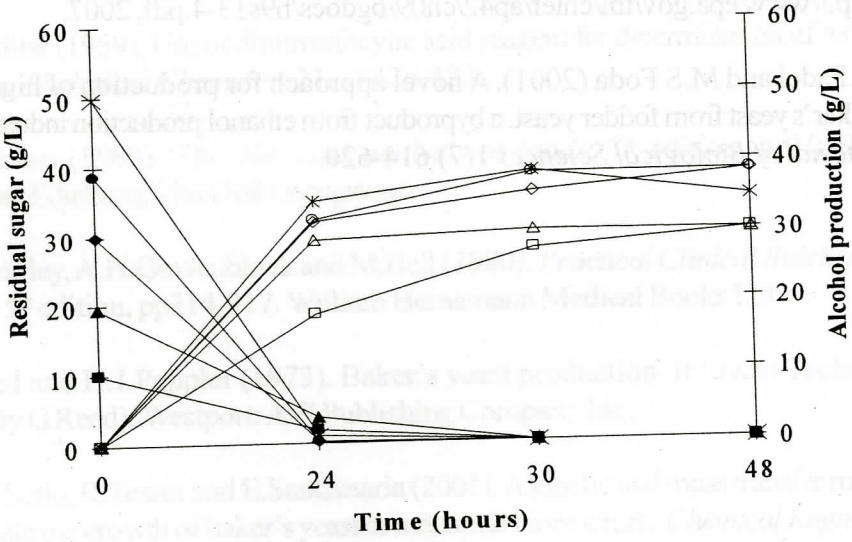
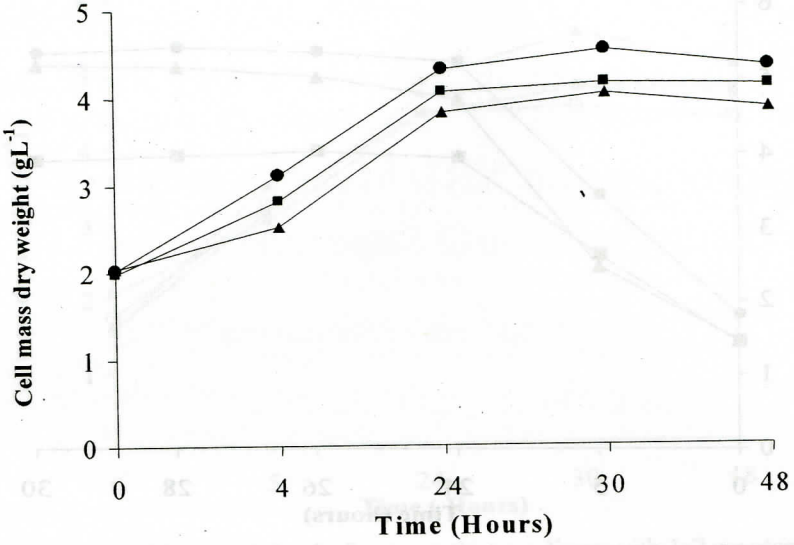


Figure 2: Residual sugar concentration (Filled symbols) and alcohol production (open symbols) in the fermentation medium with different sucrose concentrations of (—■—), 10; (—▲—), 20; (—◆—), 30; (—●—), 40 and (—✳—), 50gL^{-1} at 30°C and pH 5.0.



Figurer 3: Growth of *S. cerevisiae* under different types of agitation such as, (—■—), reciprocal shaker at 100rpm; (—▲—), mixing at 100rpm and (—●—), aeration at 100bubbles/ min at 30°C and pH 5.0.

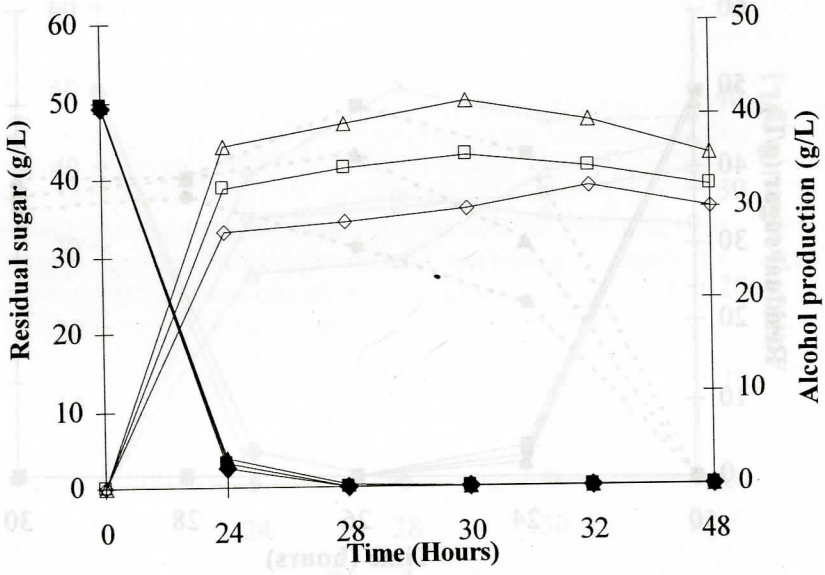
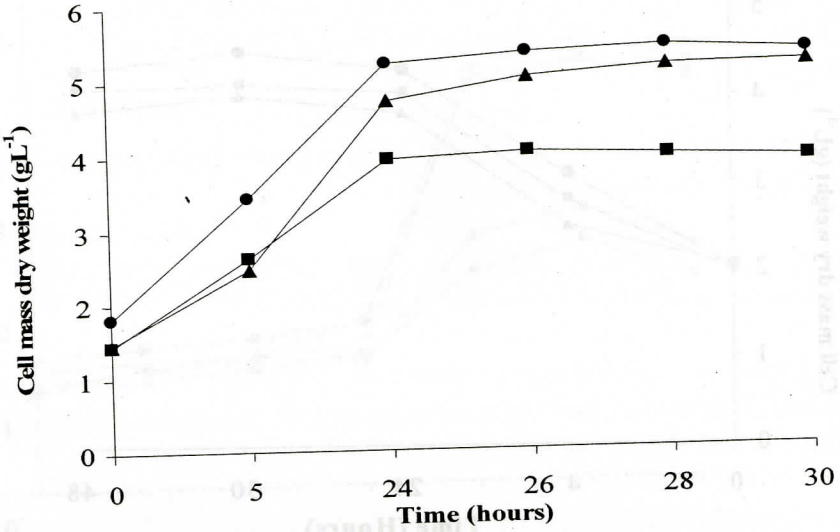


Figure 4: Residual sugar concentration (Filled symbols) and alcohol production (Open symbols) in fermentation medium under different types of agitation such as, (—■—), reciprocal shaker at 100rpm; (—▲—) mixing at 100rpm and (—●—), aeration at 100bubbles/min at 30° C and pH 5.0.



Figurer 5: Growth of *S. cerevisiae* under different rates of aeration such as, (—■—), 100bubbles/min; (—▲—), 150bubbles/min and (—●—), 200bubbles/min at 30°C and pH 5.0.

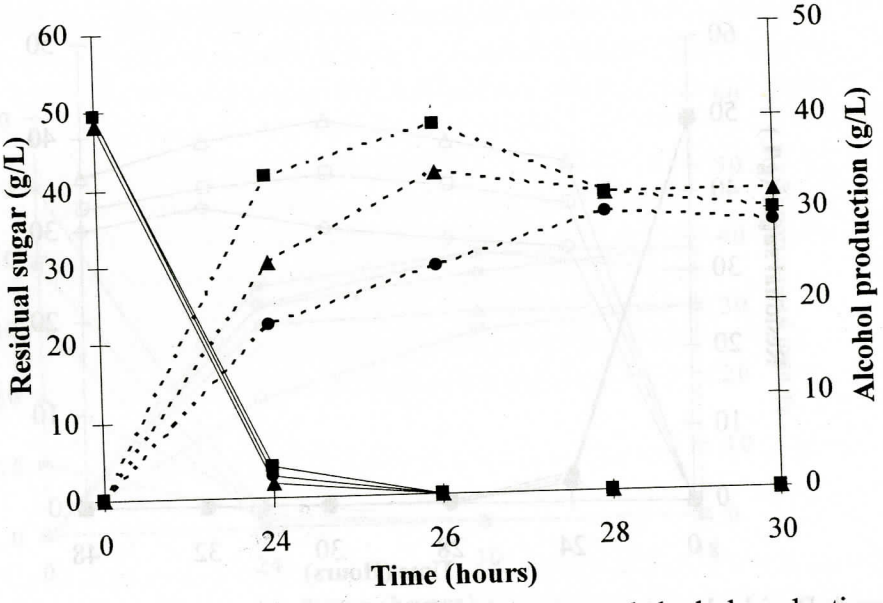


Figure 6: Residual sugar concentration (Filled symbols) and alcohol production (Open symbols) in fermentation medium under different rates of aeration such as, (—■—), 100bubbles/min; (—▲—), 150bubbles/min and (—●—), 200bubbles/min at 30°C and at pH 5.0.

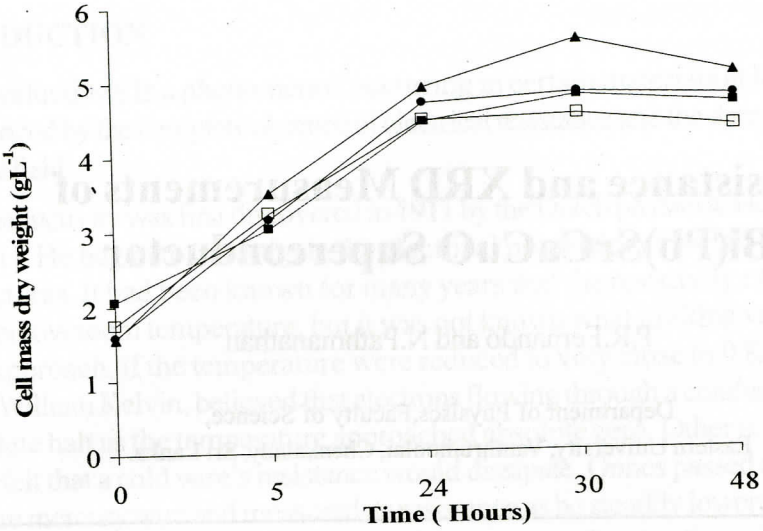


Figure 7: Growth of *S. cerevisiae* in fermentation medium with 1:2 reactor volume ratio in (—■—),1; (—▲—), 2; (—●—), 3 and (—⊟—), 5L flasks at 30°C and pH 5.0.

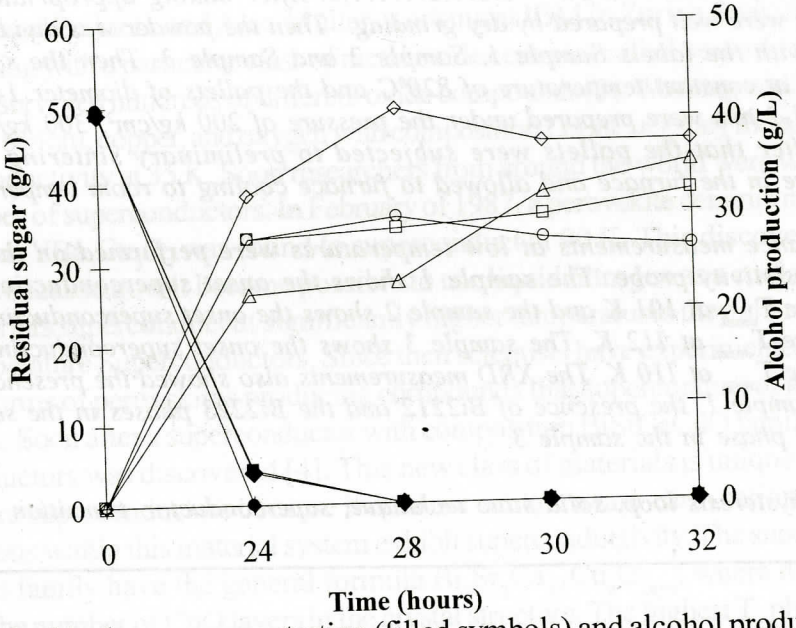


Figure 8: Residual sugar concentration (filled symbols) and alcohol production (Open symbols) in fermentation medium with 1:2 reactor volume ratio in (—■—),1; (—▲—), 2; (—●—), 3 and (—◆—), 5L flasks at 30°C and at pH 5.0