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Observation of Crabtree Effect and Diauxic Behaviour of Yeast by Using Absorption

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The aim of this study is to observe the Crabtree effect and the diauxic behaviour by using absorption. Early, Crabtree effect and diauxic behaviour have been observed either by ethanol concentration measuring or by biomass determination. Measuring ethanol concentration costs money while determining biomass costs time and suffers from low accuracy. In addition to that, diauxic behaviour can not be observed clearly by biomass curve when yeast is cultivated in low glucose concentration medium. Contrary, absorption can be used to observe Crabtree effect and diauxic behaviour because there is noticeable change in absorption value of cultivation in low glucose concentration medium within minutes. The results indicated that cultivating *Saccaromyces cerevisiea* in glucose limiting substrate within batch mode process has diauxic behaviour for high glucose concentration. This Crabtree effect can be avoided by reducing glucose concentration below 50mg/L. Above this concentration ethanol will be produced and *Saccaromyces cerevisiea* will have diauxic behaviour. Using absorption caused a reduction in the cost as there is no need to use expensive enzymatic assay for ethanol determination.

1. Introduction

Crabtree effect describes the phenomenon whereby the respiratory growth of some kinds of yeast e.g. *Saccharomyces cerevisiea,* is inhibited or repressed. The Crabtree effect is not noticeable in glucose-insensitive yeast (e.g. *candida utilis, kluyveromyces marximianus, Trichosporon cutaneum*) or in respiratory-deficient mutants (e.g. *S.cerevisiea* 'petites').Utilis, a Crabtree-negative yeast, may limit its glycolytic rate by accumulating intercellular reserve carbohydrates or the cells may exhibit altered regulation of sugar uptake (Hans Esslinger, 2009, Postama, 1989, Walker, 1998). At high glucose concentrations, *Saccharomyces cerevisiea* consumes first the glucose and then starts consuming the by-product ethanol when the glucose is depleted. That means, yeast has two different growth curves for these two substrates, which is named diauxic behaviour. Also diauxic growth was observed when bacteria are cultured in media containing more than one carbon source. Crabtree effect will happen under fully aerobic conditions and in the presence of sugars as a carbon source. Under aerobic conditions,

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yeast mitochondria are involved in ATP synthesis coupled to oxidative phosphorylation. The activities of the citric acid cycle and the respiratory chain will largely depend on the yeast species and the expression of the Crabtree effect. This is a phenomenon related that relates glucose concentrations with the particular catabolic pathway adopted by glucose-sensitive cells, in that even in the presence of oxygen fermentation predominates over respiration. Catabolism inhibition may result from the transport of a particular sugar into the cell which causes inhibition of the other sugar transport systems (Smolke, 2010; Cappuyns et al, 2009). At high glucose concentrations, *Saccharomyces cerevisiea* produces ethanol aerobically rather than producing biomass via the tricarboxylic acid. Increasing concentrations of glucose accelerates glycolysis, the breakdown of glucose, which results in the production of appreciable amounts of ATP through substrate-level phosphorylation. This reduces the need of oxidative phosphorylation done by the TCA cycle via the electron transport chain and therefore decreases oxygen consumption. In *S.cerevisiea* glucose suppression of respiration in the Crabtree effect is thought to be due to glucose repressing respiratory enzymes synthesis and/or inactivating respiratory enzymes and sugar transport activity (van den Brink, 2009). Many attempts have been done to observe these phenomena. Crabtree effect was indicated by measuring a by-product ethanol concentration through yeast growth. The diauxic growth was shown by plotting biomass against time *as Saccaromyces cerevisiea* will have two different specific growth rates (μ). Therefore, *Saccaromyces cerevisiea* will change from fast grow on glucose to slower growth on ethanol. The second growth will start when the glucose is depleted.
The aim of this study is to use absorption to observe diauxic growth and Crabtree effect.

2. Material and Methods

2.1Growth medium and cultivation

A 1L sterile bioreactor (New Brunswick) was used in cultivation of *Saccaromyces cerevisiae* (DSM No 70451), which was obtained from German bank cells. Operating conditions for cultivation were temperature of 30 ^{0}C, stirring speed of 750 rpm, pH of 5.5 and air flow rate of 1.5 L/min.L while the working volume was 750mL. Complex medium was used as growth medium by dissolving the following constituents {2.5g $(NH_4)2SO_4$, 0.5g KH_2PO_4, 0.2 g $MgSO_4.7H_2O$, 0.02 g $CaCL_2.6H_2O$, 4 mg $FeSO_4.6H_2O$, 2 mg $ZnSO_4.7H_2O$, 0.3 mg $CuSO_4.5H_2O$, 1 mg Pyridoxine, 4.4 mg Thiamine, 0.5 mg Calcium pantothenate, 20 mg myo-Inositol, 0.03 mg D-Biotin, 0.75 g L-Glutamine} in 1L bidest water. Different glucose concentrations were prepared from this medium. Cellulose acetate filter of (0.2μm) within a safety cabinet was used to sterile the medium. Operating conditions were held constant within bioreactor. Automatic control was executed to regulate pH and temperature. Heat exchanger loop and heat exchanger jacket were used to hold temperature constant. Heat exchanger loop supplied cooling water inside fermenter while heating was produced within jacket surrounding the fermenter. PH was adjusted automatically by using 1M NaOH base and 8.5 % phosphoric acid. A pre-culture of 30 mL adapted *Saccharomyces cerevisiae* on complex medium was used to inoculate the fermenter. Adaptation was achieved by cultivation *Saccharomyces cerevisiae* on complex medium each 24 h for three days. This

cultivation was done in Erlenmeyer flask within incubator at temperature of 30 ^{0}C and shaking speed of 120 rpm.
Samples (6mL) from growth culture were withdrawn and the absorptions of samples were measured within spectrophotometer (Specord 205. Analytic Jena). Growth curve of *Saccharomyces cerevisiae* was plotted by drawing absorption against process time.

3. Results and discussion

Different glucose concentrations (50 mg/L - 7g/L) were used in these experiments. Absorptions were measured to be used as indicator for observing diauxic behaviour and in order to find the minimum glucose concentration at which there is no Crabtree effect or diauxic behaviour.
By cultivation on glucose concentration of 50mg/L, the absorption was increased exponentially from 0.2 to 0.26 within 4 h. The absorption remains constant at this value till process time of 6 h. Then the absorption value started to decrease. That means *Saccaromyces cerevisiea* entered in stationary phase and starting consuming storage carbohydrate or lipids as the glucose was depleted and there is no by-product of ethanol. The results show that increasing glucose concentration from 50 mg to 100 mg per L gave a growth curve of diauxic behaviour. First growth was exponential with increasing value of absorption from 0.22 to 0.34 during the 4 h process time. Then the rate of growth was slower but exponential too. The absorption value increased from 0.34 to 0.37 till process time of 6 hours as shown in Figure 1. This second growth rate referred to that *Saccaromyces cerevisiea* starting consuming a second substrate, ethanol, after glucose depletion as the ethanol is only by-product during *Saccaromyces cerevisiea* cultivation. This hypothesis meets with others like [(Sonnleitner et al, 1986, Walker, 1998)]. They emphasized that Crabtree effect is due to a saturation of limited respiratory capacity of yeast cells.

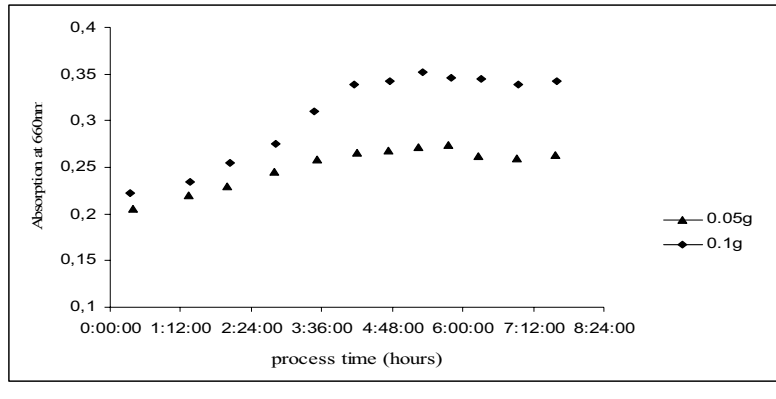

Figure 1: Cultivation of Saccharomyces cerevisiae in complex medium of 0.05 g/L and 0.1 g/L glucose concentrations at temperature of 30 ^{0}C, stirring speed of 750 rpm, pH of 5.5 and air flow rate of 1.5 L/min.

Thus, glucose-sensitive (Crabtree-positive) yeast like *S.cerevisiea* may process a limited oxidative capacity when grown on glucose which leads to an overflow reaction at pyruvate. When the respiratory capacity is saturated, ethanol is formed. *Saccaromyces cerevisiea* began to consume ethanol after synthesising the necessary enzymes. Also these results showed the usefulness of using absorption for observing Crabtree effect and diauxic behaviour. Using biomass doesn't help for such low glucose concentration (100 mg/L) as the biomass for increasing in absorption value from 0.34 to 0.37 will have the same value. Therefore plotting biomass values against process time will give one growth curve.

Yeast extract was added to see its effect on diauxic growth observation by absorption. The results indicated that diauxic behaviour can not be observed clearly by absorption if yeast extract is used as shown in Figure 2. Yeast extract accelerated growth of *Saccharomyces cerevisiae*. Therefore, the transition stage for *Saccharomyces cerevisiae* to change from glucose to ethanol consumption, stage of synthesising of necessary enzymes, was shortened as shown in Figure 2.

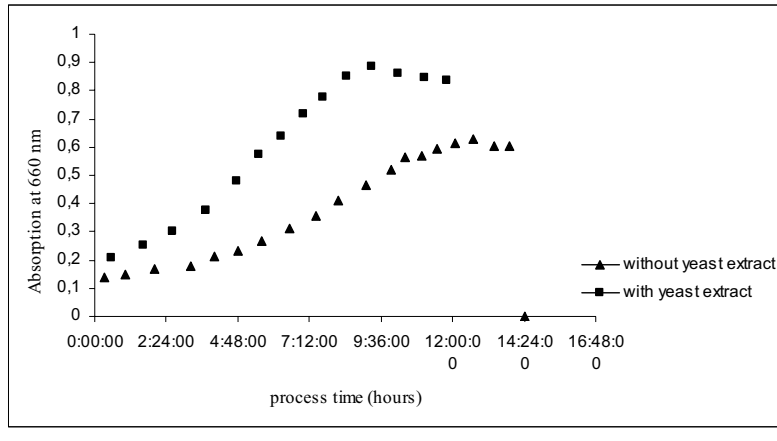

Figure 2: Effect of adding yeast extract in concentration of 0.25 g/L on cultivation of Saccharomyces cerevisiae in complex medium of 0.5 g/L glucose concentrations at temperature of 30 °C, stirring speed of 750 rpm, pH of 5.5 and air flow rate of 1.5 L/min.

Figures 3 and 4 showed the diauxic behaviour clearly for high glucose concentration. The second growth curve started at absorption values of (0.37, 0.56, 2.02, and 3.24) for the cultivation on glucose concentrations of (0.35, 0.5, 4 and 7 g/ L).

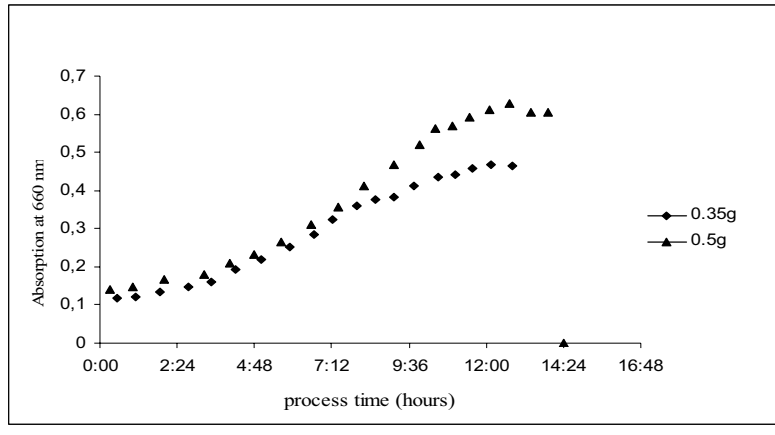

Figure 3: Cultivation of Saccharomyces cerevisiae in complex medium of 0.35 g/L and 0.5 g/L glucose concentrations at temperature of 30 °C, stirring speed of 750 rpm, pH of 5.5 and air flow rate of 1.5 L/min.

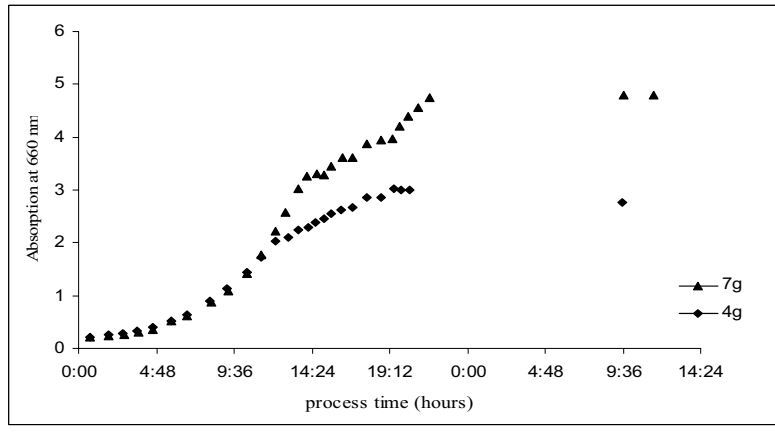

Figure 4: Cultivation of Saccharomyces cerevisiae in complex medium of 4 g/L and 7 g/L glucose concentrations at temperature of 30 °C, stirring speed of 750 rpm, pH of 5.5 and air flow rate of 1.5 L/min

4. Conclusion

Observations of diauxic growth and Crabtree effect by absorption during growth of Saccharomyces cerevisiae have many advantages over other methods. Measuring ethanol concentrations within long process time costs as much money. Biomass determination doesn't help in diauxic growth observation for low glucose concentration medium. Both ethanol measurement and biomass determination suffer from accuracy and difficulties as samples have to be withdrawn, centrifuged and diluted (if necessary). In contrary, there is no need for centrifugation step in measuring of absorption. However, most of ethanol measurements are executed by measuring the increase of absorption during enzymatic reaction of producing NADH. Therefore, it is better to use absorption value of sample in observing diauxic growth instead of using costly ethanol assay, which is executed by absorption too. The results showed the diauxic behaviour through measuring of absorption. Diauxic behaviour is an indicator of Crabtree effect. Diauxic behaviour was shown clearly for glucose concentration above 50mg/L. These results satisfy with other published results, which have used biomass, glucose and ethanol determination [(Sonnleitner et al, 1986, Kaspar von Meyenburg, 1969)]. Using absorption will reduce the cost of experiments. For instance, at least 15 samples have to be analysed during a 15 h of process time experiment. Making triple samples for accuracy will lead to analyze 45 samples for ethanol determination per experiment. This is really costly method as ethanol measurement is an expensive enzymatic assay.

Acknowledgements

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References

Cappuyns A. and Bernaert K., 2009, A dynamic Model for Diauxic Growth, Overflow Metabolism. Biotechnology and Bioengineering. 102, 280-283.

Smolke D., 2010, The Metabolic Pathway Engineering Handbook: Fundamentals. 1-16. CRC Press, Taylor& Francis Group, LLC, USA.

Hans E., 2009, Metabolic pathways during propagation and Fermentation. In Handbook of Brewing. 131 - 135.

van den Brink J., 2009, Energetic limits to metabolic flexibility: responses of Saccharomyces cerevisiae to glucose–galactose transitions. Microbiology. 155: 1340–1350

von Meyenburg K., 1969, Katabolit-Repression und der Sprossungszyklus von *Saccharomyces cerevisiae*. Dissertation. No.4279. Edig.Techn. Hochschule. Zürich

Postama E., 1989, Enzymic Analysis of the Crabtree Effect in Glucose-Limited Chemostat Cultures of *Saccharomyces cerevisiae*. Applied and Environmental Microbiology. 468-477.

Sonnleitner B. and Kappeli O., 1986, Growth of *Saccharomyces cerevisiae* is controlled by its limited respiratory capacity: formulation and verification of a hypothesis. Biotechnol. Bioeng. 28, 927-937.

Walker G., 1998, Yeast physiology and biotechnology. John Wiley & Sons. UK.