1. Starch quantification using anthrone reagent protocol

0.5 g of each sample was homogenized in hot 80% ethanol to remove sugars. Residue was precipitated by centrifugation then the residue was washed repeatedly with hot 80% ethanol until the washings did not give a colour with anthrone reagent. This residue was dried over a water bath then 2.5 ml of water and 2.5 ml of 60% perchloric acid was added to dried residues. These tubes were kept at 0 °C for 20 min for extraction of starch. Supernatant was separated by centrifugation and stored. The extraction was repeated using fresh perchloric acid and supernatant was separated by centrifugation. From the supernatant; 1 ml of extract was pipetted and 4 ml of anthrone reagent was added to each tubes. Afterwards, the test tubes were heated in a boiling water bath for 10 min then cooled to room temperature, and the absorbance at 630 nm was measured using a spectrophotometer. Glucose was used as standard for calibration graph to determine glucose concentration in samples. Starch content was calculated by multiplying the value by a factor 0.9.

![Figure 1: Standard graph for quantification of starch using anthrone reagent](image-url)
2. Fermentation ability at high temperature

Figure 2: Fermentation ability at high temperature indicated by presence of CO$_2$ in inverted Durham’s tube of yeast strains in fermentation medium at high temperature

Note: I- NCIM 3095, II- NCIM 3570, III- NCIM 3059

Explanation: Fermentation ability of yeast strains at high temperature was inspected for presence of CO$_2$ in inverted Durham’s tube in fermentation medium at various temperatures. All three yeast strain shown fermentation ability up to 40°C beyond that temperature, fermentation ability was lost. It was observed that at 45°C there was absent of CO$_2$ in Durham’s tubes. Hence, it can be concluded all three strains lost fermentation at 45°C.
3. Enzymatic hydrolysis

**Condition for enzymatic hydrolysis**

**Enzymes:**

1. Diastase 2 IU/gm at 50°C up to 24 h, 
2. Amyloglycosidase 5 IU/gm at 60°C after 24 h to 48 h.

pH: 5

Substrate load: 5% w/v

**Explanation:** it was found that sugar release increased after addition of 2 IU/gm Diastase still 21^{th} h. Further, there were no increased in sugar release. At 24^{th} h sugar release was increase due to amyloglucosidase action. The maximum sugar release (11.37 gm/l) was attended at 42^{nd} h from 50 gm/l of biomass. The conversion efficiency of total starch to reducing sugar release was found 26 % which is less compared to acid hydrolysed biomass.  

Due incompetence of enzymatic hydrolysis in this case acid hydrolysis was preferred.