Research Article

Growth kinetics and cheap substitute for industrial cultivation of the yeast *Saccharomyces cerevisiae*

Olayide F. Obidi and Wasiu A. Moyosore

Department of Microbiology, Faculty of Science, University of Lagos, Akoka-Yaba, Lagos, Nigeria

**ABSTRACT:** A cost-effective medium supplemented with sugarcane molasses (SM) and corn steep liquor (CSL) as the sole carbon and nitrogen sources respectively was formulated for the batch cultivation of *Saccharomyces cerevisiae*. The yeast growth kinetics in the medium was compared to that of conventional synthetic potato dextrose broth (PDB). Results showed the yeast growth was significantly dependent on the ratio of carbon to nitrogen sources in the media. The formulated medium supported the yeast growth with variations observed in its growth pattern at different concentrations. At 10% SM and 90% CSL (M10/C90) and (M20/C80), *S. cerevisiae* exhibited higher population density (0.38 × 10^5 to 3.10 × 10^5 CFU/ml) than in PDB (0.33 × 10^5 to 3.01 × 10^5 CFU/ml) during a 96h batch fermentation period. Analysis of variance (ANOVA) showed least significance differences at 0.05 level of probability (P< 0.05). The data obtained suggested the suitability and potentials of a locally-formulated medium for *S. cerevisiae* cultivation.

**KEYWORDS:** *Saccharomyces cerevisiae*, Corn steep Liquor, Sugarcane molasses, Growth kinetics

Correspondence: Olayide F. Obidi; laideob@yahoo.com, +2348034720933

Received: 16 January 2018; Approved: 17 May 2018.
INTRODUCTION

The yeast *Saccharomyces cerevisiae* is used in classical food fermentation such as production of beer, bread, yeast extract, wine, sake, and distilled spirits supplements in human and animal diets as well as in the production of single cell proteins (Izmirlioglu & Demirci, 2012). Yeast cells have been used as the inoculum in large scale bioethanol production (Echegaray et al., 2000). Furthermore, yeast cells have been employed in the manufacture of commercial products including hexokinase, glucose-6-phosphate dehydrogenase, invertase, and nucleic acids (Abrahao-Neto et al., 1996, 1997). The significance of yeasts in food technology as well as in human nutrition, as an alternative source of protein to meet the demands in a world of low agricultural production and rapidly increasing population makes the production of food grade yeasts extremely important (Bekatorou et al., 2006). In addition, the problem arising from the depletion of fossil fuels and the atmospheric pollution derived from their combustion can be solved by the use of ethanol in the form of bioenergy.

The most important biofuel is ethanol, which can be obtained from yeast fermentation (Echegaray et al., 2000). The cost of the fermentation medium is one of the principal factors that determine the economic viability of industrial production. It is very important that low cost medium components supply all the nutritional requirements for good growth and fermentation activity of *Saccharomyces cerevisiae*. Corn steep liquor (CSL) is a major by-product of the corn wet-milling industry and is one of the cheap sources of nitrogen (White & Johnson, 2003). In Nigeria, CSL is essentially a waste stream product of domestic or cottage industry production of 'ogi' (corn meal slurry) (Omidiji et al., 1997). Its composition depends on the corn variety, steeping condition and age of the corn kernel. Molasses is an abundant by-product of the sugar industry (Echegaray et al., 2000). It provides a cheap source of fermentable sugars and contains some nutrients (Aslam, 1999). Sugarcane molasses contains: moisture 20%, ash 8%, sugar 64%, and non-sugar 10% (Okafor, 2007).

The agro-industry generates a large amount of wastes such as molasses, grain powder by-products, whey, corn steep liquor and peels from cassava, plantain, banana, oranges and others. The feasibility of using these organic wastes to formulate cost-effective alternative culture media was studied by Anderson & Jayaraman, 2003; Brar et al., 2005a, 2005b; Keshavarzi et al., 2005; Nwabueze & Otowa, 2006; Amin et al., 2008; and Salehi et al., 2015. The ability to salvage and re-use these wastes economically also reduces environmental pollution and led to an initiative termed ‘waste to wealth’ in Nigeria. The production of a cost-effective growth medium from low-value and renewable materials is of great interest. The rationale of this work was to investigate the efficiency of sugar cane molasses and corn steep liquor as cost-effective sources of carbon and nitrogen in supporting the industrial cultivation of *Saccharomyces cerevisiae* and to evaluate the growth kinetics of the yeast at different concentrations of these wastes in the formulated medium in comparison to conventional medium.

MATERIALS AND METHODS

Collection of samples

Two canned beer samples were purchased from different retail outlets in Oshodi, Lagos Nigeria. Sugar cane molasses was obtained in sterile containers from Golden Sugar Company, a subsidiary of Flour Mills of Nigeria PLC, Apapa, Lagos. Corn steep liquor (CSL) was also collected in sterile containers from domestic producer of corn meal slurry at Agbede-Ebuwawa, Ikorodu, Lagos, Nigeria. The samples were stored aseptically at low temperature (4°C) until required. Analyses were carried out at the Federal Institute of Industrial Research Oshodi (FIIRO), Lagos, Nigeria.
Synthetic Media

The synthetic media used included potato dextrose agar (PDA) and potato dextrose broth (PDB) medium (Oxoid).

Isolation of *Saccharomyces cerevisiae*

*Saccharomyces cerevisiae* was isolated by pour plate technique as described by Nwachukwu and Akpata (2003) using PDA. A 1-ml aliquot of the beer sample was aseptically obtained using sterile pipette and transferred into a test tube containing 15 ml sterile warm (approximately 45 °C) PDA. The inoculated PDA was supplemented with 100 mg of streptomycin to prevent growing of bacteria and mixed thoroughly by creating vortexes. The whole PDA medium was poured into a sterile petri dish and allowed to spread at the base by gravity with the lid in place. The plate was incubated at room temperature (25±2 °C) for five days. All experiments were carried out in triplicates. Predominant colonies with distinct morphological differences were picked and purified by streaking three times on fresh PDA plates.

Identification of *Saccharomyces cerevisiae*

Cellular morphology was examined microscopically. *Saccharomyces cerevisiae* was identified using conventional method described by Barnet *et al.* (2000). Characterization of the isolate was carried out by subjecting the isolate to various physiological and biochemical tests which include fermentation of sugars, nitrate reduction, urease and starch test. The pure culture was maintained on PDA slants and plates at 4 °C.

Analysis of Sugarcane Molasses and Corn Steep Liquor

The sugar cane molasses and corn steep liquor were analyzed for moisture, fat, nitrogen, protein, ash and reducing sugars by the respective methods of Association of Official Analytical Chemists (2007).

Determination of Moisture Content of Molasses and Corn Steep Liquor

Five (5.0) g of each sample was placed inside a clean crucible which had been previously weighed and ignited. Then, the crucible and the samples were heated in an oven at 105°C to a constant weight for 4-6 h. The crucible was removed from the oven and cooled in a desiccator before weighing. The percentage moisture of the sample was then calculated using the formula:

\[
\text{% Moisture} = 100 \times \frac{W_2 - W_3}{W_1 - W_0}
\]

\(W_0\) = Weight of empty crucible (g)
\(W_1\) = Weight of crucible and the sample before drying (g)
\(W_2\) = Weight of crucible and the sample after drying (g) (Association of official Analytical Chemists, 2007).

Determination of Ash Content of Molasses and Corn Steep Liquor

Five (5.0) g of each sample was weighed into porcelain crucible which had been previously ignited and weighed. Organic matter was charred by igniting the samples on a hot plate in the fume cupboard. The crucible was placed in the muffle furnace and maintained at 600 °C for 3 hours, after which it was cooled in a desiccator and the ash content was weighed immediately.

The percentage ash content was then calculated as follows:

\[
\text{% Ash} = 100 \times \frac{\text{weight of crucible + ash}}{\text{weight of empty crucible}} - \text{weight of sample}
\]
Determination of Crude Fat in Molasses and Corn Steep Liquor

Five (5.0) g of each sample was put in thimbles, plugged with cotton wool. The thimbles were dried and inserted into extraction cups of a soxhlet extraction system, model HT2. The extraction cups were cleaned and weighed before extraction. Twenty five (25) ml of solvent (hexane) was introduced into each cup. The cups were inserted into the soxhlet with the samples extracted for 15 min in boiling position and 30 min in rinsing position. The extractable fat was later evaporated to dryness in an oven at 105°C for 1 hr, cooled in a desiccator and weighed (Association of Official Analytical Chemists, 2007).

The percentage fat in the sample was calculated as follows:

\[ \% \text{ Fat} = \frac{w_3-w_4 \times 100}{w_1} \]

\( w_1 \) = weight of the sample

\( w_2 \) = weight of the empty cup

\( w_3 \) = weight of cup with extracted fat (Association of official Analytical Chemists, 2007).

Determination of Crude Protein of Molasses and Corn Steep Liquor

Kjeldhal nitrogen method was used to determine the protein content of the samples. One (1) g of each sample was introduced into digestion flask. Kjeldhal catalyst tablets selenium was added to each sample. Then, 20 ml of concentrated \( \text{H}_2\text{SO}_4 \) was also added and the samples were fixed into the digester for 8 hours until a clear solution was obtained. The cooled digest was transferred into 100 ml volumetric flask and made up with distilled water. The distillation apparatus was set and rinsed for 10 min after boiling. Twenty (20) ml of 4% w/v boric acid was pipetted into conical flask, five (5) drops of methyl red was added to each flask as an indicator and the samples were diluted with 75 ml distilled water. Subsequently, 10 ml of the digest was made alkaline with 20 ml of 20% w/v \( \text{NaOH} \) and distilled. The steam exit of the distillator was closed and the colour change of boric acid to green was timed. The mixture was distilled for 15 min. The filtrate was then titrated against 0.1 N HCl to an end point (Association of Official Analytical Chemists, 2007). Distilled water was used as blank. The total protein was calculated based on the nitrogen content since proteins of typical samples contain 16% nitrogen in average (Association of Official Analytical Chemists, 2007).

\[ \% \text{ Total Nitrogen} = 0.014 \times (\text{sample titre-blank titre}) \times 0.1 \times 100 \]

\[ \% \text{ Protein} = \frac{\% \text{ Total Nitrogen} \times \text{conversion factor}}{\text{Sample weight}} \]


Determination of Glucose Content of Molasses and Corn Steep Liquor

The samples were extracted with 10 ml of 85% v/v ethanol and were boiled for 5 minutes in a water bath with constant stirring. Another 5 ml of ethanol was added and boiled for 3 minutes. The mixture was filtered using whatman 41 filter paper. The filtrate was collected and made up to 100 ml with distilled water in a volumetric flask. One (1.0) ml of the diluted filtrate was placed in a screw-capped test-tube and 2.0 ml low alkalinity copper reagent (reagent A) was added. All, including the blank which contained 1.0 ml of water and standard glucose were boiled in a boiling water bath for 10 min. The tubes were cooled at room temperature and 8.0 ml of arsenomolybdate reagent (reagent B) was added with immediate shaking. Each of the set-up was made up to 100 ml with distilled water. They were allowed
to stay for 15 min before taking readings of their absorbance at 500 nm using UV754 Hospibrand Spectrophotometer (Association of Official Analytical Chemists, 2007).

Then, the glucose content was estimated using the concentration factor

\[
\text{Glucose} = \frac{\text{Std conc} \times \text{sample Abs} \times 100 \times \text{df}}{\text{Std Abs wght of sample}}
\]


**Determination of Fructose Content of Molasses and Corn Steep Liquor**

One (1.0) ml portion of the diluted filtrate prepared above was placed into a test-tube. One (1.0) ml of resorcinol and 3.0 ml of concentrated HCl were added using a pipette. The tube was shaken thoroughly and boiled at 77 °C for 8 min. The boiling was repeated and the tube was cooled in ice-water bath at 0 °C for 5 min. The optical density of this extraction was read at 420 nm. The procedure was repeated using blank (distilled water) and standard fructose (1.0 mg/l). The fructose content in the samples was estimated using the concentration factor.

\[
\text{Fructose} = \frac{\text{Std conc} \times \text{sample Abs} \times 100 \times \text{df}}{\text{Std Abs wght of sample}}
\]


**Formulation of Local Medium for Growth Studies**

The thick brown sugarcane molasses was diluted with distilled water at ratio 1:3 (water: molasses). The diluted sugarcane molasses was mixed with CSL in different ratios in a 250 ml conical flask. The composition of the medium was varied.

**Inoculation**

Five (5.0) ml of sterile distilled water was poured into a test tube to which colonies of *Saccharomyces cerevisiae*, taken directly from the plate were emulsified. The suspension was adjusted to match 0.5 McFarland’s standard by adding sterile distilled water (Isu & Onyeagba, 2002). One (1) ml of the suspension was inoculated into each of thirteen different concentrations of the formulated medium using an automated pipette. The inoculated media were then incubated at 30 °C on shaker at 150 rpm for 96 hours, using orbital incubator shaker (MaxQ 6000 incubated stackable shaker Thermo Scientific).

**Growth Studies**

Five (5) ml each of the cultured formulated medium sample was aseptically taken into a sterile bottle using an automated pipette in every 12 hour interval until 96th hour of fermentation for various analyses. The optical densities of the resulting suspension were measured using a spectrophotometer (UV 754 Hospibrand, USA) at 620 nm every 12 h. Distilled water was used as a blank. The results were recorded and used in plotting growth curve of the organisms in different concentration of the medium (Acourene *et al*., 2007). Furthermore, a ten-fold serial dilution of the suspension was carried out using sterile distilled water as diluent to thin out high population densities of microorganisms present in the culture to a countable number of colonies. Selected dilutions including $10^{-3}$, $10^{-5}$ and $10^{-10}$ dilutions were plated out on PDA medium. This procedure was carried out in triplicate for each dilution. The PDA plates were incubated aerobically at 37 °C for seven days. Developed colonies were counted to determine the population density in colony forming units (CFU)/ml and used in plotting growth curve of the organism in different concentrations of the medium (Nwachukwu and Akpata, 2003).
Determination of the Residual Sugar Concentration of the Formulated Medium by Dinitrosalicylic Acid (DNS) Method

The residual glucose concentration in the different concentrations of the cultured formulated medium was determined by the 3, 5-dinitrosalicylic acid (DNSA) method using glucose as the standard (Miller, 1989). Standard glucose solutions were prepared in concentrations of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, and 2.0 mg/ml. One (1) ml of standard glucose solution was pipetted into a test tube and 2 ml of DNS reagent was added to it. The mixture was placed in boiling water for 5 minutes to develop the colour. After 5 minutes, the mixture was cooled at room temperature. About 2 ml of the mixture was placed in a cuvette and the absorbance measured at 520 nm using spectrophotometer (UV 754 Hospibrand, USA). A standard curve of glucose concentration and its absorbance was plotted and compared with 1 ml supernatant of each concentration of the cultured medium to determine the reducing sugar in the different concentrations of the medium.

Determination of pH of the Cultured Formulated Medium

The pH of the different concentrations of the formulated medium was determined using a pH-meter, (ADWA 8000 pH/mV). Five (5) ml of the suspension was collected at 12 hour interval into 10 ml beaker. The pH meter was calibrated using two buffers, pH 4.0 and 7.0. The reference electrode was dipped into the sample, then the reading was taken and recorded (A.O.A.C., 2007).

Determination of Dry Cell Weight of *Saccharomyces cerevisiae*

The suspensions from the different concentrations of the formulated medium were collected and centrifuged at 5000 rpm for 10 min. The supernatant was utilized for the determination of the residual sugar estimation. The remaining pellet containing the biomass was dislodged with small volume of distilled water and transferred into a known weight filter paper. The cell mass was dried in an oven at 100 °C to a constant weight, allowed to cool in a desiccator and weighed (Harrigan, 1998).

Determination of Specific Growth Rate (µ) and Mean Generation Time (ΔT) of *Saccharomyces cerevisiae*.

Specific growth rate (µ) and mean generation time (ΔT) were calculated as described by Nwachukwu & Akpata, (2003) with slight modifications. The logarithm of cell numbers was obtained at 12 h intervals and plotted against the time of fermentation to produce a straight line graph. The slope of the graph was determined and equals µ. Then, the mean generation time (ΔT) was calculated from µ, using the formula below:

\[ ΔT = \log_2/µ \]

Statistical analysis of the experiments

The data generated from this study were analysed using SPSS/20.0 software. Analysis of variance (ANOVA) was conducted to test least significance differences (LSD). Significance was accepted at 0.05 level of probability (p<0.05).

RESULTS

The selected isolates obtained from the beer were identified based on morphological (characteristic cream, oval cells, budding and ascospore formation), physiological and biochemical characterization. They were able to ferment a wide range of sugars but not lactose. These characteristics support the probable identification of the selected isolates as strains of *Saccharomyces cerevisiae* (Table 1).
Proximate analysis of the sugarcane molasses and CSL showed that molasses had a high sugar content with 7.56% and 13.70% of glucose and fructose respectively while CSL had 1.96 and 3.03% of glucose and fructose. On the other hand, sugarcane molasses had 0.007% and 0.44% of nitrogen and protein while CSL had 0.805% and 5.03% of nitrogen and protein respectively (Table 2). The mixture of molasses and corn steep liquor at different ratios (Table 3) was investigated for the batch cultivation of the isolated S. cerevisiae. The growth curve in Figure 1 showed that the locally formulated medium at all its concentrations supported the cultivation of the organism producing a growth pattern similar to the pattern observed in the conventional medium (PDB). Though, variations were observed in their growth kinetics.

<table>
<thead>
<tr>
<th>Biochemical characteristics and physiological</th>
<th>isolate-1</th>
<th>isolate-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Cream</td>
<td>Cream</td>
</tr>
<tr>
<td>Cellular Morphology</td>
<td>Oval, budding</td>
<td>Oval, budding</td>
</tr>
<tr>
<td>Motility Test</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Catalase Test</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Urease Test</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Ascospore formation</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Glucose</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Fructose</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Lactose</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Sucrose</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Maltose</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Xylose</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Galactose</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Mannitol</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Probable Identity</td>
<td>Saccharomyces cerevisiae</td>
<td>Saccharomyces cerevisiae</td>
</tr>
</tbody>
</table>

Table 1. Morphological, physiological and biochemical characteristics of isolated Saccharomyces cerevisiae

1. OBIDI et al. Substitute for industrial cultivation of the yeast Saccharomyces cerevisiae

2. Biokemistri 30(2): 73–88
Table 2. Percentage (%) Proximate Compositions of Sugarcane Molasses and Corn Steep Liquor (CSL)

<table>
<thead>
<tr>
<th></th>
<th>Sugarcane Molasses</th>
<th>Corn Steep Liquor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>36.6</td>
<td>94.8</td>
</tr>
<tr>
<td>Glucose</td>
<td>7.56</td>
<td>1.96</td>
</tr>
<tr>
<td>Fructose</td>
<td>13.70</td>
<td>3.03</td>
</tr>
<tr>
<td>Protein</td>
<td>0.44</td>
<td>5.03</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0.007</td>
<td>0.805</td>
</tr>
<tr>
<td>Ash</td>
<td>6.58</td>
<td>8.44</td>
</tr>
<tr>
<td>Fat</td>
<td>0.08</td>
<td>1.12</td>
</tr>
</tbody>
</table>

Figure 1. Optical density at 620 nm ($A_{620}$) of Saccharomyces cerevisiae in different concentrations of the medium (M = molasses; C = corn steep liquor; PDB = potato dextrose broth; 0-100 in % v/v)
Table 3: The Composition (% , v/v)-of the formulated medium

<table>
<thead>
<tr>
<th>Flask no</th>
<th>Medium</th>
<th>Composition (% , v/v)</th>
<th>Molasses</th>
<th>CSL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M100/C0</td>
<td>100</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M90/C10</td>
<td>90</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M80/C20</td>
<td>80</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M70/C30</td>
<td>70</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M60/C40</td>
<td>60</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>M50/C50</td>
<td>50</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>M40/C60</td>
<td>40</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>M30/C70</td>
<td>30</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>M20/C80</td>
<td>20</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>M10/C90</td>
<td>10</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>M0/C100</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>PDB</td>
<td>PDB</td>
<td>PDB</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. Mean population density (CFU/ml x 10^5) of *Saccharomyces cerevisiae* in different concentrations of the medium (M= molasses; C= corn steep liquor; PDB= potato dextrose broth; 0-100 in % v/v)
Figure 3. Residual sugar concentrations (g/l) of *Saccharomyces cerevisiae* at different concentrations of the medium (M= molasses; C= corn steep liquor; PDB= potato dextrose broth; 0-100 in % v/v)

Figure 4. Mean Changes in pH at different concentrations of the medium (*Saccharomyces cerevisiae*) (M= molasses; C= corn steep liquor; PDB= potato dextrose broth; 0-100 in % v/v)
Generally, growth kinetics of *S. cerevisiae* was higher in the locally formulated medium at elevated concentrations of CSL supplemented with low concentrations of sugarcane molasses. This was observed in the medium containing 90% CSL supplemented with 10% of sugarcane molasses (M10/C90), 80% CSL supplemented with 20% of sugarcane molasses (M20/C80) and 70% CSL supplemented with 30% of sugarcane molasses (M30/C70) than other concentrations. However, when compared with growth in PDB, *S. cerevisiae* showed higher growth kinetics in the locally formulated medium at (M10/C90) than PDB (Figure 1). It was further revealed that *S. cerevisiae* attained the highest population densities after 60 hrs of fermentation at M10/C90, M20/C80, and M30/C70 to yield $3.09 \times 10^5$, $2.95 \times 10^5$, and $2.69 \times 10^5$ CFU/ml respectively. These population densities dropped slightly after 72 h and did not rise to peak again at these three concentrations. Similarly, highest population density of $2.92 \times 10^5$CFU/ml was attained after 72 hours of fermentation in PDB (Figure 2).

### Table 4. Growth kinetics of *Saccharomyces cerevisiae* in the Locally Formulated Medium at Different Concentrations

<table>
<thead>
<tr>
<th>Medium</th>
<th>Specific growth rate, $\mu$ (h$^{-1}$)</th>
<th>Mean generation time, $\Delta T$ (h)</th>
<th>Mean residual sugar concentration (mg/l)</th>
<th>Dry weight (mg/l) after phase cell log</th>
</tr>
</thead>
<tbody>
<tr>
<td>M100/C0</td>
<td>0.0290</td>
<td>10.380</td>
<td>0.4935</td>
<td>8.20</td>
</tr>
<tr>
<td>M90/C10</td>
<td>0.0293</td>
<td>10.274</td>
<td>0.3810</td>
<td>8.20</td>
</tr>
<tr>
<td>M80/C20</td>
<td>0.0291</td>
<td>10.345</td>
<td>0.1778</td>
<td>7.60</td>
</tr>
<tr>
<td>M70/C30</td>
<td>0.0308</td>
<td>9.774</td>
<td>0.1103</td>
<td>8.00</td>
</tr>
<tr>
<td>M60/C40</td>
<td>0.0345</td>
<td>8.726</td>
<td>0.1035</td>
<td>6.60</td>
</tr>
<tr>
<td>M50/C50</td>
<td>0.0345</td>
<td>8.726</td>
<td>0.0338</td>
<td>6.30</td>
</tr>
<tr>
<td>M40/C60</td>
<td>0.0342</td>
<td>8.802</td>
<td>0.0000</td>
<td>3.80</td>
</tr>
<tr>
<td>M30/C70</td>
<td>0.0348</td>
<td>8.650</td>
<td>0.0000</td>
<td>4.90</td>
</tr>
<tr>
<td>M20/C80</td>
<td>0.0354</td>
<td>8.504</td>
<td>0.0000</td>
<td>6.80</td>
</tr>
<tr>
<td>M10/C90</td>
<td>0.0358</td>
<td>8.409</td>
<td>0.0000</td>
<td>7.20</td>
</tr>
<tr>
<td>M0/C100</td>
<td>0.0334</td>
<td>9.013</td>
<td>0.0000</td>
<td>3.50</td>
</tr>
<tr>
<td>PDB</td>
<td>0.0350</td>
<td>8.601</td>
<td>0.0000</td>
<td>6.00</td>
</tr>
</tbody>
</table>
Table 4 showed the growth kinetics of the organism at various concentrations of the locally formulated medium and PDB. These results showed that the organism exhibited a higher specific growth rate in the formulated medium (0.0358 h\(^{-1}\) and 0.0354 h\(^{-1}\) at M10/C90 and M20/C80 respectively) than PDB (0.0350 h\(^{-1}\)). The mean generation time, \(\Delta T\) obtained in the formulated medium was 8.409 h and 8.504 h (at M10/C90 and M20/C80 respectively) and this is lower than \(\Delta T\) obtained in PDB (8.601 h). Figure 2 showed that the residual sugar concentration decreased to zero in the conventional medium (PDB) and at lower molasses concentration (M30/C70, M20/C80, M10/C90 and M0/C100) in the formulated medium. This was observed after 36 h of fermentation in PDB, M10/C90 and M0/C100 and after 48 h and 72 h at M20/C80 and M30/C70 respectively. However at some of these points during the fermentation where residual sugar equals zero, the organism was still observed thriving in the absence of exogenous substrate. This was observed in the formulated medium at M10/C90 and M0/C100 when population density increased from 2.89 \(\times\) 10\(^5\) CFU/ml to 3.10 \(\times\) 10\(^5\) CFU/ml and 1.37 \(\times\) 10\(^5\) CFU/ml to 2.22 \(\times\) 10\(^5\) CFU/ml respectively after 36 h of fermentation and similarly in PDB (1.97 \(\times\) 10\(^5\) CFU/ml to 2.92 \(\times\) 10\(^5\) CFU/ml after 36 h fermentation). In Figure 4, it was clear that acidic pH was maintained throughout the fermentation in all the media. However the pH dropped gradually and this is continuous till the 96\(^{th}\) h of fermentation in all the media. The dry cell weight was observed to increase continuously from 0-72 h in all the media after which a general decline in dry cell weight was observed as fermentation progressed in all the media (Figure 5).
DISCUSSION

In this study, a locally-formulated medium comprising the low value agro-industrial waste materials (sugarcane molasses and corn steep liquor as natural carbon and nitrogen sources respectively) was utilized for the cultivation of *Saccharomyces cerevisiae*. The results of proximate analysis showed that sugarcane molasses contain higher amount of sugar than corn steep liquor and can serve as a good source of carbon for microbial growth. Conversely, corn steep liquor is richer in nitrogen and as such can serve as a good source of nitrogen for microbial growth. This agrees with the findings of Curtin (1983) and CRA (2006). However, molasses composition varies and is influenced by factors such as ambient temperature, moisture, season of production, variety and technology of sugar mills (Arroyo-Lopez, 2009). As a result, the sugar content of molasses produced in different countries varies according to the production technology employed. According to Curtin (1983), changes in the design of centrifuges used to separate sugar and syrup constitute one of the major advancements in the cane sugar industry. Continuous centrifugation results in more sugar extraction with a corresponding decrease in the amount of sugar left in molasses. The composition of corn steep liquor is highly variable.

Figure 6. Logarithm of cell number of *Saccharomyces cerevisiae* at different concentrations of the medium. (M= molasses; C= corn steep liquor; PDB= potato dextrose broth; 0-100 in % v/v)
and would depend on the maize variety, conditions of steeping and other factors (Okafor, 2007). Herein, the low-value agro-industrial waste materials, molasses and corn steep liquor (CSL) were used as carbon and nitrogen sources, respectively. Sluggish fermentation occurred in the test organism at elevated molasses concentrations and in the formulated medium containing CSL alone. It is interesting to note that growth rate was observed to increase with increasing concentration of CSL in the batch fermentation, but it did not increase with elevated concentrations of molasses. This is in agreement with the report of Kim & Vu, (2009) that in the presence of high sugar concentration, energy and substrate in the medium are diverted toward product formation pathway, consequently leaving less energy and substrate available for production of cell mass. Calado et al. (2003) and Kapat et al. (1997) reported that high glucose levels induce ethanol fermentation and inhibit production of cell mass. Furthermore, elevated concentrations of molasses may cause osmosis and generally microorganisms tend to maintain homeostasis in their growth environment (Hogg, 2005). Thus, the hypertonic environment may cause plasmolysis of the isolates. In contrast, the dry cell weight estimation revealed high growth rates at elevated sugar concentrations. This disagrees with many reports from literature. This observed anomalous behaviour may be due to the presence of debris in the sugarcane molasses, and consequently may alter the cell mass. This debris may be present because a westfallia separator was not available to separate the organism from the debris before estimating the dry cell weight.

Higher growth rates observed with elevated concentrations of CSL may not be unconnected with the fact that CSL is a rich source of nutrient, particularly organic nitrogen, essential minerals, and co-factors (CRA, 2006). Cane and beet molasses are not rich in basic elements like nitrogen, phosphorus, calcium and magnesium which are vital for the growth of microorganisms (Curtin, 1983). According to Gutierrez et al. (2012) nitrogen deficiencies are one of the main causes of stuck or sluggish fermentations. Thus, addition of nitrogen sources is needed in adequate amount. Hoek et al. (1998) demonstrated that, if sugars are fermented in the presence of adequate amount of nitrogen, less alcohol is formed, making the environment more favorable for the growth of the microbes.

However, microorganisms generally require carbon and nitrogen sources for growth (Nwachukwu & Akpata, 2003). If any of the required nutrients is lacking or becomes limiting, growth may be slow. This may explain the low growth kinetics observed in medium containing CSL alone (M0/C100), since the CSL contained limited amount of carbon sources.

Conclusion

The results obtained in this study revealed that a medium comprising molasses and corn steep liquor is a suitable and economically profitable for the industrial cultivation of Saccharomyces cerevisiae. The growth kinetics of this organism increases with elevated CSL concentrations supplemented with limiting amount of molasses. The formulated medium with molasses alone and CSL alone also support the yeast growth but at a reduced growth rate. Overall, in this batch cultivation of Saccharomyces cerevisiae, the locally formulated medium at concentrations of M10/C90 (containing 10% of sugarcane molasses supplemented with 90% corn steep liquor) and M20/C80 (containing 20% of sugarcane molasses supplemented with 80% corn steep liquor) showed better performance than conventional PDB medium. Theses concentrations significantly improved biomass production and will be useful for the further development of a cost-effective seed culture for bioethanol production. Furthermore, the formulated medium may be recommended as substitute for the imported conventional and synthetic PDB medium for the growth of S. cerevisiae locally, thereby conserving foreign exchange.
REFERENCES


