The Effect of pH, Temperature, and Composition of the Medium on Growth Characteristics of *Rhodotorula gracilis*

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Previous studies have shown that *Rhodotorula gracilis* is capable of forming large quantities of fat under suitable conditions (Enebo et al., 1946; Pan et al., 1949; Steinberg and Ordal, 1954a). It also has been demonstrated that growth and fat formation takes place in two distinct phases (Enebo et al., 1946). Steinberg and Ordal (1954a, 1954b) have studied the fattening phase in some detail; however, very little is known about the optimum conditions for growth of this organism.

There is, in general, a lack of accurate published in formation on the effects of pH, temperature, and aeration on the growth of yeasts. Only a few studies have appeared in the literature describing the use of pH controlling equipment in fermentation studies (Finn et al., 1950; Kempe et al., 1950; Neish and Blackwood, 1951; Maxon and Johnson, 1953; Lakata, 1954; Steinberg and Ordal, 1954a; McKee, 1955). In most cases only the initial pH was adjusted and observed and, where temperature or other conditions were variable, generally no pH control was used.

There are no temperature studies reported in the literature for *R. gracilis*. Enebo et al. (1946) used a temperature of 28°C for both growth and fat formation. Every known investigator since that time has used this temperature.

Several investigators have made quantitative studies on the effect of aeration on yeast growth (Olson and Johnson, 1949; White and Munns, 1951b; Maxon and Johnson, 1953). The only study on the effect of aeration on *R. gracilis* was made by Pan et al. (1949) who investigated the effect of air flow rate on combined growth and fattening. An increase of air flow rate from 0.1 to 1 volume of air per minute had no effect on the efficiency of sugar utilization nor on fat content. No measure of the effective oxygen-absorption rate was given.

Agarwala and Peterson (1950) and Chiao and Peterson (1953) reported on the efficiency of sugar utilization by *R. gracilis*. However, the medium used by these workers contained nonsugar carbon compounds which could be utilized by the organism, which makes any interpretation of their results equivocal.

The object of this investigation was to determine the effect of pH and temperature on total amount and rate of growth of *R. gracilis* under conditions of controlled aeration. The efficiency of glucose utilization in a chemically defined medium was also investigated.

**Materials and Methods**

**Equipment**

The fermentor assembly, which consisted of a fermentation vessel, a controlled-temperature water bath, pH control equipment, and a system for aeration and agitation, has been previously described (Steinberg and Ordal, 1954a). The maximum temperature deviation was ±0.5°C. The pH was continuously controlled within ±0.1 unit with 1 N sodium hydroxide.

Oxygen absorption rates were determined by the sulfite oxidation method of Cooper et al. (1944). Preliminary studies indicated that an oxygen-absorption rate of 3.0 g oxygen per L of medium per hr was necessary for the organism to grow exponentially until the substrate became limiting. At an oxygen-absorption rate of 1.1 g per L of medium per hr, the growth was characterized by a short logarithmic phase followed by a sloping-off phase. When these latter data were plotted on linear coordinates, a straight line was obtained indicating that the oxygen concentration was limiting for the cell concentrations studied. Foaming of the medium was controlled by the addition of a few drops of a silicone emulsion antifoam agent.

**Microbiological Methods**

The yeast used in this study was *R. gracilis* NRRL Y-1091. Stock cultures were carried on slants of the following medium: malt extract, 3 g; yeast extract, 3 g; peptone, 5 g; agar, 20 g; and water, 1000 ml. Transfers were made every 3 months and the cultures were stored in the refrigerator.

The liquid inoculum medium was a modification of that used by Enebo et al. (1946). This consisted of cerelose (glucose monohydrate), 20 g; (NH₄)₂SO₄, 4 g; yeast extract, 3 g; KH₂PO₄, 1.5 g; MgSO₄.7H₂O, 1 g; NaCl, 0.5 g; CaCl₂.2H₂O, 0.3 g; FeCl₃.6H₂O, 0.005 g; H₂C₂H₂O₂.2H₂O, 6.0 g; NaOH, 3 g; and water, 1000 ml. The cerelose was autoclaved separately to prevent excessive browning of the medium which resulted when all of the ingredients were autoclaved together.

1 Antifoam A, Dow-Corning Corp., Midland, Michigan.
The growth from a 3-day-old slant was used to inoculate a shake flask and two successive transfers were made after 18-hr intervals at 32 C on a reciprocating shaker. Preliminary experiments showed that 32 C was near the optimum temperature for growth. The resulting culture was used to inoculate the fermentor medium.

In the studies on the effects of temperature and pH on growth, the composition of the fermentor medium was the same as the inoculum medium except that the citric acid and the sodium hydroxide were omitted, since no buffer was required. The amount of cerelose was reduced from 20 to 16 g per L. The final volume of the fermentor medium plus inoculum was 9.5 L. When the glucose content of the cerelose was corrected for water of hydration and free moisture, the final glucose concentration of the medium was in the range of 1.33 to 1.46 per cent with a carbon/nitrogen ratio (C/N) of about 6/1. This was enough glucose to permit logarithmic growth to a yeast dry-weight concentration of about 0.9 g per 100 ml. At this level, when the glucose was nearly exhausted, there was a sharp break in the logarithmic growth curve.

In the experiments on efficiency of glucose utilization, the media used were either a minimal synthetic medium consisting of the fermentor medium without yeast extract, or yeast-nitrogen base medium (Wickerham 1946, 1948) which contains all of the vitamins known to be required by yeasts plus small amounts of the amino acids, L-histidine monohydrochloride, DL-methionine, and DL-tryptophane.

Dihydrostreptomycin sulfate (10 µg per ml of medium) was added to the sterile fermentor medium to control contamination. Rhodotorula species have been reported to be unaffected by streptomycin (Waksman, 1949). In addition, it was found that the growth on solid media or in broth tubes was not affected in presence of up to 5 to 10 times the streptomycin concentration used in the fermentor vessel.

Analytical Procedures

The concentration of yeast in a suspension was determined as the grams of dry weight in a 25-ml sample. Centrifuged and washed cells were dried at 100 to 105 C for 12 hr. Duplicate determinations were made on all samples.

The Somogyi-Nelson procedure was used for the determination of reducing sugar in the media containing yeast extract (Nelson, 1944; Somogyi, 1945). The anthrone reagent was used to determine the glucose concentration in the synthetic media (Roe, 1955).

Results and Discussion

Effect of pH

The range of hydrogen ion concentrations investigated extended from pH 2.5 to 7.5. In all cases, the temperature was held constant at 32 C and the aeration rate was 3.0 g of oxygen per L of medium per hr.

<table>
<thead>
<tr>
<th>pH</th>
<th>Generation Time</th>
<th>Net Dry Weight of Cells Produced</th>
<th>Glucose Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hr</td>
<td>g/100 ml</td>
<td>%</td>
</tr>
<tr>
<td>2.5</td>
<td>30.58</td>
<td>—</td>
<td>1.46</td>
</tr>
<tr>
<td>3.5</td>
<td>2.15</td>
<td>0.849</td>
<td>1.45</td>
</tr>
<tr>
<td>4.5</td>
<td>2.02</td>
<td>0.849</td>
<td>1.45</td>
</tr>
<tr>
<td>5.5</td>
<td>2.18</td>
<td>0.857</td>
<td>1.45</td>
</tr>
<tr>
<td>6.5</td>
<td>2.08</td>
<td>0.860</td>
<td>1.43</td>
</tr>
<tr>
<td>7.5</td>
<td>6.72</td>
<td>—</td>
<td>1.45</td>
</tr>
</tbody>
</table>

The effect of pH on rate of growth and total amount of growth is shown in table 1. Generation times were calculated in the logarithmic growth phase by the equation

\[ G = \frac{0.301 \ t}{\log W_2/W_1} \]

where \( G \) is the generation time, \( W_1 \) is the weight of cells present after logarithmic growth was established, and \( W_2 \) is the weight of cells at time \( t \).

These data show that the growth rate varies very little over the range pH 3.5 to 6.5 with a suggested optimum at pH 4.5. However, the net dry weight of cells produced increased slightly up to pH 6.5. There is a marked reduction in growth rate between pH 3.5 and 2.5, and between pH 6.5 and 7.5. Almost all of the glucose was consumed shortly after the end of the logarithmic growth phase, and all of the growth curves were characterized by a sharp break at that point.

These results are quite different from those reported for the effect of pH on the growth rate and total amount of growth of Saccharomyces cerevisiae. Finn and Wilson (1954) found that the most rapid growth of S. cerevisiae took place at pH 5.0. The generation times reported were 2 hr at pH 5.0 and 2.5 hr at pH 4.5, indicating that the growth rate was affected markedly by pH. Olson and Johnson (1949) also reported that the initial pH had a pronounced effect on the final yield of S. cerevisiae.

Effect of Temperature

In these studies, the pH was held constant at 4.5 while the temperature was varied in the range of 26 to 38 C. The oxygen-absorption rate was 3.0 g per L per hr. The results are shown in table 2.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Generation Time</th>
<th>Net Dry Weight of Cells Produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>hr</td>
<td>g/100 ml</td>
</tr>
<tr>
<td>26</td>
<td>2.69</td>
<td>0.846</td>
</tr>
<tr>
<td>30</td>
<td>2.27</td>
<td>0.843</td>
</tr>
<tr>
<td>32</td>
<td>2.02</td>
<td>0.849</td>
</tr>
<tr>
<td>35</td>
<td>2.30</td>
<td>0.842</td>
</tr>
<tr>
<td>38</td>
<td>No growth</td>
<td>—</td>
</tr>
</tbody>
</table>
In contrast to the effect of pH, there is an increase in growth rate with temperature with an optimum at 32°C. No growth took place at 38°C. A temperature decrease of 3°C from the optimum resulted in an 11 per cent decrease in growth rate, while a temperature increase of 3°C from 32°C resulted in a 12 per cent decrease. These results are similar to those of White and Munns (1951a) who found that the growth of S. cerevisiae increased with increasing temperature up to 30 to 36°C and then fell sharply. It also will be noted that in the range of 26 to 35°C there was no temperature effect on the net dry weight of cells produced.

**Efficiency of Glucose Utilization**

In this part of the investigation, the efficiency of glucose utilization by R. gracilis was determined in chemically defined media with glucose as the sole source of carbon and energy. The temperature, pH, and aeration rate were held constant at 32°C, pH 5.5 and 3 g of oxygen per L of medium per hr, respectively.

The three conditions investigated were as follows: (1) the inoculum produced in the inoculum medium described previously and inoculated into minimal synthetic medium containing glucose, ammonium sulfate, and mineral salts; (2) the inoculum produced in the minimal medium and the efficiency of glucose utilization determined in the same medium; and (3) the inoculum produced in yeast nitrogen base medium and the efficiency also evaluated in this medium.

As indicated in table 3, cells produced in yeast-nitrogen base medium exhibited a shorter generation time and the culture yielded a higher net dry weight of cells than did those grown in the minimal synthetic medium. Likewise, in the latter medium, the generation time was greater and the yield of cells less than that obtained when the same pH and temperature were used with the medium enriched with yeast extract (table 1, pH 5.5).

The efficiency of glucose utilization, based on the

<table>
<thead>
<tr>
<th>Medium</th>
<th>Inoculum Medium</th>
<th>Generation Time</th>
<th>Net Dry Weight of Cells Produced</th>
<th>Glucose Concentration</th>
<th>Efficiency*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal</td>
<td>Enriched</td>
<td>2.96 hr</td>
<td>0.572 g/100 ml</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>Minimal</td>
<td>3.25 hr</td>
<td>0.580 g/100 ml</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Yeast nitrogen</td>
<td>Yeast nitrogen</td>
<td>2.85 hr</td>
<td>0.661 g/100 ml</td>
<td>%</td>
<td>%</td>
</tr>
</tbody>
</table>

* (Net yield of cells) × 100.

(Glucose utilized)

Glucose consumed, in the medium enriched with 0.3 per cent yeast extract (table 1, pH 5.5) was 61.1 per cent. In the yeast nitrogen base medium, where glucose was essentially the sole source of carbon (the amino acids and vitamins in this medium collectively supplied only 0.03 g of available carbon per L), the observed efficiency was 49.7 per cent. The lower efficiencies obtained in the minimal synthetic media (43.3 and 43.0 per cent) reflected the need for added nutrients, both for a more rapid generation time and for a high efficiency in the conversion of carbon-containing compounds to cell material.

Agarwala and Peterson (1950) reported yields of 71.1 and 34.7 per cent when R. gracilis was grown on a beet molasses-corn steep liquor medium and on the synthetic medium of Olson and Johnson (1949) respectively. Chiao and Peterson (1953) obtained an efficiency of 45 per cent when this organism was cultured on a glucose-asparagine-citrate medium. These previously reported data, as well as the data presented in this paper, emphasize the importance of nonsugar carbon in the calculations of efficiency based on sugar (glucose) consumed. This has been discussed by Agarwala and Peterson (1949). The data also indicate that R. gracilis is able to convert substrate to cell material efficiently and warrant further investigation for the primary production of food or fodder yeast.

**Summary**

The effect of pH and temperature on the rate of growth and total amount of growth of Rhodotorula gracilis NRRL Y-1091 and the efficiency of glucose utilization in chemically defined media with glucose as the sole carbon source were investigated. The rate of growth did not vary significantly over the range pH 3.5 to 6.5, while the net dry weight of cells produced increased slightly over this range. The optimum temperature for growth was found to be 32°C at pH 4.5. Variations in temperature in the range 26 to 35°C had no appreciable effect on the total amount of cells produced. An efficiency of glucose utilization of 49.7 per cent was obtained in a synthetic medium supplemented with vitamins and amino acids under controlled optimum conditions of pH, temperature, and rate of aeration.

**REFERENCES**


Finn, R. K., Halvorson, H. O., and Piret, E. L. 1950
A Study of Mixed-Spore Culture and Soil-Burial Procedures in Determining Mildew Resistance of Vinyl-Coated Fabrics

A. David Baskin and Arthur M. Kaplan

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Research in these laboratories and by others (Abrams, 1948; Stahl and Pessen, 1953) has shown that fungi and bacteria can deteriorate certain plastic films, whether the films are tested alone (unsupported films) or whether they are evaluated as coatings on fabric (supported films). Films may also be deteriorated by loss of plasticizer through volatilization. However, vinyl film plasticized with equal parts of dioctylphthalate (DOP) and methyl acetylatedioctylphthalate (P4) showed no stiffening after autoclaving for 24 hr, or exposure for 8 days to oxygen, or exposure for 30 days to high relative humidity and temperature.

Our studies have shown that phthalate and phosphate plasticizers are generally resistant to fungal attack, with the aliphatic-substituted compounds showing a greater degree of resistance than the aliphatic.

Natural oils, fatty acid derivatives, and certain long-chain dicarboxylates as plasticizers are readily attacked by microflora. Structural modification of the plasticizer may make it fungus-resistant, but not necessarily resistant to bacteria (and vice versa). This was demonstrated by the response of Aspergillus versicolor and Pseudomonas aeruginosa to each of a series of homologous sebacates (Stahl and Pessen, 1953). The same authors (1954) reported that internally plasticized polymers resist degradation by fungi.

Visual rating of growth of microflora and changes in flexibility and breaking strength have been used in these laboratories as indices of deterioration of plastic films and coated fabrics. These observations were made following exposure to soil burial or by single-specie and mixed-spore suspension culture tests. These tests for evaluation of the deterioration of plastic films and coated fabrics on the basis of weight of coating and end use of the fabric have been found to be unreliable. Therefore, laboratory tests are being designed which