The effect of temperature on the solvent production by Clostridium acetobutylicum has been studied in the range 25 to 40°C. It was found that the solvent yield decreased with increasing temperature; seemingly because of a reduction in acetone production. It appeared that the yield of the other major solvent, butanol, was not affected by the temperature. Considering total solvent yield and productivity only, the optimum fermentation temperature is 35°C.

**INTRODUCTION**

The acetone-butanol fermentation underwent a rapid decline in the late 1940s due to steadily rising substrate prices, and the availability of cheap oil which allowed the inexpensive production of synthetic butanol. However, following the quadrupling of oil prices in the early 1970s, interest in the possibility of reviving the fermentation grew. Whereas in the industrial fermentation a nutritionally complex medium was invariably used (Beesch, 1952 and 1953) more recently studies have generally involved the use of chemically defined media (Gottschal and Morris, 1981 and 1983; Andersch, Bahl and Gottschalk, 1982).

It has been indicated that the temperature at which the fermentation was operated could affect solvent production in both the starch and the molasses based processes (Beesch, 1952 and 1953). The present study examines the effect of temperature upon the fermentation of Cl. acetobutylicum, including the rates of growth and solvent production in a chemically-defined medium, in an effort to determine the optimum fermentation temperature.

**MATERIALS AND METHODS**

Clostridium acetobutylicum NCIB8052 was cultivated in a 3 l stirred tank fermenter. Anaerobic conditions were maintained by sparging oxygen free nitrogen at 20 ml min⁻¹ through the vessel. The culture was stirred at 150 rpm. Medium composition is as follows: Glucose, 30 g l⁻¹; (NH₄)₂SO₄, 2 g l⁻¹; K₂PO₄, 1 g l⁻¹; MgSO₄·7H₂O, 0.1 g l⁻¹; NaCl, 0.1 g l⁻¹; Na₂MoO₄, 0.01 g l⁻¹; CaCl₂·6H₂O, 0.001 g l⁻¹; FeSO₄, 0.0015 g l⁻¹; FeSO₄·7H₂O, 0.015 g l⁻¹; Biotin, 1 mg l⁻¹; p-amino benzoic acid, 2 mg l⁻¹; Thiamine hydrochloride, 2 mg l⁻¹.

Biomass concentration was estimated by means of dry weight determination.

Glucose was estimated by the glucose oxidase/peroxidase II method (Sigma Chemical Co. Ltd.).
The concentration of NH₄ on the filtrate was determined using a
gas-sensing ion specific electrode connected to a pH meter giving a
direct reading of concentration.

Acetic and butyric acid, acetone, butanol and ethanol were measured by gas chromatographic means.

RESULTS

The temperatures examined were 25°C, 30°C, 37°C, 40°C and a
composite temperature of 40°C for the early stages of the fermentation
and 25°C for the later stages. The results of these experiments are
shown in Table 1.

DISCUSSION

Table 1 shows clearly how the solvents yield decreases with
increasing temperature. The butanol-acetone ratio, however, rises
with the temperature. It has been suggested that this may be due to
loss of acetone by evaporation in the gas stream at the higher
temperatures (Beesch, 1953). In this series of experiments, however,
the loss of solvent was minimised by the use of an effective condenser
and minimal gas flow.

Table 1 Variations in Maximum Biomass Concentration
and Total Solvent Yield and Butanol-Acetone
Ratio with Temperature

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>Biomass concentration (g/l)</th>
<th>Total solvent yield (%)</th>
<th>Butanol yield (%)</th>
<th>Acetone yield (%)</th>
<th>Effective productivity ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>2.73</td>
<td>29.1</td>
<td>21.4</td>
<td>6.2</td>
<td>0.47</td>
</tr>
<tr>
<td>30</td>
<td>2.47</td>
<td>28.4</td>
<td>21.5</td>
<td>5.8</td>
<td>0.48</td>
</tr>
<tr>
<td>37</td>
<td>2.59</td>
<td>25.5</td>
<td>20.0</td>
<td>4.3</td>
<td>0.47</td>
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<td>40</td>
<td>2.22</td>
<td>24.5</td>
<td>20.1</td>
<td>3.6</td>
<td>0.60</td>
</tr>
<tr>
<td>40/25</td>
<td>2.09</td>
<td>26.2</td>
<td>20.3</td>
<td>4.6</td>
<td></td>
</tr>
</tbody>
</table>

¹ Yield = solvents produced (g/l) glucose consumed (g/l⁻¹)

² Productivity in production phase; time between end of lag phase
and achievement of maximum solvent concentrations.

It seems, therefore, that the reduced total solvent yield at
higher temperatures is a result of a fall in the production of
acetone. It should be noted that the butanol-acetone ratio for
solvent production during the 25°C phase in the 40/25°C experiment was
3.43, which is in agreement with the ratio obtained at a constant
25°C. The production of acetone appears directly influenced by the
temperature and cell metabolism is not irreversibly affected.

Two major optimisation parameters are process yield and
productivity. The former is given in Table 1 and for purposes of
comparison, the latter can be estimated as yield divided by
fermentation hours (strictly speaking this should be concentration of
solvent divided by the number of hours. This cannot be used in this
case as the initial glucose concentration was not constant). The