Studies on continuous production of fodder yeast from molasses

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STUDIES ON CONTINUOUS PRODUCTION
OF FODDER YEAST FROM MOLASSES

A dissertation submitted to the
SWISS FEDERAL INSTITUTE OF TECHNOLOGY ZURICH
for the degree of
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presented by
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1979
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1. INTRODUCTION

Rapid increase in world population especially in developing countries has brought about nutritional problems. Protein is a major nutritional requirement of humans and animals. Protein deprivation causes long term health damages. Expansion in the production of conventional protein sources such as agricultural products, cattle and fish is limited by many factors such as the availability of land, or the climate. The need for new protein sources became more and more obvious. Besides the conventional protein sources, protein from microorganisms, or single cell protein (SCP) offered an attractive alternative.

Microbial protein can be of high nutritive value and microorganisms double their biomass within a relatively short period of time, in the magnitude of minutes to hours. They can be grown on a variety of carbon sources and it is even possible to use waste materials and substrate such as agricultural wastes, cellulosic materials and many different by-products and waste sources. The fast doubling time and the use of cheap carbon sources made SCP a potent challenger to conventional protein sources.

The economical viability of SCP is influenced by a number of factors. In addition to factors concerning the process itself such as the choice of organism and of growth medium, and details of construction, the economics of SCP are greatly affected by world market situations such as the price and availability of competing protein sources, e.g. soya and fish meal and limited foreign currency.

Factors other than purely technological or economical ones, such as geographic, political, sociological and psychological situations also have a significant influence and should not be excluded.
Because of this complexity, the economical viability for production of SCP fodder has to be taken into account on a local basis.

In this dissertation, the study of continuous production of SCP was initiated because of the availability of a surplus of molasses in Iran where about 70% of the protein demand has been met by imported fish meal and soya (data up to 1975).

Besides the design and development of new bioreactors with better mass-transfer capacities and low energy consumption, the investigation of the optimum physical and chemical culture conditions are of major importance for the improvement of biomass productivity and for efficient utilization of the substrate.

The main objective of this study was to find the effects of physical and chemical parameters, as well as medium concentration on the biomass productivity and quality. This is clearly linked to the selection of a suitable organism.

Yeasts like Saccaromyces cerevisiae, Candida tropicalis and Candida utilis have frequently been used for the production of SCP. Cultivation of these yeasts under extreme conditions, that is high medium concentrations necessary for high productivity are usually limited by biological regulatory systems like repression by glucose or oxygen. This leads to the formation of products other than biomass and therefore reduces the maximum biomass production rate. In the present work, the obligate aerobic yeast *Trichosporon cutaneum*, because of its high potential as a yeast for SCP production and its relatively high yield and protein content with no ethanol formation, was chosen and the effect of physical and chemical culture conditions on its production rate and product quality were studied.
### 2. SYMBOLS, ABBREVIATIONS AND DEFINITIONS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$B_0$</td>
<td>Contois constant for oxygen</td>
<td>(atm $1 \text{ g}^{-1}$)</td>
</tr>
<tr>
<td>$B_S$</td>
<td>Contois constant for sugar</td>
<td>(g $\text{ g}^{-1}$)</td>
</tr>
<tr>
<td>$[\text{CO}_2]$</td>
<td>Volumetric fraction of CO$_2$ in the gas stream on moisture-free basis.</td>
<td></td>
</tr>
<tr>
<td>$C_L$</td>
<td>Concentration of oxygen in the liquid phase in bulk</td>
<td>(mg $\text{ l}^{-1}$)</td>
</tr>
<tr>
<td>$C_0^*$</td>
<td>Saturation concentration of oxygen in the liquid phase in bulk</td>
<td>(mg $\text{ l}^{-1}$)</td>
</tr>
<tr>
<td>CPR</td>
<td>CO$_2$ production rate</td>
<td>(mmol $\text{ l}^{-1} \text{ h}^{-1}$)</td>
</tr>
<tr>
<td>$D$</td>
<td>Dilution rate</td>
<td>(h$^{-1}$)</td>
</tr>
<tr>
<td>$D_c$</td>
<td>Critical dilution rate</td>
<td>(h$^{-1}$)</td>
</tr>
<tr>
<td>$D_{\text{max}}$</td>
<td>Maximum dilution rate</td>
<td>(h$^{-1}$)</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxy ribonucleic acid</td>
<td></td>
</tr>
<tr>
<td>DW</td>
<td>Dry weight</td>
<td></td>
</tr>
<tr>
<td>FBT</td>
<td>Flat blade turbine</td>
<td></td>
</tr>
<tr>
<td>$H$</td>
<td>Henry's constant for oxygen</td>
<td>(mol $\text{ l}^{-1} \text{ atm}^{-1}$)</td>
</tr>
<tr>
<td>$K_{G,a}$</td>
<td>Oxygen transfer coefficient based on partial pressure driving force and total reactor volume; $K_{G,a} = H K_{L,a}$</td>
<td>(mol $\text{ l}^{-1} \text{ h}^{-1} \text{ atm}^{-1}$)</td>
</tr>
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<td>$K_{L,a}$</td>
<td>Oxygen transfer coefficient based on concentration driving force and total reactor volume</td>
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<td>$K_0$</td>
<td>Monod constant for oxygen</td>
<td>(atm)</td>
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<tr>
<td>$K_S$</td>
<td>Monod constant for sugar</td>
<td>(g $\text{ l}^{-1}$)</td>
</tr>
<tr>
<td>$l$</td>
<td>Litre</td>
<td></td>
</tr>
<tr>
<td>mm Hg</td>
<td>Millimeters of mercury</td>
<td></td>
</tr>
<tr>
<td>$m_0$</td>
<td>Maintenance coefficient for respiration</td>
<td>(mmol $\text{ g}^{-1} \text{ h}^{-1}$)</td>
</tr>
<tr>
<td>$m_S$</td>
<td>Maintenance coefficient for substrate</td>
<td>(g $\text{ g}^{-1} \text{ h}^{-1}$)</td>
</tr>
<tr>
<td>$N$</td>
<td>Normality</td>
<td></td>
</tr>
<tr>
<td>$[\text{N}_2]$</td>
<td>Volumetric fraction of nitrogen in the gas stream on moisture-free basis.</td>
<td></td>
</tr>
</tbody>
</table>

$[\text{N}_2]$$_{\text{in}}$, for inflow; $[\text{N}_2]$$_{\text{out}}$, for outflow
Normal condition

Volumetric fraction of oxygen in the gas stream on moisture-free basis. 

\[ [O_2] \text{in}, \text{ for inflow}; \ [O_2] \text{out}, \text{ for outflow} \]

Oxygen transfer rate

Oxygen uptake rate

Absolute pressure of gas in rotameter, dry basis

Partial pressure of oxygen in gas phase in bulk

Partial pressure of oxygen which is in equilibrium with bulk concentration in liquid phase \( (c^L_O) \); dissolved oxygen tension

Part per million (v/v)

Polypropylene glycol 2000

Gas (air) flow rate at normal condition

Specific CO₂ production rate

Specific oxygen uptake rate

Specific growth-limiting substrate uptake rate

Linear correlation coefficient of ordinate on abscissa

Ribonucleic acid

Revolutions per minute

Respiratory quotient

Concentration of growth-limiting substrate in bioreactor

Single cell protein

Concentration of growth-limiting substrate in the inflowing medium to the chemostat

Standard error of estimate of \( y \) on \( x \)

Substrate transfer rate

Temperature

Total reducing sugars expressed as glucose
v/v  Volume per volume
v/vm Volumetric gas flow rate (normal condition, dry basis) measured at inlet of the bioreactor
w/v  Weight per volume
w/w  Weight per weight
X    Total biomass concentration (g l⁻¹)
Yₑ   Maintenance respiration yield; endogenous yield constant on oxygen; "equivalent biomass" per oxygen (g mmol⁻¹)
Yₒmax Observed yield from oxygen (g mmol⁻¹)
Yₒmax Maximum yield coefficient for oxygen; true growth yield from oxygen (g mmol⁻¹)
Yₒ  Observed yield from sugar (g g⁻¹)
Yₒmax Maximum yield factor for sugar; true growth yield from sugar (g g⁻¹)
μ    Specific growth rate (h⁻¹)
μₑ   Specific maintenance rate (h⁻¹)
μₘ   Maximum specific growth rate (h⁻¹)
θ    Time (h)

Aerobic growth, oxidative growth, respiratory growth:
Aerobic or oxidative energy-yielding process of the living cell in which oxygen is the acceptor of hydrogen removed from the substrates

Brix scale:
A hydrometer scale used in the food industry. Brix, is an expression of density equivalent to the % by weight of sucrose in solution

Crabtree effect, respiratory repression, glucose effect, Glucose repression:
Decrease in specific activity of the enzymes of the tricarboxylic acid cycle, respiratory chain, and glyoxylate cycle
Equivalent biomass:
The biomass that could theoretically be produced with the amount of energy source used for maintenance

Henry's law:
\[ L_c^0 = H p_o^L \]

Maintenance respiration yield:
The equivalent biomass formed per unit mass of oxygen consumed for maintenance

Maintenance coefficient for respiration:
The amount of oxygen used by cells per unit time for maintenance

Maintenance coefficient for substrate:
The amount of substrate used by cells per unit time for maintenance

Maximum yield coefficient for oxygen:
The biomass theoretically formed per unit mass of oxygen consumed, if no oxygen were required for maintenance \((m_o = 0)\)

Maximum yield factor for substrate:
The biomass theoretically formed per unit mass of TRS consumed, if no energy were required for maintenance \((m_s = 0)\)

Normal condition = Normal temperature and pressure:
\(760 \text{ mm Hg} \text{ and } 0 \degree C\)

Pasteur effect:
The regulation of glycolysis in the presence of oxygen

Respiratory quotient:
\(\text{CPR} / \text{OUR}\)

Specific maintenance rate:
The equivalent biomass formed per unit biomass present per unit time

Standard error of estimate of \(y\) on \(x\):
\[
s_{y-x} = \sqrt{\frac{(Y-Y_{\text{est}})^2}{N}}
\]
3. MATERIALS AND METHODS

3.1. Strains and maintenance of cultures

Three yeast strains, *Candida tropicalis* ATCC 32113, *Candida utilis* H1074 (Institute of Microbiology, ETH, Zürich, Switzerland) and *Trichosporon cutaneum* 70698 (German Collection of Microorganisms) were used throughout this work.

The yeasts were maintained by a monthly subculture on agar slants.

3.2. Processing of the molasses

Molasses contains variable amounts of suspended particles and colloidal material and experiments have shown that they may contain toxic material (BERGANDER, 1969). It must be processed to render it a suitable substrate for microbial growth.

There are various methods of the processing. One is the hot acid method, which can be carried with a variety of acids (NEUGEBAUER et al., 1972). The hot acid treatment neutralizes the lime (used in the manufacture of sugar) sets free the volatile acids, and breaks up nitrites, thus liberating nitrogen peroxide. It also removes insoluble calcium salts and organic colloids, the main coloured products of alkaline break down of invert sugar and melanoidins, and some of the microorganisms (GORBATAYA et al., 1973).

Sulphuric acid was replaced with phosphoric acid, which can also serve as a source of phosphorus for microbial growth. The amount of sedimented material was slightly lower when phosphoric acid was used. According to PALAGINA et al., (1965), treatment of molasses with phosphoric acid increases the yield of baker's yeast by 6-7 %.
The treatment was carried out as follows:
Molasses was diluted with water in a 1:1 ratio and the pH was adjusted to 4.0-4.1 with orthophosphoric acid. The mixture was degassed in order to eliminate residual dissolved SO₂, heated for 15 min at about 80 °C, degassed and then filtered (filter housing type LAB 10SG, filter MICROWYND II type D-PPP-Y, Skan) to remove precipitated impurities.
The treated molasses was kept in containers at 4 °C. Mould growth on the surface was prevented by completely filling the containers and by occasional mixing.

Colloids which were not separated during the initial processing, appeared after sterilization of the final made up medium. These suspended particles contained about 70 % (w/w) carbon and they were not harmful to the yeasts and did not change the yield of *Tr. cutaneum*. However, they may have caused excessive foam formation, errors in biomass determination, and colour adsorption by the yeasts. Therefore they were separated by sedimentation in a continuous separating funnel.

3.3. Media

3.3.1. YEPD

This medium was used for pre-cultures and agar slants. It contained: Yeast extract (Difco), 1.0 %; Bacto-peptone, 2.0 %; Glucose, 2.0 % and, for agar slants, Oxoid agar No. 3, 1.5 %.

3.3.2. Complete molasses-salts medium (M1)

The medium was that described by FIECHTER, (1968). For 10 g TRS of sulphuric acid-treated molasses, salts, trace elements, and vitamins were:

\[(\text{NH}_4)_2\text{SO}_4, \ 2 \text{ g}; \ (\text{NH}_4)_2\text{HPO}_4, \ 0.64 \text{ g}; \ \text{KCl}, \ 0.29 \text{ g}; \ \text{MgSO}_4\cdot\text{H}_2\text{O}, \ 0.15 \text{ g}; \ \text{CaCl}_2\cdot\text{H}_2\text{O}, \ 94 \text{ mg}; \ \text{CuSO}_4\cdot\text{H}_2\text{O}, \ 0.78 \text{ mg}; \ \text{FeCl}_3\cdot6\text{H}_2\text{O}, \]
4.8 mg; ZnSO₄·7H₂O, 3 mg; MnSO₄·2H₂O, 3.5 mg; biotin, 0.01 mg; m-inositol, 20 mg; Ca-pantothenate, 10 mg; thiamine, 2 mg; pyridoxine, 0.5 mg.

3.3.3. Reduced-salts medium (M2)

Salts and vitamins for 10 g of TRS of the phosphoric acid-treated molasses were:
NH₄Cl, 2.88 g; MgSO₄·7H₂O, 0.15 g; CaCl₂·2H₂O, 73.49 mg; stock vitamin solution as in medium M1.
The concentration of NH₄Cl in medium did not exceed 10.79 g l⁻¹.

3.4. Bioreactor

The cultivations were carried out in a 7 l glass bioreactor (Fig. 1).
The impeller contained two disc turbines with 4 vertical blades extending equidistant on both sides of the disc. In most of the experiments, an aeration rate of 1 vvm was used. According to SOLOMONS (1961), air supplied in excess of this value is largely wasted and does not improve the OTR of the system. Higher aeration rates also lead to foaming difficulties and increase the rate of evaporation.
The medium was introduced through a sparger with an arrangement described by HERBERT et al., (1965).

3.5. Control instrumentation

3.5.1. Temperature and pH control

The temperature control system was an on/off system and could provide ± 0.5 °C of the set point.
The pH measurement was carried out by a steam-sterilizable electrode and was controlled by a time proportional-integral control system (type IM 276 Metrohm).
The tubes required to introduce acid and alkaline for pH
control were partially immersed in the culture medium at the impeller flow intake which prevented the retardation of mixing and consequent poor control of pH (overshoot) caused by the foam layer.

4 and 8 N solutions of $\text{PO}_4\text{H}_3$ and $\text{NH}_4\text{OH}$ were used as required.

Fig. 1 The main dimensions of the bioreactor used in the experiments. Units in mm. 1 : Baffels; 2 : Cooling coils; 3 : Air + Medium in; 4 : Sparger; 5 : FBT; effective volume : about 4 l
3.5.2. Volume control

For volume control a stand-pipe arrangement was used. If the overflow is situated in a layer of foam, the liquid so removed may have an enhanced concentration of microorganisms. In order to prevent this, the actual draw-off point was made below the culture foam level. In order to maintain a constant level, the surface of the culture must be kept flat. Therefore a cylinder with longitudinal holes, nearly six times larger in diameter, was mounted centrally on the take off tube and partly immersed in the culture media. A flow inducer (type MHRE/200, Watson-Marlow) was used to draw off all the culture fluid entering the overflow pipe. The pump was set at a rate greater than that of the medium feed pump.

At intervals during cultivation, stirring and aeration were momentarily stopped in order to note the volume of the culture for adjustment of the dilution rate and the vvm. With such an arrangement it was possible to keep the variations of the volume within \( \pm 1 \% \) for a definite gas flow and dilution rate.

Wall growth was prevented by periodic vigorous stirring for a few minutes.

3.5.3. Liquid flow control

The medium inflow was controlled by means of a diaphragm metering pump (Model Fe 211, B. Braun Melsungen) with a dosing error of less than \( \pm 2 \% \) of the full scale. The static head on the pump was kept constant by means of a level controler.
3.6. Analytical procedures

3.6.1. Pressure

Operating pressures of the bioreactor and rotameters were measured using an open U-tube manometer. Barometric pressure was measured with a closed mercury barometer. Manometer readings were corrected for standard temperature, 0 °C (WEAST, 1977).

3.6.2. Gas flow rate

Measurement and control of gas flows (compressed air, oxygen and nitrogen) were carried out by means of variable area flow meters (type DK 48/N, Rl/4 NPT, Krohne, Vögtlin) with the accuracy of ± 2 % of the full scale.

The accuracy of rotameters depends upon having gas supplied at a constant pressure. Since there were variations in the pressure of the main compressed gas pipeline, the rotameters were equipped with a flow controller (type REN, Krohne, Vögtlin) which maintains a constant mass flow of gases with constant upstream pressure and variable downstream pressures. Gas (air) flow rate at zero humidity can be calculated from the following equation.

For pressures higher than 760 mm Hg:

\[
Q = \frac{\text{Rotameter reading}}{f_t f_p f_L}
\]

\(Q\) = Equivalent air flow rate at NTP

\(f_t\) = Factor for converting the working temperature of the gas to the reference temperature (\(f_t = ((273 + t)/(273 + 20))^{1/2}\), for rotameter calibration at 20 °C).

\(f_p\) = Factor for converting the working pressure to the normal pressure, 760 mm Hg (\(f_p = (760/P)^{1/2}\)).

\(f_L\) = Factor for converting the applied gas to the standard gas (air).
3.6.3. Oxygen uptake and carbon dioxide output

The oxygen content of the effluent gas stream was measured by a paramagnetic O\textsubscript{2} -analyzer (Oxygor 3N, Maihak). The CO\textsubscript{2} content of the effluent gas stream was measured using an infrared CO\textsubscript{2} -analyzer (Unor 4N, Maihak). The instruments were calibrated before each measurement using standard mixtures of oxygen, CO\textsubscript{2} and nitrogen. The water vapour content of the effluent gas was removed by a cold water condenser, followed by an electric sample gas cooler (model CGEK, Hartmann & Braun) and a CaCl\textsubscript{2} drying column.

The oxygen uptake rate can be calculated from the relative oxygen and nitrogen concentrations in the gas phase (see also symbols and abbreviations):

\[
\text{OUR} = \text{vvm} \cdot \frac{[O_2]_{\text{in}} - [O_2]_{\text{out}}}{[N_2]_{\text{in}} - [N_2]_{\text{out}}} \quad (2)
\]

The CO\textsubscript{2} production rate can be calculated from the relative CO\textsubscript{2} and nitrogen concentrations in the gas phase:

\[
\text{CPR} = \text{vvm} \cdot \frac{[CO_2]_{\text{out}} - [CO_2]_{\text{in}}}{[N_2]_{\text{out}} - [N_2]_{\text{in}}} \quad (3)
\]

3.6.4. Dissolved oxygen tension and absorption coefficient

The dissolved oxygen tension in the bioreactor was measured by means of a membrane covered oxygen electrode (model 531, IL, Ingold).
The function of the electrode was checked to see if it could be affected by a rapid liquid flow. It was found that for the moderately rapid stirrings employed in this work, the polarographic current was independent of the flow conditions around the electrode.

Carbon dioxide did not have any effect on the electrolyte and electrode of the oxygen probe. The effect of sulphur dioxide was not checked. The electrode was calibrated using various standard gases with different oxygen contents. The recorder readings were directly proportional to the partial pressure of oxygen.

In order to prevent microbial growth on the membrane, which causes wrong measurements, almost daily cleaning of the electrode was necessary. Before measurements, the electrode was cleaned, recalibrated and sterilized using a solution of 70 % (v/v) ethanol and 1 % (v/v) concentrated HCl. Using phenol-ethylalcohol solution for this purpose showed to be harmful for electrolyte of the oxygen electrode.

A sealed polarograph cell (Rank Brothers) was also used for the in vitro determination of \( K_0 \) (the Monod constant for oxygen).

For the determination of the absorption coefficient, the rate of oxygen transfer from gas to liquid is given by:

\[
\text{OTR} = K_L a \left( c_0^* - c_L^* \right)
\]  

In terms of oxygen tensions:

\[
\text{OTR} = K_L a H \left( p_O^G - p_O^L \right) = K_G a \left( p_O^G - p_O^L \right)
\]
Or:

\[ p^L_0 = - \frac{1}{K_{Ga}} (\text{OTR}) + p^G_0 \]  

(6)

A plot of the steady state values of dissolved oxygen tension versus corresponding steady state values of OTR should give a straight line until oxygen begins to limit the respiration rate.

Fig. 2 Dissolved oxygen tension versus corresponding values of OTR under steady state conditions for the determination of \( K_{Ga} \) and \( P^G_0 \) in a continuous culture of *Tr. cutaneum*. Medium = M2 containing 4.9 g l\(^{-1}\) TRS; aeration rate = 1.0 vvm; \( t = 35 \, ^\circ C \); pH = 4; 250 ppm PPG

For an agitation rate of 1200 rpm:

\( K_{Ga} = 0.8 \, \text{mol l}^{-1} \text{ h}^{-1} \text{ atm}^{-1}, \, P^G_0 = 0.194 \, \text{atm} \)

For an agitation rate of 900 rpm:

\( K_{Ga} = 0.6 \, \text{mol l}^{-1} \text{ h}^{-1} \text{ atm}^{-1}, \, P^G_0 = 0.190 \, \text{atm} \)
$K_G a$ can be calculated from the slope of this line and $p_0^G$ from the intercept on the $P_0^L$ axis where $OTR = 0$. An example is demonstrated in Fig. 2.

It is noteworthy that the static method for the determination of overall oxygen transfer rate coefficient is independent of the probe properties and any bubble-membrane interactions.

### 3.6.5. Foam

Static measurement (HALL et al., 1973) of foam was used for comparison between the foam production by various strains and was carried out as follows:

100 ml of broth, in a 250 ml baffled shake flask, was stirred (magnetic stirrer at its maximum speed) and aerated (1 vvm) through a perforated plate at 30°C for 10 min. The solution was then put into a 250 ml graduated glass cylinder and the height of the foam formed was measured after 10 min (equilibrium time). The heights were expressed as relative values to the lowest obtained height (see Tab. 1).

Effectiveness of the various antifoams was studied by a dynamic method as follows:

Air was introduced at the rate of 1 vvm through a perforated plate at the bottom of a graduated glass cylinder containing culture after adding of 250 ppm of various antifoams. The height of the foam formed was measured after 15 min and this was compared with the height obtained with the same culture without addition of antifoam.

### 3.6.6 Viscosity

The viscosity of the broth was measured by means of a four-speed Brookfield Syncro-lectric viscosimeter.
3.6.7. Ethanol

For ethanol measurement a gas chromatograph (5830 A Hewlett Packard) was employed. A 1.8 m, 2 mm column packed with Porapak QS, 100-120 mesh was used with a flame ionization detector. The injector and detector temperatures were 300 °C and the column oven operated isothermally at 140 °C.

3.6.8. Total carbon in supernatant

The total carbon content of the supernatant was measured with a total carbon analyzer (Rapid C, Heraeus). Temperature of the combustion column: top 1000 °C, bottom 850 °C.

Titrating solution: 0.1 N-tetra butyl ammonium hydroxide in isobutanol

Absorbing solution: 150 parts N,N-dimethylformamide, 2 parts monoethanolamine and 0.2 part 1 % (w/v) thymolphthalein in N,N-dimethylformamide.

3.6.9. Measurement of total reducing sugars (TRS) in molasses

Sugars present in molasses include sucrose, the reducing (invert) sugars glucose and fructose, raffinose, and nonassimilable sugars.

The major sugar constituents of molasses are sucrose (30-40 % in cane, 48-52 % (w/w) in beet molasses) and reducing sugars (15-20 % in cane, 0.2-1.8 % (w/w) in beet molasses). Raffinose is present in relatively small amounts in beet molasses (OLBRICH, 1956; BAKER, 1968; SCHIWECK et al., 1973).

Measurements of these sugars if done separately, involves analytical methods which are time consuming and subject to interference by other medium components. Therefore a method which can determine accurately the total reducing sugars without interference by other substrates should be considered.
Various methods can be found in literature for measuring total sugars (Fehling, Anthrone, Luff-Schoorl, Picric acid, Somogyi, Phenol-sulfuric acid), but most of them lack either specificity or precision, or do not account for interfering substances.

The search for selection an appropriate method led to a modified method which was originally suggested by HOFFMANN, (1937) for the determination of glucose in blood and urine. This method is described in 3.6.9.2.

3.6.9.1. Decolourization of molasses

Molasses contains coloured materials. By fractionation of this coloured matter BUGAENKO et al., (1972) have shown 5 types of components, 4 of which were melanoidine type N-compounds containing 7-11 amino acids each and having molecular weights of 700-30000 daltons. The 5th component fraction was a mixture of N-free caramel compounds.

From the absorption spectra of molasses at different concentrations shown in Fig. 3, it is to be expected that the coloured substances will interfere with the spectrophotometric determination of sugars in molasses. The absorption curves remain similar for all the coloured products (AGARWAL et al., 1972).

If the sugar contents of the samples are more than 0.1 g l$^{-1}$, the ratio of necessary sample volume for sugar determination to the volume of diluent and/or reagent is such that the colour of the samples do not influence the results of sugar assays.

* The author wishes to thank Mr. Toshihiko Ohno for his helpful advices on this part of the work.
However, samples of lower sugar content should be decolourized in order to prevent colour interferences.

A cation exchange, for example, can remove metal ions, amino acids, protein and other interfering materials (SEQUEIRA, 1970).

Fig. 3 Absorption spectra of molasses at different concentrations (concentrations are given as equivalent TRS) using a Beckman DB-GD spectrophotometer.
For this purpose two strong and two weak types of ion-exchange-resins were employed:
- Strong acid: IR-120 from Sera
- Strong basic: IRA-93 from Sera
- Weak acid: IRC-50 from Sera
- Weak basic: IR-45 from BDH

Fig. 4 Absorbance of a molasses solution after treatment with various quantities of ion-exchange resins. Samples were measured against water at 425 nm in a Beckman DB-GD spectrophotometer using 1 cm cuvettes. Curve A, IR-120; curve B, IRA-93; curve C, IRC-50; curve D, IR-45.
The $\text{H}^+(\text{OH}^-)$ form of the cation (anion)-exchange-resins were obtained by washing the materials first with 1 N hydrochloric acid (sodiumhydroxide) and then with distilled water until the pH of the effluent was the same as that of the distilled water. Water and fines were decanted and 25 ml from a solution of molasses was added to 5 to 60 g of each one of the regenerated resins, mixed and settled for 6 min. After decanting, the absorbance of the solutions were measured at 425 nm (Fig. 4).

It is evident from Fig. 4 that amberlite IRA-93 anion-exchanger seems to have a better capacity for removing the colour matter of molasses. Therefore application of this resin alone or with IR-120 cation-exchanger (two-stage extraction) may be recommended.

If the "Colour Value" is defined as the weight of resin required to reduce the colour intensity down to a certain point, by a treatment similar to that used in water purification from phenol by carbon, the results can be interpreted according to the Freundlich isotherm formula (WEBB, 1964):

$$\log \left(\frac{\text{Ab}_o - \text{Ab}_r}{m}\right) = \log K + \frac{1}{n} \log \text{Ab}_r$$  \hspace{1cm} (7)

$\text{Ab}_o$, $\text{Ab}_r$ = Initial and residual absorbance of molasses solution
$m$ = Weight of added resin
$K$, $n$ = Constants applying to the particular resin.

If $(\text{Ab}_o - \text{Ab}_r)/m$ is plotted against $\text{Ab}_r$ on log-log paper, a straight line should result. The values of $K$ and $n$ can be calculated from the intercept and the slope of this line respectively. For IRA-93 resin, by applying Eq. 7, it can be concluded:

$$m_{cv} = 250 \text{Ab}_o - 12.5$$  \hspace{1cm} (8)
In which \( m_{cv} \) is the necessary wet weight of IRA-93 resin that can reduce the absorbance of 25 ml of a molasses solution from \( A_{0b} \) to 0.05.

By applying a two-stage extraction (IRA-93 and IR-120) not only may the amount of the required resin be reduced, but also more complete removal of the colour substances from molasses is achieved.

The recovery of sugars after treatment of the samples with ion-exchange resin was between 85-100 % and it is desirable that the standards be treated in the same manner as samples.

3.6.9.2. Manual determination of total reducing sugars in molasses

Total reducing sugars were measured as glucose based on the hexacyanoferrate (III)/(II) redox reaction.

Reagent: 0.61 g of \( K_3 Fe(CN)_6 \) dissolved in 1 L of 1 N NaOH solution.

Procedure: The reaction is conveniently carried out in graduated 10 ml test-tubes. Into these was measured 5 ml of sample (50-500 \( \mu \)g glucose) followed by 2 ml of 1 N hydrochloric acid. A standard glucose solution was prepared simultaneously. The tubes were placed in a water-bath at 70 °C for 10 min to hydrolyze the sucrose. 3 ml of the reagent was added and mixed well. After heating the samples in a boiling water-bath for 5 min and cooling in cold tap water, their volume was adjusted to 10 ml with distilled water if necessary. The optical densities of samples and standards were read, using a water blank in a suitable spectrophotometer at a wavelength of 425 nm and 1 cm cuvettes.

Comments on the method: The disappearance of the yellow colour of hexacyanoferrate (III) obeys Beer's law (Fig. 5). Sucrose, glucose, fructose, all gave quantitatively the same colour value per mole of glucose and their absorption spectra had two peaks (Fig. 6).

To avoid diluting the samples, the reagent concentration may be increased or the sample volume decreased.
Fig. 5
Determination of total reducing sugars. Method as described in text was applied to standard solutions of glucose. Samples were measured against water at 425 nm in the Beckman DB-GD spectrophotometer using 1 cm cuvettes.

Fig. 6
Absorption spectra in the hexacyanoferrate (III)/(II) redox reaction. Samples were read against water in the Beckmann DB-GD spectrophotometer, using 1 cm cuvettes.
△, 0.25 mg D-glucose
□, 0.25 mg D-fructose
○, 0.25 mg D-sucrose
Formaldehyde, glycerol, ethanol, pyruvate, acetate, benzoate and ammonium salts had no detectable effects on the measurement. Acetaldehyde caused considerable errors.
Data on the recovery of different sugars and more information can be found in JANSHEKAR et al., (1977).

3.6.9.3. Automated determination of total reducing sugars in molasses

Fig. 7A shows a continuous automated procedure adapted from the method described in 3.6.9.2.
With this technique, sugar concentrations up to $10 \text{ g l}^{-1}$ can be determined directly. Since, in this method, a decrease in colour intensity is measured (inverse colourimetric technique), the selectivity and precision of measurement at low sugar concentrations is poor, and therefore an alternative manifold should be employed (Fig. 7B).
With this technique, up to 40 samples per hour can be processed. For details see JANSHEKAR et al., (1977).

3.6.10. Biomass dry weight

The cell harvest and biomass dry weight determination were performed in duplicate by two means:
- 25 ml of culture were centrifuged in the cold at 10'000 x g for 10 min and the supernatant fluid was frozen and retained for analysis. The cells were washed 2 to 3 times by re-suspension in 25 ml of distilled water and recentrifugation. The cells were then dried at 105 ± 2 °C to constant weight.

- 10 ml of culture was filtered through a tared membrane filter (type SM 13400, Sartorius). Filter and cells were then washed with small volumes of distilled water and dried to constant weight at 105 ± 2 °C.
Fig. 7  Flow diagram with manifold for the automated assay of sugar in molasses. Dimensions of pump tubing are shown in inches i.d.

A: Flow system for sugar concentrations of 1-9 g l⁻¹
B: Flow system for sugar concentrations of 0.1-1 g l⁻¹
The purity of continuous growing cultures were checked periodically by microscopic examination and by examining the morphology of the colonies on agar plates.

3.6.11. C, H and N content of the cells

The elementary analysis of cell dry matter (C,H,N) was carried out on freeze-dried cells by the Laboratory of Organic Chemical Microanalysis (ETH).

Freeze drying of the cells was carried out by the method described by HERBERT et al., (1971) in a vacuum tray dryer (Unitrap II, Vitris).

The freeze-dried material was placed in air-tight containers and kept frozen until analyzed.

3.6.12. RNA

RNA determination was carried out on freeze-dried cells using the orcinol method as described by HERBERT et al., (1971) after extraction of acid soluble material with cold 0.25 N HClO₄, and extraction of RNA with 0.5 N HClO₄ at 37 °C (TREVELYAN et al., 1956). The time required for extraction of RNA at 37 °C was found to be 120 min for applied yeasts.

For standards, ribonucleic acid from yeast (No.109215, Boehringer) was first dissolved in 0.1 N NaOH and then treated with 0.5 N HClO₄ in the same way as the samples.

3.6.13. DNA

DNA determination was carried out on freeze-dried cells using the modification of the Burton diphenylamine method described by HERBERT et al., (1971) after extraction of acid soluble material with cold 0.25 N HClO₄, and extraction of nucleic acids with 0.5 N HClO₄ at 70 °C.
A pure DNA preparation (from calf thymus, No. 10417, Boehringer) was used as a standard.

3.6.14. Protein

The total protein content of the cells was measured according to the modified biuret method of Robinson-Hogden described by HERBERT et al., (1971). The standards were made from a protein standard solution (No. 540-10, Sigma) containing 5.0 % (w/w) human albumin and 3.0 % (w/w) human globulin (gamma).

3.7. Sterilization

The apparatus in situ, the silicon tubings, the air filters, shake flasks, agar slopes etc., and media in 150 l quantities in a vessel with a stirrer, were autoclaved at 1 atm gage (121 °C) for 20 min.

3.8. Inoculation and sampling

The inoculum build up scheme from agar slants to bioreactor inoculum was as follows:

Freshly subcultured agar slant of the yeast

\[ \text{one loop} \]

50 ml YEPD in a 250 ml baffled shake flask, incubated for 20 h on a rotary shaker at a constant temperature of 30 or 35 °C

\[ \text{5 ml} \]

100 ml of medium in a 1 l baffled shake flask, incubated for 12 h on a rotary shaker at a constant temperature of 30 or 35 °C

\[ \text{5 % (v/v) inoculum} \]

Bioreactor
The sample line terminated within an inch of the impeller, ensuring that samples were as mixed as possible. The first 20-30 ml of the sample were rejected for washing out the sampling line.

The sample pipe outlet was immersed in an antiseptic solution (see 3.6.4.).

The samples were collected in a glass container which was kept in an ice bucket.

The sampling line was used for inoculation at the start of every run.

3.9. Chemicals

The molasses was obtained from Zuckerfabrik & Raffinerie Aarberg AG., Switzerland.

Rough routine analysis:
Brix = 81.0, ash = 13.2 %, polarization = 49.9, invert sugar 0.17 %, pH = 9

Chemicals were obtained from the following:
- BDH Chem. Ltd., Poole, England
- Boehringer, Mannheim, Germany
- Difco Lab., Detroit, Mich., U.S.A
- Fluka AG., Buchs, Switzerland
- Merck AG., Darmstadt, Germany
- Oxoid Inc., London, England
- Sera Feinbiochemica, Heidelberg, Germany
- Sigma Chem. Co., St. Louis, Mo., U.S.A
3.10. Equipment

The equipment was from the following companies:
Beckman-Riic Ltd., Scotland, England
B. Braun Melsungen AG., Eschborn, Germany
Brookfield Eng. Lab., Mass., U.S.A
Hartman & Braun AG., Frankfurt, Germany
Heraeus, Hanau, Germany
Hewlett Packard, Avondale, PA., U.S.A
Ingold AG., Zürich, Switzerland
Maihak AG., Hamburg, Germany
Metrohm AG., Herisau, Switzerland
Rank Brothers, Bottisham, Cambridge, England
Sartorius, Göttingen, Germany
Skan AG., Basel, Switzerland
Technicon Ltd., Swords Co., Dublin, Ireland
Vitris Co., Gardiner, N.Y., U.S.A
Vögtlin AG., Basel, Switzerland
Watson-Marlow Ltd., Cornwall, England
4. RESULTS AND DISCUSSION

4.1. Organism selection

Selection of the appropriate organism has the most important role in the economy of industrial production of SCP.

The ideal strain for this purpose should have the following properties:
- Fast growth at high substrate concentrations
- Efficient conversion of a variety of carbon sources to biomass
- High nutritive quality
- Non toxic
- Tolerant of low pH (lower probability of bacterial contamination)
- Tolerant of high temperature (lower cooling costs)
- Easy to handle (less foam generation, more easily separable from the culture).

Bacteria and yeasts are particularly suitable for the production of SCP on a commercial scale. Yeasts grow particularly well in the pH range 3-4, while the preferred pH for bacteria is 6.5-7.5. Bacterial cultivation at higher pH leads to difficulties in maintaining sterility. Yeast cells, as they are larger than bacteria, are easier to separate from the cultivation broth. However, yeasts have a lower growth rate and protein content than bacteria. But bacteria have higher nucleic acid content and their carbohydrate requirement is higher than yeasts for the same expenditure of oxygen.

When selecting an organism, the medium to be used must also be considered.

Molasses in aqueous solutions, like many other agricultural wastes used for microbial propagation, is strongly foaming. Foam inducing components of the molasses were shown to be caramels and organic acids rather than melanoids and arabinose fractions (VLASOVA, 1958).
In addition, foam is formed as a result of the metabolism of the organism. Foaming is also a strain characteristic. The interaction between foaming agents of the nutrient medium and the metabolites of the cultured microorganisms is the main cause of the foam intensity in microbial cultures. Thus foam production should be used as a criterion for strain selection.

This section presents a comparative study of different strains of fodder yeasts grown on molasses under identical conditions. For this purpose C. tropicalis, C. utilis, and Tr. cutaneum were used and the maximum growth rate, yield of biomass, protein and nucleic acid content, ethanol production and foam intensity were used to characterize these yeasts.

From the metabolic viewpoint the first two yeasts are reported to have a better carbon balance towards biomass accumulation as compared with a glucose sensitive yeast, like S. cerevisiae (KATO et al., 1972). The third one is reported to be a strictly aerobic yeast (ANDRZEJEWSKI et al., 1974).

The results of these experiments are given in Tabs. 1 and 2. Tab. 2 shows that the yeasts tested were similar with respect to the maximum specific growth rate.

A yield of 50 % dry weight of C. tropicalis and C. utilis obtained here under aerobic condition is accepted as typical (HARRISON, 1967). FENCL et al., (1961) confirm some of their earlier findings and indicate that in the batch process the yield of C. utilis on molasses only slightly exceeds 50 % and concluded that with the exception of AGARWAL, who used sugar cane molasses, no one succeeded in obtaining significantly higher yield than 50 % on molasses.

Of the 3 yeasts in this study, the highest yield of biomass was produced by Tr. cutaneum. The range of compounds oxidized by C. tropicalis and C. utilis is more restricted. A carbon
Table 1. Comparison of the growth of 3 yeasts on molasses. The cells were incubated in 100 ml of medium M1 containing 10 g l\(^{-1}\) TRS in 1 l baffled shake flasks for 24 h at 30 °C on a rotary shaker. The initial pH of the cultures was 6 and the measurements were made after 24 h of cultivation. The evaporated ethanol is not taken into account. Data on foam are relative height.

<table>
<thead>
<tr>
<th>Organism</th>
<th>a</th>
<th>b</th>
<th>Ethanol</th>
<th>Foam</th>
<th>Protein</th>
<th>RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. tropicalis</td>
<td>0.3</td>
<td>4.5</td>
<td>2.5</td>
<td>17.5</td>
<td>41.7</td>
<td>8.5</td>
</tr>
<tr>
<td>C. utilis</td>
<td>4.8</td>
<td>5.0</td>
<td>1.8</td>
<td>1.0</td>
<td>47.9</td>
<td>10.5</td>
</tr>
<tr>
<td>Tr. cutaneum</td>
<td>5.3</td>
<td>5.9</td>
<td>&lt;0.3</td>
<td>3.6</td>
<td>47.1</td>
<td>7.9</td>
</tr>
</tbody>
</table>

recovery averaging 99.9 % with a standard deviation of 4.2 confirms the correctness of the results (GSCHWEND, 1977a).

Comparison of the biomass end-concentration in the culture before and after complete elimination of foam (Tab. 1) shows that the inclination of yeasts to concentrate in the foam phase is different.

The RNA content of Tr. cutaneum was found to be slightly lower than that of the two other yeasts. C. tropicalis generated the most foam and its protein content was slightly less. The protein content found here is in the same order as those reported for fodder yeasts grown on molasses (IUPAC, 1963).
Table 2. Comparison of the growth of 3 yeasts on molasses.
Medium = M1 containing 50 g l⁻¹ TRS; aeration rate = 1.0 vvm; agitation rate = 1000 rpm; t = 30 °C; pH = 5.
Maximum specific growth rates were measured in the period of sugar utilization. Yield is the ratio of produced biomass to utilized sugar throughout the batch. Ethanol is the maximum detected in the batch without considering its evaporation. The values given are average of 1-4 determinations.

Fig. 8 shows the result from the continuous cultivation of C. tropicalis under aerobic conditions.
By increasing the sugar concentration in the medium, the ethanol production increased and occurred at lower dilution rates, causing a decrease in the yield coefficient and the productivity. In this experiment, dissolved oxygen tension in the broth was not measured. However, similar results are also reported for C. utilis when dissolved oxygen tension was kept above 50 % of saturation (MIAN et al., 1976).

GSCHWEND (1977b), compared the growth of C. utilis and Tr. cutaneum in a chemostat under similar cultivation conditions. He found that the specific rate of sugar consumption, Qₛ, by C. utilis can increase to 180 % that of Tr. cutaneum for dilution rates above 0.25 h⁻¹.
Fig. 8  Effect of substrate concentration and dilution rate on the ethanol production by \( \text{C. tropicalis} \).
Medium = M1 containing 10-70 g l\(^{-1}\) TRS; aeration rate = 3-6 vvm; agitation rate = 2000 rpm. Other culture conditions were as for Tab. 2.

According to KNOEPFEL, (1972), the repression in \( \text{C. tropicalis} \) appears only at high substrat concentrations and oxygen limitation. MIAN et al., (1976) reported that only sugar concentration beyond a threshold level resulted in the Crabtree effect. BOIDOL et al., (1976) reported the Crabtree effect in \( \text{C. utilis} \) when the specific growth rate exceeded 0.27 h\(^{-1}\), irrespective of the sugar feed rate. According to FENCL et al., (1961), excess of oxygen and according to NYIRI, (1975) the proper C/N ratio of the growth medium can prevent such repression.
Although in the above mentioned literature there is some controversy concerning the cause of this repression, all verify the existence of a respiratory repression in *C. tropicalis* and *C. utilis*.

As it can be seen from Tabs. 1 and 2 and will be shown later in continuous cultivation of *Tr. cutaneum*, the assimilation of carbon by this yeast even under oxygen limitation is strictly oxidative.

The amino acid and vitamin analysis of *Tr. cutaneum* can be found in TORSTEN, (1972). A toxicological study of this strain has not yet been done (WINDISCH, 1976).

### 4.2. Nutritional requirements

There is a considerable degree of variation between the types of molasses according to the country of origin, processing factory, season and condition of storage. Because of these differences in the composition and properties of various molasses, it is not possible to develop a medium which can be used as a "basal or standard" medium. The design of medium, which will be reported here, does not lead to the "most economical" medium but to a medium which supplies nutrients for optimal growth of *Tr. cutaneum*.

Tab. 3 shows the analysis of phosphoric acid-treated molasses for elements essential for microbial growth.

Tab. 3 and medium M1 (see 3.3.2.) show that the molasses used supplies enough phosphorous and potassium but not sufficient nitrogen, magnesium or calcium.
Table 3. Essential elements of treated molasses per 10 g of TRS.

Therefore ammonium chloride, magnesium sulphate, and calcium chloride were also included. The variety of trace elements in molasses is considerable (SUOMALAINEN et al., 1970) and there is no need for addition of them. Beet molasses is generally deficient in biotin, other growth factors like vitamins and amino acids are different in various molasses and therefore they were added according to medium M1. Further analysis is necessary to reduce the amount and number of these vitamins.

The designed medium was used in a batch culture. At the end of the growth, every one of the reduced or eliminated components were added to the culture and the reaction of the culture to any of these additions was followed. Besides the N-source, the culture did not react upon addition of any one of the reduced or eliminated components, showing the enrichment of the medium by these elements.

A nitrogen balance in the fluid phase of the chemostat, using this nitrogen limited medium, showed that the nitrogen content of the cells came only from the nitrogen added to the medium. Therefore it was concluded that the nitrogen of molasses was not assimilated by cells and should not be taken into account in medium design. Composition of the new designed medium (M2) is shown in 3.3.3.
A positive effect of glutamic or asparatic acid on the growth of *S. cerevisiae* (FIECHTER, 1968) and a stimulatory effect of a combination of glycine-proline-arginine on growth of *Tr. cutaneum* (SHIRALKAR et al., 1976) have been reported. Therefore the possibility of increasing the yield and maximum specific growth rate by addition of these amino acids to the culture medium was studied. But no effect was observed in batch experiments showing the adequacy of growth factors in the medium.

Finally, in order to establish experimentally that in the designed medium, carbon was limiting and that all other nutrient were present in excess, it was shown that at a fixed dilution rate, the microbial population density is directly proportional to the sugar concentration in the reservoir. Fig. 9 shows the relationship obtained between cell concentration and $S_R$.

In order to achieve high productivity, high concentrations of the substrate are used. This necessitates increasing the amount of supplemental nutrients accordingly. For many nutrients, there is a wide concentration range over which the specific growth rate does not significantly differ. There are, however some obvious exceptions of practical consequence, and all sufficiently soluble nutrients suppress growth at very high concentrations.

Special experiments were done to determine the optimal concentrations of other nutrients in more concentrated media. To check for toxicity of high supplement concentrations, the concentration of sugar was kept constant (5 g l$^{-1}$) and the concentrations of other ingredients of the medium were increased up to the concentration used for 100 g l$^{-1}$ sugar as follow:
Effect of different levels of TRS on the steady state concentration of *Tr. cutaneum* in a chemostat culture. Medium = 5-25 g l⁻¹, TRS; MgSO₄·7H₂O, 0.22 g l⁻¹; CaCl₂·2H₂O, 110 mg l⁻¹; biotin, 0.015 mg l⁻¹; m-inositol, 30 mg l⁻¹; Ca-pantothenate, 15 mg l⁻¹; thiamine, 3 mg l⁻¹; pyridoxine, 0.75 mg l⁻¹; and two different concentrations of nitrogen supply. Aeration rate = 1.0 vvm (air + O₂, [O₂] in = 0.42); agitation rate = 900 rpm; t = 30 °C; pH = 5; D = 0.26 h⁻¹.
Medium A: contained 5 g l\(^{-1}\) TRS and other supplements according to medium M2.

Medium B: The same as A, but the concentrations of magnesium and calcium salts and vitamins were increased by a factor of 20.

Medium C: The same as B, but the concentration of ammonium chloride was increased by a factor of 7.5.

Medium D: The same as B, but the concentration of ammonium chloride was increased by a factor of 10.

Medium E: The same as B, but the concentration of ammonium chloride was increased by a factor of 15.

The chemostat was operated at a high dilution rate (0.5 h\(^{-1}\)) so that the variations in effective yield (X/S\(_R\)) and maximum dilution rate (which both influence the productivity) could be observed at the same time. The results are presented in Tab. 4.

It can be seen that the cell biomass concentration for medium A, B, and C remained constant, therefore it can be concluded that an increase in the amounts of magnesium and calcium salts by a factor of 20 and for ammonium chloride by a factor of 7.5 did not have any adverse effect on the growth of cells. A further increase in the ammonium chloride concentration led to a retardation in growth and wash out.

The solubility of oxygen can be reduced by increasing the salt concentration in the medium without dissolved oxygen tension being affected. This may cause the culture to become oxygen deficient and can lead to wash out. Generally speaking, at increased salt concentration, the reduction in oxygen solubility can compensate for the increase in the oxygen transfer rate coefficient so that the oxygen transfer rate remains unaffected (ZLOKARNIK, 1978). However, considering the probable reduction of oxygen solubility caused by addition of slats in above mentioned mediums and dissolved oxygen tension data (Tab. 4), it can be shown that the oxygen has been sufficient for growth and could not be the cause of X reduction.
Table 4. Effect of the concentrations of supplemented salts on growth of *Tr. cutaneum*.

Composition of the mediums are given in text. Aeration rate = 1.0 vvm; agitation rate = 1200 rpm; 
$t = 35\, ^\circ C; \text{pH} = 4; D = 0.5 \, h^{-1}$. Data on $p_O^L$ are equivalent to 84-91 % air saturation.

The result may be due to the toxic effect of chloride (YADAV et al., 1973). It was not necessary to replace the ammonium chloride by other ammonium salts. By regulating the pH with ammonia, there was no probability of nitrogen limitation at high cell densities, this was evident from the nitrogen balance in the liquid phase.

Increasing the salt concentrations caused an increase in specific oxygen uptake and specific CO$_2$ production rates showing an inefficiency in carbon and oxygen consumption. This may be attributed to the increased maintenance energy required as the ionic strength of the medium increases (STOUTHAMER, 1973).

Another interesting feature of the results is that the protein and RNA content of the cells increased by 23 and 41 % respectively when the concentration of supplemented salts was increased. The reason for such a phenomenon is not clear and remains to be investigated.
4.3. Foam destruction

4.3.1. Choice of method and means

High effective yield and productivity in SCP production requires high rates of aeration and vigorous stirring. These conditions and the concentrated nutrient solutions commonly employed provide an ideal situation for foam formation. Foaming usually is not a problem with synthetic media because they do not contain any protein or relatively high molecular weight peptides. Formation of foam, however, is one of the recurring problems in working with molasses. Foam formation, by changing the agitation pattern of the culture, prolongs the time required to reach steady state conditions.

Foam produced by cultivation of yeasts on molasses is not well drained. Mechanical foam breakers, when employed, must be rotated at high speeds to be effective. This tends to increase the power consumption of the system considerably. Further, overflow volume control of the culture is much more difficult when mechanical foam breakers are used.

Because of these problems with mechanical foam breakers, chemical anti-foam agents were employed. The following were tested:

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silicon DB-31 emulsion</td>
<td>Dow Corning, Belgium</td>
</tr>
<tr>
<td>Silicon F 1265 fluid</td>
<td>&quot;</td>
</tr>
<tr>
<td>Silicon DC MC A compound</td>
<td>&quot;</td>
</tr>
<tr>
<td>Silicon RD emulsion</td>
<td>&quot;</td>
</tr>
<tr>
<td>Silicon 426 R</td>
<td>Rhodorsil, France</td>
</tr>
<tr>
<td>Polypropylen glycol 2000</td>
<td>Fluka, Switzerland</td>
</tr>
</tbody>
</table>
Tr. cutaneum was grown in molasses containing 10 g l\(^{-1}\) TRS in 1 l baffled shake flasks on a rotary shaker at 30 °C. After 12 h, towards the end of growth when the culture had its most foam, the pH was adjusted to 4 and foam intensity was measured (see 3.6.5). Of the anti-foams tested, only polypropylene glycol (PPG) 2000 completely destroyed the foam. Therefore it was chosen as the defoaming agent for the rest of the experiments.

PPG 2000 was not metabolized by the yeast, therefore it remained effective longer. It does not contain lipid peroxides which according to PARTESHKO et al., (1973) can reduce both the levels of vitamins and the yield of yeast. PPG 2000 does not accumulate on the surface of the microorganism as silicon anti-foam agents do. Thus it does not cause later difficulties during recovery of the product from the broth. It works well in the acid range used in biomass production whereas according to SOLOMONS, (1967) silicon anti-foams are more effective at alkaline pH values.

In continuous cultivations anti-foam was added to the incoming medium. Less anti-foam is required when it is added continuously. Furthermore, rapid changes in mass-transfer conditions in the chemostat are avoided (SOLOMONS, 1967).

No additional automatic equipment was used.

4.3.2. The effect of anti-foam on the growth of Tr. cutaneum

Increasing the concentration of molasses strongly increases the stability of foam by increasing its viscosity value. It was noticed that at a high concentration of molasses (more than 10 g l\(^{-1}\) TRS content), 250 ppm of PPG 2000 was not sufficient to destroy the foam completely, hence application of more anti-foam was necessary.
Fig. 10 Effect of the addition of anti-foam on the growth of *Tr. cutaneum* in a carbon limited chemostat culture. Medium = M2 containing 10 g l\(^{-1}\) TRS; aeration rate = 1.0 vvm; agitation rate = 900 rpm; \(t = 35\, ^{\circ}\)C; pH = 4; \(D = 0.4\, h^{-1}\).
Anti-foam agents, particularly those based on PPG or silicons, are generally considered nontoxic. This is not to say that they are without effect on microorganisms. Anti-foams are surface active agents and may be expected to influence the membrane functions of cells.

In order to study the effect of anti-foam on the metabolism of *Tr. cutaneum*, the concentration of anti-foam in the ingoing medium was increased from 250 to 1750 ppm. In the chemostat at a constant dilution rate, the yield, productivity and respiration of the cells, as well as their protein and RNA content were measured. The results indicate that under the conditions used the anti-foam had no effect on the cells (Fig. 10).

4.3.3. The effect of anti-foam on the oxygen transfer rate

There are various reports concerning the effect of anti-foam on the overall oxygen transfer rate coefficient (\(K_a\)) and its consequent influence on the oxygen transfer rate (OTR). It is accepted that the addition of anti-foam may cause an increase in bubble size and reduction of the interfacial area and consequent reduction of \(K_a\). It can also increase resistance to diffusion at gas-liquid interface. The net effect of the addition of anti-foam agent depends on the limiting step in the oxygen supply process.

In order to study the effect of addition of anti-foam (in the range employed in these experiments) on the overall oxygen transfer rate coefficient, the \(K_a\) was measured by the steady state method in the experiments described in 4.3.2. It was observed that the overall oxygen transfer rate remained almost constant when the concentration of anti-foam increased from 250 to 1750 ppm (Fig. 11).
Fig. 11 Effect of the concentration of anti-foam on the overall oxygen transfer rate coefficient measured by steady state method. Culture conditions were as for Fig. 10.

In highly agitated and aerated systems like the one used in this study, the anti-foam effect on the interfacial area is reduced. It is probable that most of the gas bubbles are not in close enough contact for the anti-foam to cause them to coalesce (HALL et al., 1973). It is possible that an anti-foam effect on $K_Ga$ would be seen if lower anti-foam concentrations were used (AIBA et al., 1973a).
4.4. Effect of temperature

4.4.1. Introduction

Temperature is one of the important engineering parameters in SCP production. Thermophilic or thermodurant strains are more suitable for production in tropical countries. Thermotolerant yeasts are reported to have an increase acidotolerance (SATTLER, 1966). Thermophily among the yeasts is a rather unusual phenomenon.

There are different reports concerning the effect of temperature on growth and metabolism rates of the cells. LOGINOVA, (1966) reported that the yeasts growing at high temperatures have higher growth and metabolism rates (see also ISAKOVA et al., 1974).

Temperature effects the nucleic acid content of the cells. Although in some cases a high nucleic acid content is desirable (as in flavour enhancing nucleotide production), a low nucleic acid content is necessary in SCP for human consumption.

Temperature affects the solubility of oxygen in the medium. It may also indirectly influence the mass-transfer rate of the system.

Few studies have taken into account all of these factors at the same time in continuous production of SCP (POZMOGOVA et al., 1966; COONEY et al., 1976). In this section the effect of temperature on growth characteristics of Tr. cutaneum in continuous culture and on the in vivo $K_G$ are described.
4.4.2. Experimental procedure

Cell characteristics like yield, respiration activity, composition, and physiology are functions of both environmental factors, like temperature and pH, and of growth rate.

A study of the true concentration of growth temperature on the above mentioned parameters, as distinct from those caused by alteration in growth rate, is possible only when a chemostat operating at a fixed dilution rate is employed. The maximum specific growth rate has been shown to change with temperature according to the Arrhenius relation. Therefore, in a chemostat at constant dilution rate, the relative dilution rate \( D/\mu_{\text{max}} \) changes with the temperature. If the cell concentration, yield, respiration activity and composition of the cells are also a function of \( \mu_{\text{max}} \), the determination of the temperature effect will not be possible in a chemostat operating at a fixed dilution rate.

However, for this study, a chemostat with \( D = 0.4 \text{ h}^{-1} \) which is the maximum dilution rate of \( \text{Tr. cutaneum} \) at 30 °C (Fig. 12) was used because:

- The temperature could be changed only within the range favourable for high growth rates (\( \mu \geq 0.4 \text{ h}^{-1} \)).
- A part of the substrate would be left unused at 30 °C and \( D = 0.4 \text{ h}^{-1} \). An increase in cell density would lower the residual substrate found. This decrease could be used to calculate a higher \( \mu_{\text{max}} \). Conversely, higher substrate concentration and lower cell density would indicate that \( \mu_{\text{max}} \) and hence maximum productivity was adversely affected by the temperature change.
- As washout occurs as soon as \( \mu_{\text{max}} \) drops below 0.4 h\(^{-1}\), the range of decrease of \( \mu_{\text{max}} \) is more limited at \( D = 0.4 \text{ h}^{-1} \) than at a lower \( D \). The effect of the relative growth rate on cell characteristics in this case may be assumed negligible.

With the chemostat so arranged, the temperature could be varied between 27 and 40 °C.
Fig. 12 Continuous growth of *Tr. cutaneum* at different temperatures. pH = 5.

The temperature of the culture was raised by 2 °C and the culture was allowed to reach its new steady state before the temperature was increased again. The steady state was determined from the dissolved oxygen tension in the broth, the biomass concentration and $Q_{O_2}$ and $Q_{CO_2}$ measurements. The dissolved oxygen tension never fell below 65 mm Hg. For the calculation of the yield from oxygen, assuming negligible endogenous respiration, the ratio of dilution rate to $Q_{O_2}$ was used.
4.4.3. Effect of temperature on growth and composition of 
Tr. cutaneum

Fig. 13 shows the effect of temperature on productivity, respiration, and growth yield from sugar and oxygen. A pronounced increase in biomass concentration was seen when the temperature was raised from 27 to 30 °C. From 30 to about 37 °C there was a slight increase in biomass which was followed by a sharp fall when the temperature exceeded 37 °C. At 40 °C, complete washout occurred.

The specific rate of oxygen consumption, $Q_{O_2}$, was markedly stimulated above 30 °C causing a decrease of $Y_O$. It is reported that the cytochrome content in C. tropicalis cells increases with increasing temperature (Loginova, 1966). Hence the respiratory rate of the cells could be correlated with their cytochrome content.

The observed yield from sugar remained constant between 27 and 37 °C. Above this temperature range, $Y_S$ decreased due to inefficient use of sugar. Decrease in yield was accompanied by a decrease in $Q_{O_2}$ and a sharp increase in RQ. This decrease in $Y_S$ could be due to increased cell morbidity or altered metabolism (as indicated by the change in RQ values). Temperature can affect the synthesis of individual enzymes as shown by Hunter et al., (1972). Alternatively the synthesis of intracellular and/or extracellular polysaccharide may be increased at high temperatures as predicted by Lodder, (1970). Such an effect has been observed by Brown et al., (1969) for C. utilis grown at a fixed rate under glucose limitation when the growth temperature was lowered from 30 to 15 °C. However, our present knowledge of the molecular basis of the temperature effect on polysaccharide synthesis in microorganisms is rather limited.
Fig. 13 Effect of temperature on the growth of \textit{Tr. cutaneum}.
Medium = M2 containing 10 g l\textsuperscript{-1} TRS; aeration rate = 1.0 vvm; agitation rate = 900 rpm; pH = 5; anti-foam = 250 ppm PPG; D = 0.4 h\textsuperscript{-1}.
More information regarding the responses to temperature changes was obtained by studying the cell composition (Fig. 14). The protein and RNA content of the cells decreased steadily 22 and 46% as the temperature was increased from 27 to 37°C respectively. Above this limit, they increased slightly. The bottom curve in Fig. 14 shows that the (P/NA)D ratio of the cells increases by 58% in the range 27 to 37°C and then falls sharply. Thus the "efficiency of nucleic acid in protein synthesis" at higher temperatures decreases, a further indication of altered metabolism. The above pattern of changes in RNA is similar to those reported by TEMPEST et al., (1965) for the growth of Aerobacter aerogenes grown in a chemostat under glycerol limitation. They assumed that the rate of protein synthesis per unit of ribosome is constant at a particular temperature. Increasing the temperature increases ribosomal activity so that in order to maintain a constant growth rate the cell requires less ribosomal RNA. This explanation could explain the decrease in RNA up to 37°C and the increasing "efficiency" of protein synthesis in the same range.

In contrast to TEMPEST et al., (1965) who found no change in DNA content with temperature, a gradual increase (more than 50% change) in DNA content was found. This may be due to reduced cell size at the intermediate temperature, but size distribution has not been studied in detail.

To summarize, above 37°C, the RQ of the culture rises significantly and the observed protein synthesis efficiency and $Y_S$ falls drastically. This is consistent with altered metabolism at higher temperatures.
Fig. 14 Effect of growth temperature on steady state composition of *Tr. cutaneum*. Culture conditions were as for Fig. 13.

P, protein; NA, total nucleic acid (DNA + RNA). Values in % of dry weight. $(P/NA)D = 0.4$ h$^{-1}$. 

$(P/NA)D = \text{efficiency of nucleic acid in protein synthesis.}$
4.4.4. Effect of temperature on the overall oxygen transfer rate coefficient

According to the semi-theoretical equation derived by O'CONNOR, (1956) on the basis of oxygen transfer bubble aeration in the activated-sludge process, $K_L a$ is proportional to the root of absolute temperature: $K_L a \propto (T)^{1/2}$.

AIBA et al., (1973 b) noted that this equation has not yet been applied under actual working conditions in bioreactors.

In order to study the effect of temperature on the overall mass transfer rate coefficient, $K_G a$ was measured via steady state oxygen balance as the temperature was changed between 27 and 39 °C (Fig. 15). The linear regression line shown has a correlation coefficient of 0.8 and slope of $-0.01 \text{ mol l}^{-1} \text{ h}^{-1} \text{ atm}^{-1} \text{ °C}^{-1}$.

Since the cultivation was in a dilute medium, the $K_G a$ is unlikely to be directly affected by a decrease in viscosity as the temperature increases (HATTORI et al., 1972). The effect may be attributed to the increased evaporation losses and rate of thinning which shortens the life of fluid foam (the proportion of gas dispersed in the liquid, fine dispersion of gas bubbles) as the temperature increases (BIKERMAN, 1973).
Fig. 15 Effect of temperature on the overall oxygen transfer rate coefficient. Culture conditions were as for Fig. 13.
4.5. Effect of pH

4.5.1. Introduction

pH is another important factor in SCP production. This parameter may be used to reduce the possibility of bacterial contamination which clearly may disrupt successful sterile continuous operation.

pH can alter the metabolism of the microorganism and cause undesired metabolic products. For example, yeast produces ethanol or glycerol and acetic acid from sugar if the pH is in acidic or alkaline respectively.

SHVETS et al., (1973) by growing four different strains of yeasts at various pH values noticed that a lowering of the pH led to a greater formation of organic acid and to fewer higher alcohols whereas this did not exert any influence upon the esters and aldehydes.

The effect of pH was studied by changing the pH from 5 towards 3, and 3 towards 6 at $D = 0.45 \text{ h}^{-1}$ allowing the culture to reach its new steady state before again changing the pH.

4.5.2. Effect of pH on growth and composition of Tr. cutaneum

Tr. cutaneum grown at a fixed dilution rate of $0.45 \text{ h}^{-1}$ increased its respiration activity when the pH was lowered from 6 to 3.5. At a pH of 3 the cells washed out, showing that their maximum growth rate decreased at this pH (Fig. 16). According to HARRISON et al., (1971) the reason for the increase in respiration activity can be attributed to the increase in ATP maintenance energy required.

The yield from sugar was also decreased at lower pH (Fig. 16).
Fig. 16  Effect of pH on the growth of *Tr. cutaneum*. 
Culture conditions were as for Fig. 13.
t = 35 °C; D = 0.45 h⁻¹.
Fig. 17 Effect of pH on steady state composition of *Tr. cutaneum*. Culture conditions were as for Fig. 16.

P, protein; NA, total nucleic acid (DNA + RNA). Values in % of dry weight. \( \frac{P}{NA}D \) = efficiency of nucleic acid in protein synthesis. \( D = 0.45 \text{ h}^{-1} \).
The cells grown at lower pH contained more protein and RNA but the "efficiency of nucleic acid in protein synthesis" remained almost constant (Fig. 17). This result is similar to that reported by ALROY et al., (1973) for C. utilis.

4.5.3. Effect of pH on the overall oxygen transfer rate coefficient

The effect of pH on $K_Ga$ has not yet been studied. Steady state measurement of the overall oxygen transfer rate coefficient showed a decrease with increasing pH of the culture (Fig. 18). This effect can be attributed to the influence of pH on the consistency of the liquid foam. SZARKA et al., (1969) and HALL et al., (1973) found that the stability of protein foams increases at lower pH, which can explain the observed increase in the liquid foam volume at lower pH (Fig. 18).

Fig. 18 Effect of pH on the overall oxygen transfer rate coefficient. Culture conditions were as for Fig. 16. A is the percentage of the increase in the actual working volume (proportional to the decrease in liquid foam volume) calculated on the basis of the actual working volume at pH of 3.5.
4.6. Continuous culture of Tr. cutaneum

4.6.1. Introduction

The maximum substrate utilization for the formation of biological matter with the highest yield is usually attained in continuous culture.

The maximum mass-transfer performance in a bioreactor is achieved only for a short time if a batch process is applied. In continuous culture, a constant use of a bioreactor's peak of performance becomes possible. Thus the use of continuous culture is a virtual necessity for economic industrial scale SCP production.

For biomass production a single-stage process is usually preferable. When the carbon source is a complex mixture (e.g. sulphite waste liquors), two-stage operation may be advantageous. FENCL et al., (1961) have studied the optimum conditions for the production of the yeast Ç. utilis. They have demonstrated that in cases where the yeast utilizes a single source of carbon or when different sources of carbon are utilized simultaneously and at equal rates, a single-stage chemostat is more economical than a multistage or semi-continuous one. As the maximum growth rates of Tr. cutaneum on glucose, fructose, and sucrose (the main sugars in molasses) are almost the same, a single-stage continuous culture was selected for this study.

4.6.2. Growth on different medium concentrations with a constant oxygen supply

The influence of substrate concentration and dilution rate on biomass output was assessed by growing Tr. cutaneum at substrate concentrations of 4.9, 48.5 and 97.0 g l⁻¹ TRS (Figs. 19, 20 and 21). The aeration and agitation rates were kept constant for all three experiments (1.0 vvm, 1200 rpm).
Fig. 19 Effect of dilution rate on the growth and metabolism of *Tr. cutaneum* in carbon limited chemostat culture. Medium = M2 containing 4.9 g l\(^{-1}\) TRS; aeration rate = 1.0 vvm; agitation rate = 1200 rpm; \(t = 35^\circ\text{C}\); pH = 4; anti-foam = 750 ppm of PPG. \(r\) is the coefficient of the least square regression line of \(Q_{O_2}\) (\(Q_S\)) against \(D\).
Fig. 20 Effect of dilution rate on the growth and metabolism of *Tr. cutaneum* medium = M2 containing 48.5 g l⁻¹ TRS; culture conditions were as for Fig. 19.

\( r \) is the coefficient of correlation of the least square regression line of \( Q_{O_2} \) (or \( Q_S \)) against \( D \).
Fig. 21 Effect of dilution rate on the growth and metabolism of Tr. cutaneum
Medium = M2 containing 97.0 g l\(^{-1}\) TRS; culture conditions were as for
Fig. 19.

\( r \) is the coefficient of correlation of the least square regression
line of \( Q_{O_2} \) (\( Q_s \)) against \( D \).
At the substrate concentration of 4.9 g l\(^{-1}\) TRS (Fig. 19), the kinetics of growth were according to Monod, (1950) as will be shown in section 4.7.

\(Q_{O_2}\) and \(Q_{CO_2}\) increased linearly as the dilution rate was increased. The RQ was constant at 1.04 as was expected from earlier experiments.

The biomass productivity reached a maximum value of 1.4 g l\(^{-1}\) h\(^{-1}\) at \(D_{\text{max}} = 0.49 - 0.51\) h\(^{-1}\) and then decreased sharply.

At substrate concentrations of 48.5 and 97.0 g l\(^{-1}\) TRS (Figs. 20 and 21 resp.) the growth was oxygen limited under the conditions applied. At substrate concentration of 48.5 g l\(^{-1}\) the oxygen limitation started at \(D=0.23\) h\(^{-1}\) (Fig. 20). At \(S_R\) of 97.0 g l\(^{-1}\), oxygen was limited at even lower dilution rates (approx. 0.1 h\(^{-1}\)). In vitro measurement of the critical oxygen tension gave a value of 4.7 ± 0.7 mm Hg which is consistent with the 4 mm Hg previously reported for yeast (FINN, 1954).

The \(Q_{O_2}\) and \(Q_{CO_2}\) increased linearly with increasing dilution rate, this was also observed when oxygen was not limiting (Fig. 19). The rate of increase of \(Q_{O_2}\) with respect to dilution rate was depending on the substrate concentration and was 17.36, 19.30 and 27.71 mmol oxygen g\(^{-1}\) for the respective substrate concentrations of 4.9, 48.5 and 97.0 g l\(^{-1}\). Since the \(Y_{Ox}^\text{max}\) is the reciprocal of the slope of \(Q_{O_2}\) versus \(D\), this represents a 10 and 37 % reduction of \(Y_{Ox}^\text{max}\) at 48.5 and 97.0 g l\(^{-1}\) TRS respectively when compared with the \(Y_{Ox}^\text{max}\) at a substrate concentration of 4.9 g l\(^{-1}\).

The specific substrate uptake rate (\(Q_S\)) increased linearly with increasing dilution rate in all cases. The slope of the regression line of \(Q_S\) against \(D\) increased from 8.74 to 11.34 mmol glucose g\(^{-1}\) for \(S_R\) values of 4.9 to 97.0 g l\(^{-1}\). This corresponds to a 23 % reduction in the maximum yield value from sugar (\(Y_{S}^\text{max}\)).
The productivity was linked to the oxygen limitation. No further increase in productivity occurred when oxygen became limiting. The maximum biomass productivity for an $S_R$ of 48.5 g l$^{-1}$ was 8.3 and for 97.0 g l$^{-1}$ it was 7.1 g l$^{-1}$ h$^{-1}$. This difference is probably due to the lower value of $Y_{0}^{\text{max}}$ at higher substrate concentration.

Under oxygen limiting conditions, accumulation of the substrate in the broth took place and increased with increasing dilution rate. No ethanol production was detected (less than 0.3 g l$^{-1}$). The carbon recovery in the biomass and in CO$_2$ was 100.7 ± 3 % which confirms that no appreciable amounts of products other than biomass and CO$_2$ were formed. This was in accordance with the RQ value which remained constant at 1.04 and confirmed that the metabolism of *Tr. cutaneum* is strictly aerobic.

4.6.3 Growth on different medium concentrations with an excess oxygen supply

Since the biomass production rate was linked to the oxygen availability, as shown in 4.6.2, further experiments were carried out under excess oxygen supply. The oxygen transfer rate of the system was improved by increasing the agitation rate and by supplementing the inflowing air with oxygen. The results are shown in Figs. 22 and 23.

In spite of the excess oxygen supply, the maximum dilution rate which had been attained for an $S_R$ of 4.9 g l$^{-1}$ (e.g. $D_{\text{max}}$ = 0.49 - 0.51 h$^{-1}$) was not achieved. The maximum dilution rate was reduced to 0.36 - 0.38 h$^{-1}$ and 0.30 - 0.32 h$^{-1}$ for $S_R$ values of 48.5 and 97.0 g l$^{-1}$ respectively. An unexpected drop in biomass concentration was coincident with a drastic rise in substrate concentration. The reduction in $D_{\text{max}}$ at high medium concentration was not prevented by changing the agitation rate or the amount of oxygen in the inlet air (Tab. 5). In the next chapter (4.7), it
Fig. 22 Effect of dilution rate on the growth and metabolism of Tr. cutaneum. Medium = M2 containing 48.5 g 1^-1 TRS; aeration rate = 1.0 vvm (air + oxygen, [O_2]_in = 0.42); agitation rate = 1600 rpm; t = 35 °C; pH = 4; anti-foam = 750 ppm of PPG. r is the coefficient of the least square regression line of Q_{O_2} (Q_S) against D.
Effect of dilution rate on the growth and metabolism of *T. cutaneum*. Medium = M2 containing 97.0 g l⁻¹ TRS; culture conditions were as for Fig. 22. 

*r* is the coefficient of the least square regression line of \( Q_\text{O}_2 \) (\( Q_S \)) against \( D \).

*Fig. 23* Effect of dilution rate on the growth and metabolism of *T. cutaneum*. Medium = M2 containing 97.0 g l⁻¹ TRS; culture conditions were as for Fig. 22. 

*r* is the coefficient of the least square regression line of \( Q_\text{O}_2 \) (\( Q_S \)) against \( D \).
will be shown theoretically that even a mass-transfer rate coefficient higher than 1.5 mol 1\(^{-1}\) h\(^{-1}\) atm\(^{-1}\) which corresponds to a agitation rate greater than 1600 rpm, does not improve the biomass output rate.

<table>
<thead>
<tr>
<th>No.</th>
<th>RPM</th>
<th>([O_2]_{in})</th>
<th>(D_{max}) h(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>850</td>
<td>0.38</td>
<td>0.36 - 0.38</td>
</tr>
<tr>
<td>2</td>
<td>850</td>
<td>0.58</td>
<td>0.36 - 0.38</td>
</tr>
<tr>
<td>3</td>
<td>1200</td>
<td>0.38</td>
<td>0.36 - 0.38</td>
</tr>
<tr>
<td>4*</td>
<td>1600</td>
<td>0.42</td>
<td>0.36 - 0.38</td>
</tr>
</tbody>
</table>

Table 5. Effect of agitation rate and of oxygen enrichment on \(D_{max}\) in continuous culture of Tr. cutaneum.

- \(S_R = 48.5\ g\ 1^{-1}\) TRS; total volumetric gas flow rate = 1.0 vvm; \(t = 35\ ^\circ\)C; \(pH = 4\).
- \(K_{Ga} = 1.5\ mol\ 1^{-1}\ h^{-1}\ atm^{-1}\)

As observed under oxygen limited conditions, the rate of the increase of \(Q_{O2}\) with respect to dilution rate was higher at substrate concentrations of 48.5 and 97.0 g 1\(^{-1}\) compared with 4.9 g 1\(^{-1}\) TRS. The slope of the \(Q_{O2}\) versus D regression lines increased from 17.36 mmol g\(^{-1}\) to 20.26 and 26.71 for the respective substrate concentrations. That represents a 14 and 35 % reduction in the \(Y_{O}\) value.

The maximum yield coefficient for oxygen of a culture at two different oxygen supplies was compared (Tab. 6). The comparison showed that the \(Y_{O}^{max}\) was unlikely to be affected by the oxygen supply.
supply but was strongly affected by the medium concentration. Irrespective to the oxygen supply rate (limiting or in excess) a 10 times increased medium concentration reduced the $\gamma_{\text{max}}$ by 10 to 14%. A 20 fold increase caused a 35 to 37 % reduction in $\gamma_{\text{max}}$.

<table>
<thead>
<tr>
<th>$S_R$</th>
<th>4.9</th>
<th>48.5</th>
<th>97.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRS g l$^{-1}$</td>
<td>57.6</td>
<td>49.4</td>
<td>37.4</td>
</tr>
<tr>
<td>oxygen</td>
<td>4.9</td>
<td>48.5</td>
<td>97.0</td>
</tr>
<tr>
<td>in excess</td>
<td>57.6</td>
<td>49.4</td>
<td>37.4</td>
</tr>
<tr>
<td>limited</td>
<td>51.8</td>
<td>36.1</td>
<td></td>
</tr>
</tbody>
</table>

Table 6. Effect of substrate concentration and oxygen supply on the maximum yield coefficient for oxygen.

The slope of the regression line of $Q_S$ against $D$ for an $S_R$ of 97.0 g l$^{-1}$ (11.31 mmol g$^{-1}$, Fig. 23) was higher than that for 4.9 g l$^{-1}$ as had been already observed in the experiment under oxygen limiting condition (Fig. 21). The increase corresponded to a 23 % reduction of the $\gamma_{\text{max}}$ value. It may be concluded that, as for $\gamma_{\text{max}}$, $\gamma_{\text{max}}$ is affected by substrate concentration rather than by the oxygen supply.

In Tab. 7 the maximum measured values of $Q_{O_2}$ and $Q_S$ for every medium concentration are presented.
It seems that at each different medium concentration the average value of $Q_{O_2}^{\max}$ and $Q_S^{\max}$ was achieved ($Q_{O_2}^{\max}$ about 13 mmol g\(^{-1}\) h\(^{-1}\), $Q_S^{\max}$ about 5 mmol g\(^{-1}\) h\(^{-1}\)) and these values were apparently not influenced by substrate concentration. However, it is not clear whether the growth restriction sets in at different values of $Q_{O_2}$ and $Q_S$ or these values decrease at high medium concentration as is found for *S. cerevisiae* (KNOEPFEL, 1972).

<table>
<thead>
<tr>
<th>$S_R$ (g) (^{-1}) TRS</th>
<th>$D_{\max}$ (h)^{-1}</th>
<th>$Q_{O_2}^{\max}$ mmol (g)^{-1} h(^{-1})</th>
<th>$Q_S^{\max}$ mmol (g)^{-1} h(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.9</td>
<td>0.49 - 0.51</td>
<td>12.9</td>
<td>5.1 - 5.2</td>
</tr>
<tr>
<td>48.5</td>
<td>0.35 - 0.38</td>
<td>11.3 - 12.8</td>
<td>4.5 - 4.9</td>
</tr>
<tr>
<td>97.0</td>
<td>0.30 - 0.32</td>
<td>12.9 - 14.5</td>
<td>4.7 - 5.7</td>
</tr>
</tbody>
</table>

Table 7. Maximum measured values of oxygen and substrate uptake rates of *Tr. cutaneum* on different medium concentrations.

Considering that the cell mass and substrate concentration near "wash out" are sensitive to slight changes in dilution rate, it may be concluded that the $Q_{O_2}^{\max}$ and $Q_S^{\max}$ values are the same at different medium concentrations.

The dilution rate above which the substrate concentration in the culture increases, and the corresponding values of $Q_{O_2}$ and $Q_S$ are shown in Tab. 8. It may be concluded that the limitation which prevented complete utilization of substrate started at lower dilution rates than $D_{\max}$ (e.g. $D = 0.12 - 0.17$ h\(^{-1}\)) and it existed even for dilute medium ($S_R = 4.9$ g \(1\)^{-1}).
Table 8. Dilution rate for the start of the substrate concentration rise in chemostat culture of *Tr. cutaneum* on different medium concentrations.

$Q_o$ and $Q_s$ are the specific oxygen and substrate uptake rate at dilution rate of $D$; $S_{\text{min}}$ is the minimum measured substrate concentration in the culture.

The observed yield from sugar as a function of dilution rate for different medium concentrations is shown in Fig. 24.

<table>
<thead>
<tr>
<th>$S_R$ (g l$^{-1}$ TRS)</th>
<th>$D$ (h$^{-1}$)</th>
<th>$S_{\text{min}}$ (g l$^{-1}$ TRS)</th>
<th>$Q_o$ (mmol g$^{-1}$ h$^{-1}$)</th>
<th>$Q_s$ (mmol g$^{-1}$ h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.9</td>
<td>0.10 - 0.15</td>
<td>0.1</td>
<td>3.5 - 4.4</td>
<td>1.0 - 1.5</td>
</tr>
<tr>
<td>48.5</td>
<td>0.10 - 0.15</td>
<td>1.3</td>
<td>3.0 - 4.6</td>
<td>1.0 - 1.4</td>
</tr>
<tr>
<td>97.0</td>
<td>0.12 - 0.17</td>
<td>3.2</td>
<td>3.7 - 5.0</td>
<td>1.1 - 1.7</td>
</tr>
</tbody>
</table>

Fig. 24 Observed yield from molasses as a function of dilution rate for different medium concentrations with an excess oxygen supply.
The yield from sugar increased initially with the dilution rate but the increase was less at a high substrate concentration. The drop in yield as $D$ was increased further, coincided with the substrate accumulation in the culture and both phenomenon may be correlated.

The decrease in maximum dilution rate at high medium concentration prevented the proportional increase in maximum productivity by increasing $S_R$ (Fig. 25). The change of $(DX)_{max}$ per unit change of $S_R$ decreased. The $(DX)_{max}$ reached the maximum value of about $14 \text{ g l}^{-1} \text{ h}^{-1}$ at a substrate concentration of $100 - 120 \text{ g l}^{-1}$ and then decreased. Because of the higher yield at cultivation temperature of $30 ^\circ \text{C}$ and pH of 5, this value ($14 \text{ g l}^{-1} \text{ h}^{-1}$) can be increased to about $15 \text{ g l}^{-1} \text{ h}^{-1}$. As a consequent of the $D_{max}$ reduction by increased $S_R$, the maximum possible production rate per unit of expended substrate, which is proportional to the economical efficiency of the system, dropped by 7 and 20 % for a 10 and 20 fold increase in medium concentration respectively.
Fig. 25 Maximum values of biomass production and dilution rates as a function of input substrate concentration in continuous culture of *Tr. cutaneum*.

A: $D_{\text{max}}$

B: Expected value for $(DX)_{\text{max}}$ which is the extrapolation of maximum productivity for $S_R = 4.9 \text{ g l}^{-1}$.

c: Obtained $(DX)_{\text{max}}$. The dotted part of this curve is calculated from the extrapolated values of $D_{\text{max}}$ (A).
4.6.4. Effect of medium concentration and oxygen supply on protein and RNA content of the cells

Protein content of the cells as a function of dilution rate at two different medium concentrations and oxygen supplies are shown in Fig. 26. The percentage protein content of the cells increased with dilution rate and medium concentration. The cells grown under excess oxygen supply had less protein. This is in contrast to the

![Graph showing protein content of Tr. cutaneum as a function of dilution rate at different medium concentration and oxygen supply.]

**Fig. 26** Protein content of *Tr. cutaneum* as a function of dilution rate at different medium concentration and oxygen supply.

A: $S_R = 48.5$ g l$^{-1}$ TRS; aeration rate = 0.36 vvm air; agitation rate = 1200 rpm

B: $S_R = 48.5$ g l$^{-1}$ TRS; aeration rate = 1.0 vvm (air + O$_2$, [O$_2$]$_{in} = 0.42$); agitation rate = 1600 rpm

C: $S_R = 97.0$ g l$^{-1}$ TRS; aeration rate = 1.0 vvm; agitation rate = 1200 rpm

D: $S_R = 97.0$ g l$^{-1}$ TRS; aeration and agitation rates as for B;

t = 35 °C; pH = 4
results of MEDVEDEVA et al. (1966) who found that an increase in aeration leads to more formation of proteinaceous substances in the yeast biomass.

The RNA content of the cells showed a positive correlation with dilution rate (Fig. 27). It increased with increasing medium concentration. Unlike the protein, there was no apparent relationship between the RNA content of the cells and the oxygen supply rate.

It is worth mentioning that the increase in protein and RNA content of the cells had been already observed when the concentrations of supplementary salts to the medium were increased (see Tab. 4).

![Fig. 27](image_url) **RNA content of Tr. cutaneum as a function of dilution rate for different concentrations of medium.**

\[ t = 35 \, ^\circ C; \, pH = 4 \]
4.6.5. Discussion

Existence of some sort of restriction in the complete conversion of substrate to biomass is evident in the cultivations under study. The result of this restriction is not only substrate loss, but also biomass concentration drop which limits the maximum biomass production rate. The restriction intensifies at high growth rate and at high medium concentrations.

This phenomenon is unlikely to be the result of external restrictions since:
- The biomass concentration drop is linked with increases in substrate and oxygen concentration in the culture.
- $Q_{O_2}$ and $Q_S$ increase linearly with increasing dilution rate and achieve the same maximum value for all concentrations of medium tested.
- There is higher maximum productivity on 97.0 g l$^{-1}$ than on 48.5 g l$^{-1}$ medium. The maximum productivity for an $S_R$ of 48.5 g l$^{-1}$ is 9.6 g l$^{-1}$ h$^{-1}$. The expected value is 14 g l$^{-1}$ h$^{-1}$ (10 times the maximum productivity when $S_R$ is 4.9 g l$^{-1}$). For this much productivity, 293 and 126 mmol l$^{-1}$ h$^{-1}$ oxygen and substrate respectively are required. The mass-transfer capacity of the bioreactor is greater than these values. In continuous culture on a medium of 97.0 g l$^{-1}$ TRS, the bioreactor under the same condition of aeration and agitation was able to provide 382 and 143 mmol l$^{-1}$ h$^{-1}$ of oxygen and substrate respectively. The only difference in culture conditions between the former and the latter case was the higher concentration of medium and biomass in the latter one. Hence for culture on 48.5 g l$^{-1}$ TRS, one may expect even higher mass-transfer rates if the intercellular distance should bring about mass-transfer problems.

The phenomenon may be due to the limitations intrinsic in the medium or the organisms. The demand of the organism is the product of cell concentration and $Q_{O_2}$ (resp. $Q_S$). Factors which in
this case may affect the performance of the cells are accumulated growth related toxic substances (various metabolites, CO₂) or the medium itself via a salt effect, glucose effect or oxygen effect.

Formation of growth related toxic substances

In these experiments, 96 to 100 % of the carbon used was recovered as biomass and CO₂, thus one may assume a perfect conversion of substrate to biomass, CO₂, and H₂O. However, the existence of a metabolic product of low carbon content can not be ruled out.

Concerning CO₂-inhibition, according to CHEN et al., (1976), inhibition of yeast growth is negligible below 40 % (v/v) CO₂ (1.19 x 10⁻² M). The maximum CO₂ concentration measured among all of the experiments was 13.14 % (v/v) which is far below the concentration considered necessary for growth inhibition.

Medium

There is some evidence that a number of inhibitors may be present in molasses. These include sulphur dioxide (the level is reduced during the heating for sterilization), hydroxymethyl furfural (usually in cane), potassium imidodisulphonate and fatty acids (HARRISON, 1971).

According to SCHIWECK et al., (1973), substances which inhibit yeast formation could not be found in beet molasses. It has been found that the toxic substances of molasses accumulate in the trub particles of molasses and are separated during treatment of molasses (NEUGEBAUER et al., 1972).

Moreover, restricted productivity has been observed for cultivations on glucose-salts media. KARRER, (1978), has reported 12.5 g l⁻¹ h⁻¹ as the maximum production rate of Tr. cutaneum on 10 % glucose-salts media under an oxygen partial pressure of 0.40 atm.
Oxygen may have a toxic effect on organisms. Excessive oxygen may cause cessation of growth. This was checked by increasing the oxygen volumetric fraction in the inflowing gas from 0.42 to 0.70 while the dilution rate was kept constant at 0.40 h\(^{-1}\). No growth cessation was detected. Applying pure oxygen caused the cells to wash out. In none of the other experiments, however, did the oxygen volumetric fraction in the inflowing gas exceed 0.70.

Apart from toxicity, oxygen may act as a metabolic regulator. Inhibition of glycolysis by oxygen, the Pasteur effect, is manifested as a reduction of \(Q_S\) in the presence of excess oxygen. From the comparison of \(Q_S\)-values under low and high dissolved oxygen tension, the regulation of glycolysis by oxygen in \textit{Tr. cutaneum} can be excluded.

Glucose and salt effects are other possible growth restrictors. In the glucose effect, the respiratory pathways are inhibited by glucose or metabolites and the specific oxygen uptake rate of the cells decreases. This is in contrast with the results reported here for \(Q_{O_2}\) at different medium concentrations.

Possible effect of salts cannot be eliminated. It is worth mentioning, however, that according to Tab. 4 the \(Q_{O_2}\), \(Q_{CO_2}\), protein, and RNA content of the cells increased when the concentration of the supplementary salts to the medium was raised. The same observation was made during continuous cultivation at different medium concentrations.

The medium need not necessarily influence the metabolic activity of the cells, as it does in glucose effect. Its inhibitory activity on cells may be more direct. Alteration in the cell's permeability, or blockage of facilitated transport mechanisms are examples of direct effects of media on cells (HEIDEL et al., 1978). In this way, the flux of different elements and components into and out of cells may be reduced. Besides data on \(Q_{O_2}\), \(Q_{CO_2}\),
and hence there is no information on the flux of elements participating in cell metabolism.

The decrease in $Y_x^\text{max}$ at high medium concentration which was repeatedly observed both under unsufficient and excess oxygen supply may be explained in two ways:

If the lower cell yield is causally connected with higher maintenance requirements, then one may conclude that the lesser portion of the biologically available energy, produced by catabolism, would be available for maintenance purposes and that more ATP is used for the maintenance of concentration gradients between the cell and the medium at higher medium concentrations.

If the lower cell yield from oxygen is not due to the increased maintenance requirement, then it may be presumed that either the ATP requirement for biomass production at high medium concentration increases, or that less efficient oxidative phosphorylation takes place (STOUTHAMER, 1976).
4.7. Modelling of growth kinetics

4.7.1. Introduction

In the preceding chapters, it was shown how physical and biological characteristics of the system were changed under different culture conditions. In this chapter, the aim was not to develop a model for a successful prediction of the behaviour of the chemostat, but rather, by the help of mathematical treatment of the system, to clarify the effect of the change of kinetic, stoichiometric, and mass-transfer parameters on biomass output.

For this purpose, it was necessary to apply a simple deterministic kinetic model with biologically meaningful parameters. In this way, it was possible to test the model experimentally and to explain the deviations between model predictions and experimental observations.

4.7.2. Formulation of the models and basic assumption

A number of mathematical treatments of microbial growth have been presented for studies in both batch and continuous cultures. The Monod model is a frequently used example of such a formulation to describe microbial growth in continuous culture. The Monod model considers that the growth rate is limited by the availability of a single substrate and is merely a function of substrate concentration as seen in enzymatic reactions in vitro. In this model, the affinity of the cells for substrate uptake and maximum growth rate are constant \( K_S \) and \( \mu_{\text{max}} \). The monod model was used here as a reference model for kinetic studies at dilute culture.

From the form of \( X - D \) diagrams for different medium concentrations (Figs. 19, 22, 23) one may recognize that the apparent value of \( K_S \), at least, did not remain constant. Therefore it was necessary to consider an alternative model in which \( K_S \) appears as a variable.
The Contois model, (1959) is one of the models in which without participation of too many parameters, the variability of \( K_S \) is taken into account. Contois assumed that the apparent value of \( K_S \) is simply a linear function of input medium concentration. That is under assumption of constant maximum yield: \( K_S = B_S X \), \( B_S \) is a constant value under defined conditions. Hence, the relation between the growth rate and the substrate concentration according to Contois will be:

\[
\mu = \frac{\mu_{\text{max}} S}{B_S X + S}
\]  

(9)

At high substrate concentrations the microbial population increases. The increased microbial population increases the total oxygen demand of the culture. Oxygen availability rate may then limit the growth. Thus besides the substrate, oxygen concentration has also to be considered as a probable limiting factor in the kinetic model of yeast growth. This may be done by assuming a simple type of interactive model of substrate limitation (BADER, 1978). This type of model has been used by COONEY et al., (1971) for the growth of \( A. \) aerogenes under carbon-nitrogen and phosphorous-nitrogen and by SINCLAIR et al., (1975) for the growth of \( C. \) utilis under glycerol-oxygen limiting condition. They concluded that the model could describe the behaviour of their system reasonably well.

Provided that the carbon or oxygen limitation can be described by the Monod or the Contois model, four possible models may be suggested:
Monod-Monod  \[ \mu = \frac{\mu_{\max} S}{(K_s + S)} \frac{P^L_0}{(K_0 + P^L_0)} \]  

(II)

Contois-Monod  \[ \mu = \frac{\mu_{\max} S}{(B_sX + S)} \frac{P^L_0}{(K_0 + P^L_0)} \]  

(I)

Monod-Contois  \[ \mu = \frac{\mu_{\max} S}{(K_s + S)} \frac{P^L_0}{(B_0X + P^L_0)} \]  

(III)

Contois-Contois  \[ \mu = \frac{\mu_{\max} S}{(B_sX + S)} \frac{P^L_0}{(B_0X + P^L_0)} \]  

(IV)

With excess substrate or oxygen, every one of the above mentioned interactive models reduces to the simple, substrate or oxygen, limited growth kinetic.

Application of one of the above mentioned models together with equations of biomass, substrate, and oxygen balance can be used to estimate biomass, substrate, and oxygen concentrations if the values of the necessary parameters are determined.

For derivation of mass-balances the following assumptions are made:

- That there is complete mixing. The criterion for complete mixing is that the composition of the stream leaving the bioreactor is the same as that of any sample drawn from the interior of the bioreactor.
- That no products are formed other than biomass, CO₂, and H₂O.
That the specific maintenance rate ($\mu_e$) is independent of the limiting substrate, substrate concentration value, and the specific growth rate.

That other growth characteristics like $\mu_{\text{max}}$, $Y_{S}^{\text{max}}$, $Y_{O}^{\text{max}}$, $Y_e$ are all constant and independent of the limiting substrate and substrate concentration value.

That the cells are not flexible to any adaptation phenomenon.

When the above assumptions are made, the biomass balance according to Herbert's model of maintenance (HERBERT, 1958):

$$\frac{dX}{d\theta} = (\mu - D - \mu_e)X \quad (10)$$

According to this model of maintenance, the organism uses up part of its own biomass in order to maintain its viability and ability to function. It is assumed that no exogeneous substrate is consumed for maintenance. Substrate balance:

$$\frac{dS}{d\theta} = D(S_R - S) - \frac{\mu}{Y_{S}^{\text{max}}} X \quad (11)$$

Oxygen balance in liquid phase:

$$\frac{dC_O^L}{d\theta} = \frac{D}{H} \left[ (p_{0}^L)_{\text{in}} - (p_{0}^L)_{\text{out}} \right] + K_{a}^G (p_{0}^G - p_{0}^L) - \frac{\mu}{Y_{O}^{\text{max}}} X - \frac{\mu_e}{Y_e} X \quad (12)$$

The rate of oxygen introduction into the culture in the ingoing medium, $(D/H)(p_{0}^L)_{\text{in}}$, is negligible compared to the rate of transfer from gas to liquid (OTR). Also the rate of the out coming oxygen through the medium, $(D/H)(p_{0}^L)_{\text{out}}$, is negligible compared
with the rate of the oxygen taken up by cell (OUR). Hence, the Eq. (12) becomes:

\[
\frac{dc_0}{d\Theta} = K_G a (p_O^G - p_O^L) - \frac{\mu}{Y_0^{max}} X - \frac{\mu_e}{Y_e} X
\]  

(13)

Steady state form of mass-balance Eqs:

\[
\text{Eq. (10)} \quad \mu = D + \mu_e
\]  

(14)

\[
\text{Eq. (11)} \quad X = \frac{\frac{S_R - S}{\mu}}{\mu} D
\]  

(15)

\[
\text{Eq. (13)} \quad p_O^L = p_O^G - \frac{\frac{X}{K_G a}}{\frac{\mu}{Y_0^{max}} + \frac{\mu_e}{Y_e}}
\]  

(16)

4.7.3. Determination of parameters

The parameters in the above mentioned equations, i.e., Eqs. I-IV and 14 - 16 are of three types. These are kinetic (\(\mu_{max}', \mu_e',\ K_S, \ K_0, \ B_S, \ B_0\)); stochiometric (\(Y_S^{max}, \ Y_0^{max}, \ Y_e\)); and mass transfer (\(K_G a, \ p_O^G\)) parameters. When the values of these parameters are determined, steady state values of biomass and substrate concentration as well as dissolved oxygen tension for any value of input substrate concentration can be calculated.
No matter what the kinetic model of growth is, parameters $\mu_e$, $Y_e$, $Y_{S_{\text{max}}}$, $Y_{O_{\text{max}}}$, $K_a$, and $p_{G}^{G}$ can be estimated independently.

From Eqs. (14) and (15):

$$\frac{(S_R - S)}{X} = \frac{1}{Y_S} = \frac{\mu_e}{Y_{S_{\text{max}}}} + \frac{1}{D} + \frac{1}{Y_{S_{\text{max}}}}$$  \hspace{1cm} (17)

Or:

$$Q_S = \frac{1}{D} + \frac{\mu_e}{Y_{S_{\text{max}}}} + \frac{\mu_e}{Y_{S_{\text{max}}}}$$  \hspace{1cm} (18)

It is already known, and can be seen from Eq. (17), that the observed yield increases at high growth rates. This fact shows that for the single-cell protein production, the cultures should be grown at the highest practicable dilution rate ($D_{\text{max}}$) in order to obtain maximum cell yield for the smallest expenditure of substrate.

Eq. (18) can be used for the determination of $\mu_e$ and $Y_{S_{\text{max}}}^{\text{max}}$. Fitting a least square line to the data of the experiment presented in Fig. 19, assuming $D$ is an independent variable, will give:

$$Y_{S_{\text{max}}} = 0.64 \text{ g g}^{-1}$$

$$m_S = \frac{\mu_e}{Y_{S_{\text{max}}}} = 0.03 \pm 0.02 \text{ g g}^{-1} \text{ h}^{-1}$$

$$\mu_e = 0.02 \pm 0.01 \text{ h}^{-1}$$
The maintenance coefficient of *C. utilis* on prickly pear juice attained a value of 0.09 g of sugar per g biomass per h (PAREDES-LOPEZ et al., 1976). A specific maintenance rate of 0.012 h\(^{-1}\) is reported by VAN UDEN, (1970) for *S. cerevisiae* in a glucose limited chemostat culture.

\( Y_0^{\text{max}} \) and \( Y_e \) can be determined from the oxygen uptake rate data. The oxygen uptake rate of the culture in the steady state condition is expressed by (see also Eq. 12):

\[
\text{OUR} = \frac{\mu}{Y_0^{\text{max}}} X + \frac{\mu_e}{Y_e} X
\]  

(19)

From Eqs. (14) and (19):

\[
\frac{\text{OUR}}{X} = \frac{Q_{O_2}}{X} = \frac{1}{Y_0^{\text{max}}} D + \frac{\mu_e}{Y_e} \left( \frac{1}{Y_0^{\text{max}}} + \frac{1}{Y_e} \right)
\]  

(20)

The least square regression line of \( Q_{O_2} \) against \( D \) gives a straight line with a slope of \( 1/Y_0^{\text{max}} \) and intercept of \( \mu_e(1/Y_0^{\text{max}} + 1/Y_e) \). This plot is presented in Fig. 19. From it, the values of \( Y_0^{\text{max}} \) and of the maintenance coefficient for respiration (\( m_0 \)) can be calculated:

\( Y_0^{\text{max}} = 0.058 \text{ g mmol}^{-1} \)

\( m_0 = \mu_e \left( \frac{1}{Y_0^{\text{max}}} + \frac{1}{Y_e} \right) = 1.80 \pm 0.78 \text{ mmol g}^{-1} \text{ h}^{-1} \)

Assuming \( \mu_e = 0.02 \pm 0.01:\)

\( Y_e = 0.032 \pm 0.028 \text{ g mmol}^{-1} \)
Maximum yield coefficients of oxygen of 0.042 and 0.049 g DW per mmol oxygen are reported for \textit{C. \textit{utilis}} grown on glucose (JOHNSON, 1967a) and \textit{S. \textit{cerevisiae}} on beet molasses (HOSPODKA, 1966).

The maintenance coefficient for respiration found by BUTTON et al., (1966) for \textit{C. \textit{utilis}} grown on glycerol was 1.75 mmol oxygen per g DW per h.

$K_G$, $a$ and $p_0^G$ can be determined as described in 3.6.4.

The values of $K_S$, $B_S$, $K_O$, and $B_O$ were determined under wholly carbon or oxygen-limiting conditions according to the approach applied by SINCLAIR et al., (1975).

According to BADER et al., (1975) this approach can not be a general one but will probably work well in many chemostat situations.

If model I or III are valid under condition of excess oxygen, these two models reduce to:

$$P = P_{max} \frac{S}{(K_S + S)}$$

(21)

Substitution of $\mu$ from Eq. (14) and rearrangement:

$$\frac{1}{(D + \mu_e)} = \frac{K_S}{\mu_{max}} \frac{1}{S} + \frac{1}{\mu_{max}}$$

(22)

The reciprocal plot of the $(D + \mu_e)$ and sugar concentration in the supernatant should give a straight line with a slope of $K_S/\mu_{max}$ and an intercept of $1/\mu_{max}$ (Fig. 28).
From Fig. 28:

\[ \mu_{\text{max}} = 0.67 \pm 0.06 \text{ h}^{-1} \]

\[ K_s = 0.24 \pm 0.04 \text{ g l}^{-1} \]

A value of 0.012 g l\(^{-1}\) is reported for \( K_s \) of \( C. \) tropicalis on glucose (Knoepfel, 1972). Malek et al., (1966) has reported a value of 0.3 g l\(^{-1}\) for \( C. \) utilis on xylose.

The \( K_s \) value obtained here is rather high. This is because of the substrate concentration rise in the culture which had already begun at low dilution rates (see Tab. 8). Furthermore not all of the reducing substances may be assimilated. The value of \( K_s \), hence, should be considered as an apparent or effective value.

---

**Fig. 28** Regression of reciprocals of the growth rate against reciprocals of the TRS in the bioreactor for continuous culture of *Tr. cutaneum*.

Data from Fig. 19; \( \mu_e = 0.02 \pm 0.01; \) \( r = 1.00; \)

\( s_{y-x} = \pm 0.125 \)
If model II or IV are valid, in the case of excess oxygen these two models reduce to:

\[
\mu = \mu_{\text{max}} \frac{S}{(B_S X + S)}
\]  

(23)

Substitution of \( \mu \) from Eq. (14) and rearrangement:

\[
\frac{1}{(D + \mu_e)} = \frac{B_S}{\mu_{\text{max}}} \frac{X}{S} + \frac{1}{\mu_{\text{max}}}
\]  

(24)

Fig. 29 Regression of the reciprocals of the growth rate against \( X/S \) in continuous culture of *Tr. cutaneum*. Data from Fig. 19; \( \mu_e = 0.02 \pm 0.01; r = 1.00; s_{y-x} = 0.12 \)
A plot of $1/(D + \mu_e)$ versus $X/S$ should give a straight line with a slope of $B_S/\mu_{max}$ and an intercept of $1/\mu_{max}$ (Fig. 29).

From Fig. 29:

$$\mu_{max} = 0.68 \pm 0.06 \text{ h}^{-1}$$

$$B_S = 0.095 \pm 0.015 \text{ g g}^{-1}$$

$K_0$ was measured by two methods:

(a) **In vitro**, by means of a sealed polarograph cell (see 3.6.4.). 50 μl of the culture was transferred from the chemostat operating at a dilution rate of 0.45 h$^{-1}$ under the condition described in Fig. 20. It was incubated in the chamber of a polarograph cell containing 4 ml of the same medium as used in the chemostat. The decrease of dissolved oxygen tension with time was monitored (Fig. 30). $K_0$ was determined as the dissolved oxygen tension at which the slope of the tangent is half that of the linear portion. The results gave a mean value of $1.52 \pm 0.23 \text{ mmHg}$.

(b) **In situ**, with a chemostat operating under oxygen limiting conditions. For this purpose some authors like JOHNSON, (1967b) and SINCLAIR et al., (1975) have used a chemostat without aeration. The only oxygen that the organisms received was that already dissolved in the inflowing medium. Because of the low solubility of oxygen in broth, the concentration of biomass formed is low. The effect of biomass concentration on growth rate (Contois model of growth for oxygen limitation) can not be studied.
Fig. 30 Plot of the decrease in dissolved oxygen tension with time for *Tr. cutaneum* cells which were initially growing in unlimited supplies of nutrient.

Oxygen limiting condition can also be created by increasing the substrate concentration while the oxygen supply is maintained constant. The culture becomes oxygen limited at growth rates at which the oxygen demand rate exceeds the oxygen supply rate. This situation occurs in the experiment shown in Fig. 20 when $D \geq 0.23 \text{ h}^{-1}$. 
When excess substrate is present the model I or II reduce to:

\[ \mu = \frac{\mu_{\text{max}} L}{p_0} \frac{1}{(K_0 + p_0^L)} \]  

(25)

or:

\[ \frac{1}{(D + \mu_e)} = \frac{K_0}{\mu_{\text{max}}} \frac{1}{\mu_{\text{max}}} \frac{p_0^L}{p_0} \]  

(26)

A plot of \(1/(D + \mu_e)\) versus \(1/p_0^L\) should give a straight line with the slope of \(K_0/\mu_{\text{max}}\) and intercept of \(1/\mu_{\text{max}}\) (Fig. 31).

From Fig. 31:

\[ \mu_{\text{max}} = 0.54 \pm 0.05 \text{ h}^{-1} \]

\[ K_0 = 1.24 \pm 0.14 \text{ mm Hg} \]

The value of \(K_0\) determined here is not unlike that measured by the dynamic method in (a). JOHNSON, (1967b) has obtained a value of \(1.3 \cdot 10^{-6} \text{ mol l}^{-1}\) for \(K_0\) of \(C. \text{utilis}\) which is equivalent to 0.95 mm Hg.

For the determination of \(B_0\), under excess substrate concentration models III and IV reduce to:

\[ \mu = \frac{\mu_{\text{max}} L}{p_0} \frac{1}{(B_0X + p_0^L)} \]  

(27)
Fig. 31 Regression of reciprocals of the growth rate against reciprocals of the dissolved oxygen tension in continuous culture of Tr. cutaneum. Data from Fig. 20 when \( D \geq 0.23 \, \text{h}^{-1} \); \( \mu_e = 0.02 \pm 0.01 \, \text{h}^{-1} \); \( r = 0.98 \); \( s_{y-x} = \pm 0.14 \).

or:

\[
\frac{1}{(D + \mu_e)^{-1}} = \frac{B_0}{\mu_{\text{max}}} \left( \frac{X}{P_0^L} \right) + \frac{1}{\mu_{\text{max}}}
\]  

(27)

A plot of \( 1/(D + \mu_e) \) versus \( X/P_0^L \) should give a straight line with the slope of \( B_0/\mu_{\text{max}} \) and intercept of \( 1/\mu_{\text{max}} \) (Fig. 32).
Fig. 32 Regression of reciprocals of the growth rate against $X/p_{O}^{L}$.  
Data from Fig. 20 when $D \geq 0.23$ $h^{-1}$; $\mu_e = 0.02 \pm 0.01$ $h^{-1}$; $r = 0.99$; $s_{y-x} = 0.09$

From Fig. 32:

$\mu_{max} = 0.51 \pm 0.03$ $h^{-1}$

$B_0 = 0.04 \pm 0.003$ mm Hg l g$^{-1}$

The $\mu_{max}$ calculated here, i.e., from the chemostat with an $S_R$ of 48.5 g l$^{-1}$ has an average value of 0.53 $h^{-1}$ which is lower than the 0.68 $h^{-1}$ calculated for the chemostat with an $S_R$ of 4.9 g l$^{-1}$
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Table 9. Parameter values

** The Lineweaver-Burk plot of growth model deviates from linearity at increased substrate concentrations (S-shaped curve).

$P_{max}$ was calculated from the linear correlation of data.
It may be concluded that the $\mu_{\text{max}}$ as defined by MONOD, (1942) is not constant and decreases at high substrate concentration. This is consistent with the conclusion obtained from the specific oxygen uptake rate studies at different medium concentrations. Variation in $\mu_{\text{max}}$ values is also reported by STANLEY, (1964) for C. utilis and by MARTINEZ-PEINADO et al., (1977) for S. cerevisiae.

After this observation, $\mu_{\text{max}}$ was not considered as a constant parameter in model construction.

In Tab. 9 the determined parameter values are summarized.

By the knowledge of the characteristic ($\mu_{\text{max}}, \mu_e, K_g, Y_g, ...$) and control ($S_R, D, K_g, a, p_0^L$) variables, the steady state values of state variables ($X, S, p_0^L$) can be calculated using one of the four suggested models (I-IV) and Eqs. 14, 15 and 16 (see appendix).

4.7.4. Differences in Monod and Contois growth kinetics, effect of parameter values

Fig. 33 shows the differences between predicted values of biomass concentration according to the Monod or Contois models under carbon and oxygen-limiting conditions.

Under carbon-limiting condition (curves 1 and 2) Contois' model predicts lower values for $X$ and respectively higher values for $S$ and $p_0^L$ than Monod's.

Under oxygen-limiting condition (curve 3), the predicted curves are almost the same for the Monod or the Contois model. This is because of the relatively low value of $B_0$. When the oxygen supply is unsufficient, the $X$ value is also low, especially at high growth rates causing a low value for $B_0 X$. For the same value of $B_0$, it can be calculated that the difference between Monod and Contois predicted values may be significant for $X$ values above 30 g l$^{-1}$. 
Fig. 33 Comparison of biomass production with substrate-limited (curves 1 and 2) and oxygen-limited (curve 3) growth in steady states of chemostat culture.

$S_0 = 48.5 \, \text{g} \, \text{l}^{-1}$; for carbon-limited: $K_G a = 1.5 \, \text{mol} \, \text{l}^{-1} \, \text{h}^{-1} \, \text{atm}^{-1}$ and $p_O^G = 0.369 \, \text{atm}$; for oxygen-limited: $K_G a = 0.2 \, \text{mol} \, \text{l}^{-1} \, \text{h}^{-1} \, \text{atm}^{-1}$ and $p_O^G = 0.110 \, \text{atm}$, other parameters as in Tab. 9.
In the following, the effect of the change in biological and mass transfer (physical) parameters on predicted steady state values of state variables \( (X, S, p^L_0) \) are considered. For the sake of simplicity:
- Oxygen and substrate-limiting conditions are considered separately.
- Only one parameter is changed at a time independently of the others.
- Only one of the predicted values of state variables \( (X, S, p^L_0) \) is presented as a curve. The change of the other two and related conclusions can be found in the legend of every figure.
- The presented results are according to Monod kinetics. The general difference between the Monod predicted and Contois predicted values remains the same as was shown in Fig. 33.

Figs. 34 - 41 show the changes in state variables as the result of the change in characteristic and physical parameters for oxygen-limiting conditions. The same for substrate-limiting conditions are presented in Figs. 42 - 48.
Fig. 34 Effects of change in specific maintenance rate on steady state biomass concentration in an oxygen-limited chemostate culture.

Control parameters:
\[ S_R = 48.5 \, \text{g} \, \text{l}^{-1}; \, K_a = 0.2 \, \text{mol} \, \text{l}^{-1} \, \text{h}^{-1} \, \text{atm}^{-1}; \, p_O = 0.110 \, \text{atm}; \]  
other parameters as in Tab. 9.

When the specific maintenance rate increases: \( X \) decreases, \( S \) increases, \( p_O \) does not change, \( D_c \) decreases, oxygen limitation occurs at lower dilution rates.
Fig. 35 Effects of change in the endogenous yield constant on oxygen on steady state biomass concentration in an oxygen-limited chemostat culture. Control parameters as in Fig. 34; other parameters as in Tab. 9. When the endogenous yield constant on oxygen decreases: $X$ decreases, $S$ increases, $p_0^L$ and $D_c$ do not change, oxygen limitation occurs at lower dilution rates.
Fig. 36 Effects of change in the maximum yield factor for substrate on steady state substrate concentration in the bioreactor in an oxygen-limited chemostat culture.

Control parameters as in Fig. 34; other parameters as in Tab. 9.
When the maximum yield factor for substrate decreases: $X$, $p^L$, and $D_c$ do not change; $S$ decreases; oxygen limitation occurs at higher dilution rates.

Fig. 37 Effects of change in maximum yield coefficient for oxygen on steady state biomass concentration in an oxygen-limited chemostat culture.

Control parameters as in Fig. 34; other parameters as in Tab. 9.
When the maximum yield coefficient for oxygen decreases: $X$ decreases; $S$ increases; $p^L$, $D_c$, and the dilution rate for oxygen limitation do not change.
Fig. 38 Effects of change in the Monod constant for oxygen on steady state biomass concentration in an oxygen-limited chemostat culture.
Control parameters as in Fig. 34; other parameters as in Tab. 9.
When $K_o$ increases: $X$ and $D_C$ decrease; $S$ and $p_{O}^L$ increase.

Fig. 39 Effects of change in the Contois constant for oxygen on steady state biomass concentration in an oxygen-limited chemostat culture.
Control parameters as in Fig. 34; other parameters as in Tab. 9.
Fig. 40 Effects of change in the oxygen-transfer coefficient on steady state biomass concentration in an oxygen-limited chemostat culture. Other control parameters as in Fig. 34; characteristic parameters as in Tab. 9.

When the oxygen-transfer coefficient increases: $X$ increases; $S$ decreases; $P_q^L$ (in the oxygen limiting phase) and $D_c$ do not change; oxygen limitation occurs at higher dilution rates.

Fig. 41 Effects of change in partial pressure of oxygen in the gas phase in bulk on steady state biomass concentration in an oxygen-limited chemostat culture. Other control parameters as in Fig. 34; characteristic parameters as in Tab. 9. For comments see Fig. 40.
Effects of change in specific maintenance rate on steady state biomass concentration in a carbon-limited chemostat culture.

Control parameters: \( S_R = 48.5 \, g \, l^{-1} \); \( K_{a} = 1.5 \, mol \, l^{-1} \, h^{-1} \, atm^{-1} \); \( P_{O}^{G} = 0.369 \, atm \); other parameters as in Tab. 9.

When the specific maintenance rate increases: \( X \), \( P_{O}^{L} \), and \( D_{c} \) decrease; \( S \) does not change.
Fig. 43 Effects of change in endogenous yield constant on oxygen on steady state dissolved oxygen tension in a carbon-limited chemostat culture. Control parameters as in Fig. 42; other parameters as in Tab. 9. When the endogenous yield constant on oxygen decreases: $X$, $S$, and $D_c$ do not change; $p_O^L$ decreases.
Fig. 44 Effects of change in the maximum yield factor for substrate on steady state biomass concentration in a carbon-limited chemostat culture. Control parameters as in Fig. 42; other parameters as in Tab. 9. When the maximum yield factor for substrate decreases: $X$ decreases; $S$ and $D_c$ do not change; $p_0^L$ increases.

Fig. 45 Effects of change in the maximum yield coefficient for oxygen on steady state dissolved oxygen tension in a carbon-limited chemostat culture. Control parameters as in Fig. 42; other parameters as in Tab. 9. When the maximum yield coefficient for oxygen decreases: $X$, $S$, and $D_c$ do not change; $p_0^L$ decreases.
Fig. 46 Effects of change in the Monod constant for substrate on steady state biomass concentration in a carbon-limited chemostat culture. Control parameters as in Fig. 42; other parameters as in Tab. 9. When $K_s$ increases: $X$ and $D_c$ decrease; $S$ and $P_0^L$ increase.

Fig. 47 Effects of change in the Contois constant for substrate on steady state biomass concentration in a carbon-limited chemostat culture. Control parameters as in Fig. 42; other parameters as in Tab. 9. For comments see Fig. 46.
Fig. 48 Effects of change in the oxygen-transfer coefficient on steady state dissolved oxygen tension in a carbon-limited chemostat culture.

Other control parameters as in Fig. 42; characteristic parameters as in Tab. 9.

When the oxygen-transfer coefficient increases: $X$, $S$, and $D_c$ do not change; $p_O^L$ increases. A similar effect is seen when the partial pressure of oxygen in the gas phase is increased.
From the study of these figures it can be concluded that:

Accumulation of substrate in the culture can happen either when the culture becomes oxygen limited or when the effective values of \( K_S \) or \( B_S \) increase. Increase in \( B_S \) can be caused by decrease in the yield value, which itself is related to the maintenance requirements. Substrate accumulation caused by oxygen limitation can be simply distinguished from that caused by \( K_S \) or \( B_S \) increase. There is a basic difference between change of substrate concentration with dilution rate (\( dS/dD \)) for each case. For oxygen limitation, this derivative decreases by \( D \) increase whereas under sufficient oxygen it increases. Therefore, in addition to \( p_o^L \) values, S-D diagrams shown in Figs. 22 and 23, also prove that the oxygen has not been a limiting factor in these two experiments since the change in \( S \) per unit change of dilution rate increases by \( D \) increase.

When the oxygen is sufficient, substrate concentration in the culture is not directly affected by the change in yield factor. Decrease in yield is linked with \( B_S \) increase and this causes an accumulation of unused substrate in the culture (combination of Figs. 44 and 47).

Biomass output rate can be decreased, if the oxygen is limiting, by decrease of \( Y_e \), \( Y_{max}^m \), \( K_g^a \) and \( p_o^G \), or increase of \( \mu_e \) and \( K_o \) or \( B_o \). When the culture is not oxygen limited, biomass output rate can be decreased by increasing \( \mu_e \) and \( K_S \) or \( B_S \) or by decreasing \( Y_{max}^S \) but is not affected by mass-transfer parameters.

The amount of biomass reduced for maintenance reasons is dependent on the growth rate and biomass concentration. Assuming that the specific maintenance rate is constant, then when the dilution rate is low, and the biomass density high, maintenance will cost a great deal of energy and biomass concentration will fall. Losses may be as great as 100 %.
Fig. 42 shows that a maintenance rate of 0.03 h\(^{-1}\) which is only about 6\% of the maximum specific growth rate, can reduce biomass output rate by 10 to 100\%.

The endogenous yield constant also has a considerable effect on state variables of the chemostat. Fig. 35 shows that change in Y\(_e\) within its maximum and minimum determined values can affect X by up to 22\% under oxygen limiting condition. Under carbon limitation, it can cause up to 70\% change in the dissolved oxygen tension (Fig. 43).

The critical dilution rate, or wash-out point can be reduced when the specific maintenance rate or the effective value of the saturation constant (K\(_S\) or K\(_Q\)) increases. When oxygen is not the limiting factor, mass-transfer parameters do not have any effect on wash-out point (Fig. 48). Under oxygen limitation, however, depending on the oxygen uptake rate and the maximum specific growth rate of the cells, mass-transfer parameters may or may not affect the wash-out point (Fig. 40).

4.7.5. Testing of the models

For different cultivations, the biomass concentration was calculated over the whole range of dilution rate. This was done for each one of the interactive models. The standard errors of deviations between calculated and experimental values are presented in Tab. 10.

For an S\(_R\) of 4.9 g l\(^{-1}\) (column 1), all the models give approximately equal estimations. The reason is that the most of the characteristic parameters have been determined in this experiment. Here, both Monod and Contois kinetic models fit reasonably. Good linear correlation between plotted data presented in Figs. 28 and 29 confirms also that both of these models hold reasonably well for this experiment.
Table 10. Standard deviation between predicted X versus D diagrams and experimental results

<table>
<thead>
<tr>
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<th>2</th>
<th>3</th>
<th>4</th>
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<tr>
<td>$s_{y-X}$, g l$^{-1}$</td>
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<td></td>
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<tr>
<td>$S_R$, g l$^{-1}$</td>
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<td>48.5</td>
<td>97.0</td>
<td>97.0</td>
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<tr>
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<td>0.36</td>
<td>1.0</td>
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<tr>
<td>$[O_2]_{in}$</td>
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<td>0.21</td>
<td>0.42</td>
<td>0.21</td>
<td>0.42</td>
</tr>
<tr>
<td>rpm</td>
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<td>1200</td>
<td>1600</td>
<td>1200</td>
<td>1600</td>
</tr>
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<td>-5.1</td>
<td>-3.5</td>
<td>-18.4</td>
</tr>
<tr>
<td>Model II</td>
<td>±0.46</td>
<td>-2.4</td>
<td>±1.4</td>
<td>-4.0</td>
<td>-6.9</td>
</tr>
<tr>
<td>Model III</td>
<td>±0.42</td>
<td>-3.5</td>
<td>-5.1</td>
<td>-3.3</td>
<td>-17.5</td>
</tr>
<tr>
<td>Model IV</td>
<td>±0.47</td>
<td>-3.0</td>
<td>±1.3</td>
<td>-2.9</td>
<td>-6.8</td>
</tr>
</tbody>
</table>

Columns 2 and 4 (Tab. 10) belong to the experiments under insufficient oxygen supply. As was explained in 4.7.4., under oxygen limitation, there is little difference between the Monod or the Contois model in expressing the kinetics of oxygen consumption. Therefore the standard errors are not considerably different (columns 2 and 4).

The difference between models becomes visible at high substrate concentration and excess oxygen supply (columns 3 and 5). Here, models II and IV which both contain Contois form for substrate utilization give less deviation. For an $S_R$ of 48.5 g l$^{-1}$, the predicted X value by these models has an error of about 1 g l$^{-1}$ compared to a 5 g l$^{-1}$ error of model I or III (both containing the Monod model for substrate utilization). For an $S_R$ of 97.0 g l$^{-1}$, this difference becomes more clear, i.e. an error of about 7 g l$^{-1}$.
compared to 18 g l⁻¹. Hence it can be concluded that predictions by model II or IV are nearer to reality than those by models I and III.

By considering concluding points in 4.7.4. and experimental observations in 4.6.2. and 4.6.3., it is possible to give an explanation for the better estimation by the Contois model. As was shown in Fig. 33, curves 1 and 2, the Contois estimates higher values for S (lower X) than does the Monod model. This difference becomes greater at high Sᵢ values since BₛX is proportion to Sᵢ whereas Kₛ, in the Monod model, is constant. This property compensates implicitly the error which arises from the decrease in yield factor at high substrate concentration which was not considered in model construction.

It should be noted that not only the Contois model, but models based on growth inhibition, (see PIRT, 1975) or diffusion and permeability limited kinetics (see POWELL, 1967) which may serve as a correction to saturation constant would give a better fit than the Monod model.

Although the model was constructed using 10 different parameters, it is still too simple to consider all the biological variations taking place when the cells are growing on different medium concentrations and oxygen supplies. For example the correlation between substrate concentration, \( \mu_{\text{max}} \), and yields are not known. In other words, simple substitution of \( Kₛ \) by \( BₛX \) does not explain the more complicated interrelation of growth parameters. Because of these inadequancies, the model cannot predict the state variables precisely (see Figs. 49 and 50, Tab. 10 for models II and IV).
Fig. 49 Biomass, substrate and dissolved oxygen tension as a function of dilution rate. Experimental values and curves predicted by the Contois-Monod interactive model:

$$\mu = \frac{\mu_{\text{max}} S}{(B_S X + S)}$$

$$S_R = 97.0 \text{ g l}^{-1}$$; culture conditions see Fig. 21
Fig. 50 Biomass, substrate and dissolved oxygen tension as a function of dilution rate. Experimental values and curves predicted by the Contois-Monod interactive model:

\[ \mu = \mu_{\text{max}} \frac{S}{(B_X + S)} \frac{p_0^L}{(K_0 + p_0^L)} \]

\[ S_R = 97.0 \text{ g l}^{-1} \]; culture conditions see Fig. 23.
5. SUMMARY

The main objective of this study was SCP production from molasses. The effects of physical and chemical parameters, as well as the medium concentration on the production rate and composition of biomass were investigated.

For the determination of sugars in molasses (TRS), a method based on the hexacyanoferrate (III)/(II) redox reaction was modified. Suggestions for the decolourization of molasses were made. A continuous automated procedure was optimized which minimized the errors and interferences.

A study comparing the growth of Candida tropicalis, Candida utilis and Trichosporon cutaneum on molasses media showed that the Tr. cutaneum has a high potential as a yeast for SCP production. It is characterized by a high yield and protein content, strictly aerobic growth, and relatively low foam formation. The growth medium was optimized with respect to the salts concentration per gramme of treated molasses.

For the prevention of extensive foam formation during cultivation on molasses, polypropylene glycol (PPG) 2000 was chosen as anti-foam agent. No influence on the growth was detected at PPG concentrations up to 1750 ppm.

In the temperature range 27 to 37 °C, the yield of biomass from sugar ($Y_S$) remained constant. The specific rate of oxygen consumption ($Q_{O_2}$) was stimulated at high temperatures causing a decrease in yield from oxygen ($Y_O$). At temperatures higher than 37 °C, $Y_S$ decreased whereas the $Y_O$ increased. Both yields increased when the pH was changed from 3 to 6. They decreased on concentrated media (from 4.9 to 97.0 g l$^{-1}$ TRS) but were unlikely to have been affected by the oxygen supply.
Productivity increased markedly when the temperature was raised from 27 to 30 °C. It rose slightly from 30 to 37 °C and dropped sharply when the temperature exceeded 37 °C. Productivity increased rapidly as pH was raised from 3 to 4 and remained almost constant up to pH 6. At high medium concentrations (48.5 and 97.0 g l\(^{-1}\) TRS) and adequate oxygen supply, the maximum dilution rate \(D_{\text{max}}\) decreased which limited the maximum attainable biomass productivity (15.43 g l\(^{-1}\) h\(^{-1}\)).

The protein and RNA content of the cells decreased steadily as the temperature was increased from 27 to 37 °C and the pH from 3 to 6. The protein and RNA contents of the cells were higher in more concentrated media. The protein content of the cells grown under lower oxygen supply was higher but the RNA content was little affected.

The overall oxygen-transfer rate coefficient \((K_o a)\) was decreased by increasing the temperature and pH. The addition of 250 to 1750 ppm of PPG did not change the \(K_o a\) of the system.

The effect of the change of kinetic, stoichiometric, and mass-transfer parameters on biomass output rate was analysed by mathematical treatment of the system. Applying Monod and Contois models, it was shown that the kinetic expression for growth rate dependence on substrate concentration gives predicted values closer to measured data if the Contois formulation is used. There was no superiority between the Monod and Contois models in expressing the kinetics of oxygen consumption. The models were used for growth studies under different medium concentrations and oxygen supplies. It was concluded that, even an interactive model based on the Contois model for carbon and the Monod or Contois model for oxygen consumption, is still too simple for successful prediction of the behaviour of the chemostat under the wide range of flow rate, substrate, and oxygen conditions.
Das Ziel dieser Arbeit war, die Grundlagen für die Herstellung von Mikrobenprotein (SCP) aus Melasse zu erarbeiten. Der Einfluss chemischer und physikalischer Parameter, sowie der Mediumskonzentration auf die Produktivität und die Zusammensetzung der Biomasse wurde bestimmt.

Für die Zuckerbestimmung in Melasse (TRS) wurde die Hexacyanoferrat-Methode modifiziert. Sie beruht auf der Reduktion von Fe$^{3+}$ zu Fe$^{2+}$ durch reduzierende Zucker. Ein Verfahren zur Entfärbung der Melasse wurde erarbeitet und die Zuckerbestimmung nach Minimalisierung der Messfehler und der Farbinterferenzen automatisiert.

Der Vergleich des Wachstums von *C. tropicalis*, *C. utilis* und *Tr. cutaneum* auf Melasse zeigte, dass *Tr. cutaneum* sich für die SCP-Herstellung am besten eignet. *Tr. cutaneum* zeichnet sich durch hohe Ausbeute und hohen Proteingehalt, obligat respiratives Wachstum und relativ geringe Schaumbildung aus. Die Zusammensetzung des Mediums wurde optimiert und die Zugabe der Medienkomponenten pro Gramm behandelter Melasse berechnet. Der Schaumbildung wurde mit Polypropylenglykol (PPG) 2000 vorgebeugt. Unter 1750 ppm PPG wurde das Wachstum von *Tr. cutaneum* durch das Antischäummittel nicht beeinflusst.

Die Ausbeute von *Tr. cutaneum* bezüglich Melasse ($Y_S$) war konstant bei Temperaturen zwischen 27 und 37 °C. Die spezifische Sauerstoffaufnahmerate ($Q_{O_{2}}$) wurde durch die höheren Temperaturen stimuliert, was einem Rückgang der Ausbeute bezüglich Sauerstoff ($Y_{O}$) gleichkommt. Bei Temperaturen über 37 °C sank $Y_S$ während $Y_{O}$ zunahm. $Y_S$ und $Y_{O}$ stiegen im pH-Bereich zwischen 3 bis 6 mit ansteigendem pH, sanken jedoch im konzentrierten Medium (von 4.9 bis 97.0 g l$^{-1}$ TRS). Es wurde gezeigt, dass die Verringerung der Ausbeute im konzentrierten Medium nicht auf die Sauerstoffversorgung der Zellen zurückzuführen war.
Eine ausgeprägte Erhöhung der Produktivität wurde beobachtet, wenn die Züchtungstemperatur von 27 °C auf 30 °C erhöht wurde. Über 30 °C war die Zunahme gering und bei Temperaturen über 37 °C sank die Produktivität stark. Bei Kultivierungen zwischen pH 3 und 4 nahm die Produktivität mit steigendem pH zu und blieb dann konstant bis pH 6. Bei hohen Medienkonzentrationen (48.5 bzw. 97.0 g l⁻¹ TRS) und angemessener Sauerstoffsättigung sank die maximale Verdünnungsgeschwindigkeit, was die Limitierung der maximalen Produktivität auf 15.43 g l⁻¹ h⁻¹ bedeutete.

Der Protein- und Nukleinsäuregehalt sank sowohl mit zunehmender Temperatur im Bereich zwischen 27 °C und 37 °C als auch bei steigenden pH-Werten zwischen 3 und 6, war jedoch höher in konzentrierten Medien. Bei Sauerstofflimitation stieg der Proteingehalt, während der Nukleinsäuregehalt keine Abhängigkeit zeigte.

Der Sauerstoffübergangs-Koeffizient ($K_a$) sank mit zunehmender Temperatur und steigendem pH. Die Zugabe von PPG (250-1750 ppm) hatte im verwendeten System keinen Einfluss auf den $K_a$.

Substrate and dissolved oxygen tension can be calculated from the following equations:

\[ S = S_R - BX \]

\[ p_L^{G} = p_G^{O} - \frac{1}{A} \times X \]

where:

\[ A = \frac{K_{G}^{a} \gamma_{O}^{\max}}{D + \mu_{e} (1 + \frac{\gamma_{O}^{\max}}{Y_{e}})} \]

\[ B = \frac{D + \mu_{e}}{D \cdot \gamma_{S}^{\max}} \]
Biomass concentration, $X$, is the real and positive root of one of the four following equations:

For Monod-Monod kinetics (model I):

$$X^2 \left[ (1 - C) \frac{B}{A} \right] +$$

$$X \left[ (C - 1) \left( \frac{1}{A} S_R + BF^G_0 \right) - \frac{1}{A} K_S - B K_O \right] +$$

$$1 \left[ (1 - C) \left( P^G_0 S_R + K_S P^G_0 + K^O_S R + K^O_K S \right) \right] = 0$$

For Contois-Monod kinetics (model II):

$$X^2 \left[ - \frac{1}{A} B_S + (1 - C) \frac{B}{A} \right] +$$

$$X \left[ B_S P^G_0 + K^O B_S + (C - 1) \left( \frac{1}{A} S_R + B P^G_0 \right) - B K_O \right] +$$

$$1 \left[ (1 - C) \left( P^G_0 S_R + K^O S_R \right) \right] = 0$$
For Monod–Contois kinetics (model III):

\[
x^2 \left[ -B B_0 + (1 - C) \frac{B}{A} \right] + \\
X \left[ B O S_R + B_0 K_S + (C - 1) \left( \frac{1}{A} S_R + B P_0^G \right) - \frac{1}{A} K_S \right] + \\
1 \left[ (1 - C) P_0^G S_R + K_S P_0^G \right] = 0
\]

For Contois–Contois kinetics (model IV):

\[
x^2 \left[ B O S_R - \frac{1}{A} B_S - B B_0 + (1 - C) \frac{B}{A} \right] + \\
X \left[ B S P_0^G + B O S_R + (C - 1) \left( \frac{1}{A} S_R + B P_0^G \right) \right] + \\
1 \left[ (1 - C) P_0^G S_R \right] = 0
\]

where:

\[
C = \frac{\mu_{\text{max}}}{D + \mu_e}
\]
C EINLESEN DER BEHÖRDTEN KONSTANTEN WERTE

DIMENSION 5

INPUT(I)= INPUT, TAPE1= INPUT, TAPE2= OUTPUT

PROGRAM HOSEIN

**Gleichung quadratischen Berechnens**

\[ \text{CONTINUE110} \]

\[ \text{GO TO 133} \]

\[ \text{CONTINUE120} \]

\[ \text{CONTINUE133} \]

\[ \text{1-3.} \]

\[ \text{IF} (\text{IALPHI,J).EQ.0.) \text{GO TO 122} \]

\[ \text{IF} (\text{ICALFETE1I,J).LT.0.) \text{GO TO 122} \]

\[ \text{M} = \text{J} \cdot \text{i} \]

\[ \text{J} = 1, \ldots, \text{c} \]

\[ \text{GLEICHUNG QUADRATISCHE KoEFFIZIENTEN BERECHNEN} \]

\[ \text{CONTINUE100} \]

\[ \text{GO TO 133} \]

\[ \text{CONTINUE110} \]

\[ \text{GO TO 133} \]

**Gleichungen**

\[ \text{GAMMA(1,1)} = \text{XV1} = \text{ICDII} - \text{H} \cdot \text{ISR}/\text{ADOII} \]

\[ \text{GAMMA(1,2)} = \text{XV2} = \text{ICDII} - \text{H} \cdot \text{ISR}/\text{ADOII} \]

\[ \text{GAMMA(1,3)} = \text{XV3} = \text{ICDII} - \text{H} \cdot \text{ISR}/\text{ADOII} \]

\[ \text{ALPHI,1} = \text{XV1} - \text{ICDII}/\text{ADOII} \]

\[ \text{ALPHI,2} = \text{XV1} - \text{ICDII}/\text{ADOII} \]

\[ \text{ALPHI,3} = \text{XV1} - \text{ICDII}/\text{ADOII} \]

**MODELL**

\[ \text{K} = \text{ICDII} \]

\[ \text{GAMMA(1,1)} = \text{XV1} - \text{ICDII}/\text{ADOII} \]

\[ \text{GAMMA(1,2)} = \text{XV2} - \text{ICDII}/\text{ADOII} \]

**MODELL**

\[ \text{K} = \text{ICDII} \]

\[ \text{GAMMA(1,1)} = \text{XV1} - \text{ICDII}/\text{ADOII} \]

\[ \text{GAMMA(1,2)} = \text{XV2} - \text{ICDII}/\text{ADOII} \]

**MODELL**

\[ \text{K} = \text{ICDII} \]

\[ \text{GAMMA(1,1)} = \text{XV1} - \text{ICDII}/\text{ADOII} \]

\[ \text{GAMMA(1,2)} = \text{XV2} - \text{ICDII}/\text{ADOII} \]

\[ \text{GAMMA(1,3)} = \text{XV3} - \text{ICDII}/\text{ADOII} \]

\[ \text{GAMMA(2,1)} = \text{XV1} - \text{ICDII}/\text{ADOII} \]

\[ \text{GAMMA(2,2)} = \text{XV2} - \text{ICDII}/\text{ADOII} \]

\[ \text{GAMMA(2,3)} = \text{XV3} - \text{ICDII}/\text{ADOII} \]

\[ \text{GAMMA(3,1)} = \text{XV1} - \text{ICDII}/\text{ADOII} \]

\[ \text{GAMMA(3,2)} = \text{XV2} - \text{ICDII}/\text{ADOII} \]

\[ \text{GAMMA(3,3)} = \text{XV3} - \text{ICDII}/\text{ADOII} \]

**PROGRAMM**

**MODELL**

\[ \text{K} = \text{ICDII} \]

\[ \text{GAMMA(1,1)} = \text{XV1} - \text{ICDII}/\text{ADOII} \]

\[ \text{GAMMA(1,2)} = \text{XV2} - \text{ICDII}/\text{ADOII} \]

\[ \text{GAMMA(1,3)} = \text{XV3} - \text{ICDII}/\text{ADOII} \]

\[ \text{GAMMA(2,1)} = \text{XV1} - \text{ICDII}/\text{ADOII} \]

\[ \text{GAMMA(2,2)} = \text{XV2} - \text{ICDII}/\text{ADOII} \]

\[ \text{GAMMA(2,3)} = \text{XV3} - \text{ICDII}/\text{ADOII} \]

\[ \text{GAMMA(3,1)} = \text{XV1} - \text{ICDII}/\text{ADOII} \]

\[ \text{GAMMA(3,2)} = \text{XV2} - \text{ICDII}/\text{ADOII} \]

\[ \text{GAMMA(3,3)} = \text{XV3} - \text{ICDII}/\text{ADOII} \]

**MODELL**

\[ \text{K} = \text{ICDII} \]

\[ \text{GAMMA(1,1)} = \text{XV1} - \text{ICDII}/\text{ADOII} \]

\[ \text{GAMMA(1,2)} = \text{XV2} - \text{ICDII}/\text{ADOII} \]

\[ \text{GAMMA(1,3)} = \text{XV3} - \text{ICDII}/\text{ADOII} \]

\[ \text{GAMMA(2,1)} = \text{XV1} - \text{ICDII}/\text{ADOII} \]

\[ \text{GAMMA(2,2)} = \text{XV2} - \text{ICDII}/\text{ADOII} \]

\[ \text{GAMMA(2,3)} = \text{XV3} - \text{ICDII}/\text{ADOII} \]

\[ \text{GAMMA(3,1)} = \text{XV1} - \text{ICDII}/\text{ADOII} \]

\[ \text{GAMMA(3,2)} = \text{XV2} - \text{ICDII}/\text{ADOII} \]

\[ \text{GAMMA(3,3)} = \text{XV3} - \text{ICDII}/\text{ADOII} \]
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