

OXYGEN TRANSFER IN FILAMENTOUS BIOcultures

WA Pretorius • P Wille

WRC Report No. 331/1/01



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OXYGEN TRANSFER IN FILAMENTOUS BIOCULTURES

Final Report

to the Water Research Commission

by

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WRC Report No. 331/1/01
ISBN 1 86845 785 0

JULY 2001

EXECUTIVE SUMMARY

BACKGROUND:

1. INTRODUCTION

The Water Utilisation Division of the University of Pretoria has developed a micro-screen method for the selective cultivation of essentially mono cultures of filamentous algae and fungi.

Because of the screening action of the micro-screen, it is possible to increase the biomass in a continuous culture of filamentous fungi to higher concentrations than what is usually achieved by other means. In general, higher concentrations of biomass means that smaller reactor volumes are required to effect a particular bio-mediated reaction.

Although there are advantages in by increasing the biomass concentration in a bioreactor, one serious disadvantage observed with filamentous biomass is that an increase in biomass concentration leads to a decrease in the efficiency of oxygen transfer in such cultures. Since oxygen is an essential (and costly) nutrient for aerobic biochemical processes, optimisation of oxygen transfer in filamentous fungi was identified as essential for the successful application of the micro-screen selective cultivation method for the treatment of some specific industrial wastewater streams.

This project deals with the development of improved cultivation techniques of filamentous fungi (growing on a fatty acid containing industrial effluent) with special emphasis on oxygen transfer in such biocultures.

2. OXYGEN TRANSFER IN (WASTE) WATER AND METHODS

A literature survey was made of the various methods used for determining the oxygen transfer rates obtainable from aeration devices. Aeration devices are usually tested under "standard" conditions and the benefit of these tests are that different aeration devices can be compared with each other.

The real test of aeration devices lies in its performance under "field" conditions. As it is rather difficult to test aeration devices under these conditions, the Standard test results are usually modified in an attempt to simulate field conditions. At best, this is only an approximation of the real situation.

In wastewater treatment, Chemical Oxygen Demand (COD) balances are used to determine the oxygen requirement of a particular biological wastewater treatment process. This same method, although very cumbersome, could be used to determine the oxygen transfer efficiency in a biological growth system. This was the method used in this study to determine the aeration efficiency under various experimental conditions.

3. EXPERIMENTS AND RESULTS

The experiments done can be classified as Background experiments and Aeration comparative experiments.

In the Background experiments conditions were established under which meaningful comparative experiments could be performed. A comparison of the traditional chemostat method with the newer auxostat method for the continuous cultivation of micro-organisms was made. The auxostat or pH controlled feed-on-demand method was found to be better for our purposes.

The auxostat method was then applied to two reactors in series operation. In this reactor arrangement, the first bioreactor supplies a constant concentration and physiological age of micro-organisms to the second bioreactor. Different experimental conditions are then superimposed on the second bioreactor and its effect measured. This arrangement works quite well.

The following aeration conditions were tested:

- The effect of mechanical mixing with diffused air aeration on oxygen transfer;
- The effect of air flow rate on oxygen transfer; and
- The effect of diffuser submergence on oxygen transfer.

Virtually the same maximum oxygen transfer rates of about 1100 mg O₂/l.h could be achieved by the various actions imposed on the system. However, there are some practical limits which should not be exceeded. These limits are:

- The concentration of filamentous biomass should preferably be less than 6000 mg/l, otherwise oxygen transfer is limited in an exponential order with increase in biomass concentration;
- The maximum aeration rate should be limited to about 1 m³ air/m³ liquid.min. Higher aeration rates caused severe foaming and could cause structural damage to the bioreactor.
- The optimum number of mechanical air bubble breakers are two. Less or more mechanical bubble breakers had a diminishing effect on oxygen transfer rates.

Lastly, it was found that there exists a linear relationship between the submergence depth of the diffuser and the rate of oxygen transfer. This means that the deeper the aeration depth, the better.

CONCLUSIONS

This study has produced a few new concepts with respect to the cultivation of filamentous fungi that will make its industrial application practical and economically feasible.

ACKNOWLEDGEMENTS

The authors are grateful to the Water Research Commission for its financial support without which this research would have been impossible.

CONTENTS

Page

EXECUTIVE SUMMARY	i
BACKGROUND	i
1. INTRODUCTION	i
2. OXYGEN TRANSFER IN (WASTE) WATER AND METHODS	i
3. EXPERIMENTS AND RESULTS	ii
CONCLUSIONS	iii
ACKNOWLEDGEMENTS	iii
CONTENTS	iv
LIST OF TABLES	viii
LIST OF FIGURES	ix
INTRODUCTION	1
OXYGEN TRANSFER IN BIO-CULTURES: AN OVERVIEW	2
Measurement of oxygen transfer rates in clean (tap water)	3
OXYGEN TRANSFER IN WASTEWATER	4
OXYGEN TRANSFER IN MYCELIAL FUNGI CULTURES	5
Effect of organics	5
Effect of surface active agents	6
Mycelial fungi	6
MEASUREMENT OF OXYGEN TRANSFER IN WATER	7
Measurement of oxygen transfer in clean water	7
Measurement of oxygen transfer in wastewater	7

	Page
BACKGROUND TO THIS STUDY	9
Essential background research	9
Main aeration research	9
EXPERIMENTAL	10
Common parameters to all experiments	10
Feedstock	10
Fungus	11
Analysis and control	11
Oxygen transfer rates	11
Bioreactors	12
Micro-screen selector with cell recycle	12
Reactor without cell recycle	12
Flow configuration and pH control	13
Selector micro-screen reactor with cell recycle	13
Manual feedrate control	13
Automatic pH controlled feed-on-demand	13
Reactor without selector and without recycle	14
BACKGROUND, DESIGN, OPERATION AND RESULTS OF SPECIFIC EXPERIMENTS¹⁵	
Experiment 1: Chemostat vs autostat operation	15
Background	15
Operation	15
Results and discussion	15
Experiment 2: Partial neutralisation of feedstock	16
Background	16
Operation	17
Results and discussion	17

	Page
COMPARATIVE STUDIES ON OXYGEN TRANSFER RATE	18
Background	18
Experiment 3: Effect of the number of rotors on a shaft	19
Operation	19
Results and discussion	19
Experiment 4: Effect of rotating speed of rotor on oxygen transfer rate	20
Operation	20
Results and discussion	20
Experiment 5: Effect of airflow rate on the oxygen transfer rate	21
Operation	21
Results and discussion	21
Experiment 6: Effect of reactor depth on the oxygen transfer rate	22
Operation	22
Results and discussion	22
Experiment 7: Effect of biomass concentration on oxygen transfer and viscosity of the medium	23
Operation	23
Results and discussion	23
SUMMARY AND CONCLUSION	24
BIBLIOGRAPHY	25

APPENDIX 1	29
APPENDIX 2	30
APPENDIX 3	31
APPENDIX 4	31
APPENDIX 5	31
APPENDIX 6	32
APPENDIX 7	32
APPENDIX 8	33

LIST OF TABLES

- Table 1:** Theoretical COD values for various empirical microbial cell formulations.
- Table 2:** Composition and concentration of undiluted feedstock.
- Table 3:** Nutrient addition to undiluted feedstock.
- Table 4:** Feed and effluent concentration of a selective micro-screen operated as chemo and auxostat respectively.
- Table 5:** Spacing of rotors on shaft.

LIST OF FIGURES

PAGE

- Figure 1: Decrease of k_La values depending on mycelial concentration in broth; The value determined polarographically is plotted against mould concentration, %.
- Figure 2: Rushton rotor.
- Figure 3: Reactor with manual feed control.
- Figure 4: pH controlled feed-on-demand.
- Figure 5: Two-reactor in series combination.
- Figure 6: Effect of partial neutralisation on oxygen transfer.
- Figure 7: Effect of number of rotors on the oxygen transfer rate.
- Figure 8: Effect of the rotating speed of two rotors on oxygen transfer rate.
- Figure 9: Effect of airflow rate on the oxygen transfer rate.
- Figure 10: Effect of reactor depth on oxygen transfer rate.
- Figure 11: Effect of biomass concentration on oxygen transfer and viscosity of reactor content.

INTRODUCTION

The Water Utilisation Division of the University of Pretoria has developed a micro-screen method for the selective cultivation of essentially mono cultures of filamentous algae (Pretorius and Hensman, 1984) and fungi (Kühn and Pretorius, 1988 and 1989; and Pretorius and Lempert, 1993). In this process the micro-screen also acts as a cell separator, similar to a secondary clarifier in activated sludge.

For activated sludge processes with secondary clarifiers, a maximum design value for the biomass concentration (or Mixed Liquor Suspended Solids (MLSS)) of about 4000 mg MLSS/l is recommended (Water Research Commission, 1984). The MLSS value of 4000 mg MLSS/l is arbitrary chosen to ensure a biomass free effluent. Biomass in the effluent results in poor effluent quality.

One of the results of this relatively low sustainable concentration of biomass is that relatively large bioreactors are required to accommodate the required biomass for the activated sludge process.

Because of its very nature of screening, the micro-screen selector/separator has not the same biomass concentration limitations as conventional gravity separators. Virtually any desirable concentrations of filamentous biomass can be maintained in such a micro-screen bioreactor. This means that as the biomass concentration is increased, the size of the bioreactor can then be proportionately decreased.

Although there are advantages by increasing the biomass concentration in a bioreactor, one serious disadvantage observed with filamentous biomass is that an increase in biomass concentration leads to a decrease in the efficiency of oxygen transfer in such cultures. Since oxygen is an essential (and costly) nutrient required for activated sludge type wastewater treatment processes (Rieth *et al.*, 1995) and for aerobic biochemical processes (Chisti, 1989), optimisation of oxygen transfer in cultures of filamentous fungi was identified as essential for the successful application of the micro-screen selective cultivation method.

This report deals with the development of improved cultivation techniques of filamentous fungi (growing on a fatty acid containing industrial effluent) with special emphasis on oxygen transfer in such bio-cultures.

OXYGEN TRANSFER IN BIO-CULTURES: AN OVERVIEW

Solubility of oxygen in water

The solubility of oxygen in water is determined by the partial pressure of oxygen, temperature of the water and the concentration of dissolved impurities in the water (ASCE, 1983).

An empirical formula (Barnes *et al.*, 1981) that gives the effect of temperature between 5°C and 25°C and salinity on the solubility in clean water from air at sea level (total pressure = 101.325 kPa, or oxygen partial pressure = 21.24 kPa) is:

$$C_s = \frac{482.5 - S[0.003 - (2.6T \times 10^{-5})]}{32.6 + T} \quad (1)$$

where C_s is the saturation concentration of oxygen (mg/l); S is the salinity (mg/l); and T is the temperature (°C).

Equation (1) gives approximate values at sea level and a correction must be made for altitude:

$$C_{sh} = C_{so} (0.99)^{h/88} \quad (2)$$

where C_{sh} is the saturation concentration at an elevation of h metres; C_{so} is the saturation concentration at sea level.

The maximum concentration of oxygen from air in tap water (salinity, $S = 400$ mg/l) and at 25°C in Pretoria ($h = 1400$ m) is given by Eq. (1) and (2) as approximately 7.13 mg O/l.

Aerobic micro-organisms require a specific minimum concentration of oxygen to grow. If the oxygen concentration is below this minimum concentration, no aerobic growth will

occur. This critical oxygen concentration lies in the range of 0.96 to 1.6 mg O/l (Bailey and Ollis, 1986).

The maximum volumetric oxygen transfer rate that can be achieved economically on a sustained basis in activated sludge is around 100 mg O/l.h (Grady *et al.*, 1999) and in production fermentation between 200 mg and 1000 mg O/l.h (Chisti, 1989).

From the relatively low solubility of oxygen obtained from air, to the relatively high oxygen demand of micro-organisms grown in productive fermentation, it is clear that huge quantities of oxygen (air) must be supplied to overcome the critical oxygen concentration required for unrestricted growth.

Measurement of oxygen transfer rates in clean (tap water)

Aerators and other oxygen transfer devices are calibrated by measuring its oxygen transfer capability under standard conditions. Standard conditions in the United States are taken as the use of clean (tap) water at 20°C, at sea level and at zero dissolved oxygen (ASCE, 1983 and 1984). In Germany and Austria the guidelines for oxygen transfer measurements in clean water allow the addition of 5 g/m³ of an arbitrary anionic surfactant into the clean water (Wagner and Popel, 1996).

The basic model recommended for the analysis in clean water is (ASCE, 1984):

$$C = C^*_{\infty} - (C^*_{\infty} - C_0) \exp(-K_L a t) \quad (3)$$

Where:

C^*_{∞} = Average dissolved oxygen (DO) saturation concentration attained at infinite time, mgO/l.

$K_L a$ = Apparent volumetric mass transfer coefficient, t⁻¹. K_L is the transfer coefficient for unit area (kg/s.m²); a is the area of air-water surface (m²). Neither K_L nor a can readily be measured, but the composite transfer coefficient $K_L a$ can be inferred from the test.

C_0 = DO concentration at $t = 0$, estimated from the model, mg O//

C = Effective average DO concentration in the liquid phase, mg O//

t = Time, usually minutes

Non-linear regression is employed to fit Eq. (3) to the measured DO profile determined during the oxygenation test. Test procedures are fully described elsewhere (ASCE, 1983 and 1984). Non-linear regression curve fitting is required to determine C_{∞}^* and $K_L a$ from the experimental data. A special computer program (see ASCE 1983 and 1984) is required for the best determination of these constants. However, these constants can easily be determined with a spreadsheet as shown in Appendix 1.

Once $K_L a$ and C_{∞}^* are known the rate of oxygen mass transfer can be calculated by:

$$K_L a = \frac{\text{rate of mass transfer per unit volume}}{C_{\infty}^* - C} \quad (4)$$

Since it is usually not possible to perform the oxygen transfer test under the standard conditions described above, it is necessary to compensate for the difference in temperature and barometric pressure as shown elsewhere (ASCE, 1984) to obtain a Standard Oxygen Transfer Rate (SOTR).

OXYGEN TRANSFER IN WASTEWATER

Many factors such as wastewater contaminants, temperature, DO concentrations, turbulence and basin geometry, all affect oxygen transfer rate. Since these factors make field application unique, it has become standard practice to develop additional techniques for adjusting rates at "standard conditions" to rates at field (wastewater) conditions (ASCE, 1993).

Field oxygen transfer rates, OTR_f , are calculated from SOTR, through the use of alpha, α , beta, β and theta, θ factors. The α factor is to accommodate the effect of impurities on $K_L a$; the β factor compensates for the average DO saturation concentration, C_{∞}^* , attained

at infinite time; and the θ factor compensates for temperature. OTR_f can be calculated from SOTR:

$$OTR_f = \frac{\alpha(SOTR)\theta^{(T-20)}}{C^*_{\infty 20}}(\beta C^*_{\infty ww} - C_R) \quad (5)$$

where $C^*_{\infty ww}$ indicating wastewater and C_R = desired DO concentration under normal operation, mg O/l.

From the above it is clear that the direct measurement of oxygen transfer in wastewater is effected by many factors, which should be taken in account when oxygen transfer in mycelial fungi cultures is measured.

OXYGEN TRANSFER IN MYCELIAL FUNGI CULTURES

Effect of organics

The concentration of impurities like dissolved organics is generally much higher in fermentation broths than in wastewater. Some of these dissolved organics have a detrimental effect while other have a beneficial effect on oxygen transfer.

Eckenfelder and Barnhart (1961) found that the K_La value decrease to 40% of its clean water value when 10 g/l peptone was added. Similarly, surface active agents usually decrease the K_La values to such an extent that compensation for it is made in the K_La obtainable in wastewater.

In contrast to the above, Zieminshi *et al.* (1960) found that the value of K_La in distilled water increased by 50 to 100% with the addition of 20 mg/l of substances like n-buthyl and iso-amylacetate.

Fermentation nutrients and fermentation products thus can substantially change the K_La as measured in clean water.

Effect of surface active agents

The effect of surface active agents on $K_L a$ is well known in wastewater aeration. This effect is compensated by the α factor on $K_L a$. Studies by Wagner and Pöpel (1996) showed that the presence of 5 mg surfactant/l decreases $K_L a$ by 55%. The effect of surfactant was greater on fine bubble aeration (α values in range between 0.4 and 0.7), than on mechanical aeration systems ($\alpha = 0.9$ to 0.95). Also, non-ionic surfactants reduce the oxygen transfer more strongly than anionic surfactants.

Mycelial fungi

While most bacteria and yeasts give rise to a Newtonian suspension with the suspending fluid having nearly the same viscosity as water, some bacteria and yeast fermentation systems can be highly viscous and non-Newtonian. The latter behaviour is largely due to various polymers secreted by the cells into the surrounding medium (Chisti, 1989). Most mycelial fungi have a thread like form which form large flocs in suspended growth systems (Kühn and Pretorius, 1987; Pretorius and Lempert, 1993). This mycelial form viscous fluids with non-Newtonian rheological properties. Brierley and Steel (1959) study the effect of the mycelial concentration of *Aspergillus niger* on the reduction of $K_L a$ as shown in Figure 1.

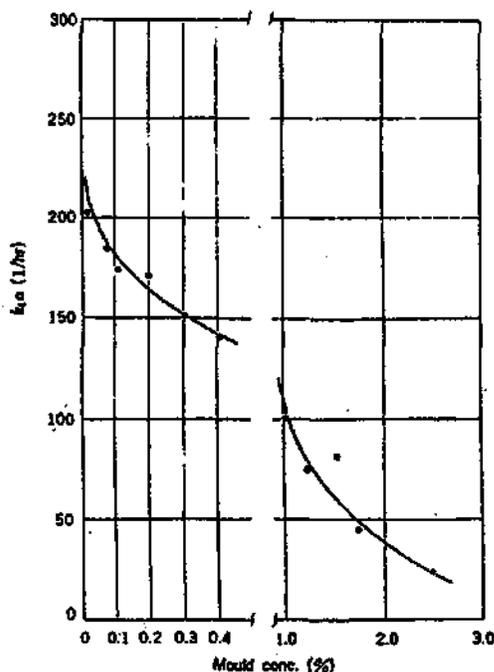


Fig. 1. Decrease of $k_L a$ values depending on mycelial concentration in broth; The value determined polarographically is plotted against mould concentration, % (*Aspergillus niger*).

In summary it can be said that although there are similarities, there are also quite substantial differences between oxygen transfer in clean and wastewater on the one hand and mycelial fungi cultures on the other.

MEASUREMENT OF OXYGEN TRANSFER IN WATER

Measurement of oxygen transfer in clean water

An in-detail description of the apparatus and methods to be used for the determination of oxygen transfer in clean (tap) water is given by ASCE (1994). ASCE (1994) recommended that the dissolved oxygen be chemically removed by sulphite (Na_2SO_3). The sulphite deoxygenation reaction is catalysed by cobalt (CoCl_2 or CoSO_4). Oxygenation is then started and oxygen measurements taken at various prescribed points. The unsteady state oxygen transfer test data is then fitted to the oxygen uptake model given in Eq. 1.

In laboratory experiments, deoxygenation can also be obtained by nitrogen stripping of the oxygen in the test water (ASCE 1993).

Measurement of oxygen transfer in wastewater

Alpha, α and beta, β values are necessary for describing the influence of dissolved and suspended solids on the transfer capability of aeration equipment in clean water. In general, α and β measurements are made only when the field transfer rates are to be compared to standardised transfer rates developed under clean water conditions (ASCE, 1993).

Under field conditions, i.e. activated sludge systems which contain respiring biomass, three test methods have been used which do not require a direct measure of oxygen uptake rate, namely:

- (i) the mass balance method which requires data on the net change in oxidation level between all entering and exiting liquid fluids;

- (ii) the off-gas method which is simply a mass balance on oxygen that includes both the liquid and gas streams: and
- (ii) the tracer method which indirectly measures the rate of oxygen transfer by determining the rate of transfer of a radioactive tracer.

The mass balance method is most commonly used and can be performed on continuous flow (steady state) and batch (unsteady state) reactors.

The energy in a compound is related to the electrons available for transfer, which can be related to the amount of oxygen required to completely oxidise the compound or the oxygen demand (OD) of the compound (Grady and Lim, 1990). The amount of OD removed from the medium must equal the oxygen actually used by the biomass, plus the OD of the biomass (cells) formed. The steady state oxygen requirement of a bioculture can be calculated by a mass balance on OD. The oxygen demand of organic matter can be measured by the Chemical Oxygen Demand (COD) test (Water Research Commission, 1984).

The COD (or OD) of microbial cells depends on the empirical formula of cell material, which can vary between 1.10 to 1.33 g COD/g TSS as shown in Table 1.

Table 1: Theoretical COD values for various empirical microbial cell formulations. (McCarty, 1964).

Bacterial formulation	Molecular weight	Theoretical COD in grams	
		Per g TSS	Per g VSS
C ₅ H ₇ O ₂ N	113	1.28	1.42
C ₅ H ₉ O ₃ N	131	1.10	1.22
C ₇ H ₁₀ O ₃ N	156	1.33	1.48
C ₅ H ₈ O ₂ N	168	1.32	1.47

Grady and Lim (1980) called the COD of ash-free cells (VSS) β and the oxygen uptake rate, r_o , to make a COD balance over a bioreactor growing at steady state:

$$FS_o - FS - F\beta X + (-r_oV) = 0 \tag{6}$$

Where: F = flow rate
 S_o = COD concentration of inflowing substrate

S	=	COD concentration of effluent
X	=	ash-free biomass
V	=	volume of the reactor

The mass oxygen transfer rate r_o (mg O₂/l.h) can readily be calculated from Eq. 6.

Because of the difficulty to measure K/a in a fermentation broth, especially one with mycelial growth, this method of COD mass balances was used in this study.

BACKGROUND TO THIS STUDY

The use of continuous stirred tank reactors (CSTR) with cell recycle for biochemical operations is not new (Grady and Lim, 1960). The use of a micro-screen as species selector and cell separator is new. The result is that most of the work done thus far (Pretorius and Hensman, 1984; Kühn and Pretorius, 1988) were of an exploratory nature.

Planning this research, it becomes clear that the exploratory studies have identified a few issues which first should be answered, before meaningful experiments on oxygen transfer could be attempted.

Essential background research

As a fatty acid containing industrial wastewater supplemented with nutrients was used as basic feedstock, pH control was a problem as large quantities of neutralising agent was required to keep the pH within acceptable limits. Experiments were done to see if it was possible to reduce the amount of neutralising agent required.

Main aeration research

Grady and Lim (1980) describing the use of a chain of CSTRs with multiple feed points mentioned that in a multistream system the micro-organisms in the second stage can be operated at any practical rate without the danger of washout in the system. The characteristics of such a two-stage system makes it ideally suitable for maintaining a rapid growing culture for a long period.

This two-stage reactor set-up and flow configuration were used for all comparative experiments.

In summary, the experiments are as follows:

1. Essential background research

The development of a pH-controlled, feed-on-demand fungi growth system with the purpose of reducing the amount of fatty acid neutralising agent and improving oxygen transfer rates. This is described in Experiments 1 and 2 respectively.

2. Comparative aeration studies

A series of comparative aeration studies are described in Experiments 3 to 7 respectively.

EXPERIMENTAL

Common parameters to all experiments

Feedstock

A fatty acid containing effluent from a synthetic petroleum producing industry was used as feedstock for all experiments in this study. The composition and average concentration of the undiluted industrial effluent is shown in Table 2.

Table 2: Composition and concentration of undiluted feedstock.

Constituents	Concentration (mg/l)	COD mg/l
Acetic acid	8748	9323
Propionic acid	2564	3877
i-Butyric acid	321	584
n-Butyric acid	979	1779
i-Valeric acid	212	432
n-Valeric acid	283	575
Average COD concentration		16570*
Coefficient of variation		900

- * Some batches were as high as 21 300 mg COD/l.
pH of undiluted feedstock = 3.8.

Nutrients were added to the undiluted feedstock at concentrations as shown in Table 3.

Table 3: Nutrient addition to undiluted feedstock.

Nutrient	Mass (g) added per liter
NH ₃	1.0
H ₃ PO ₄	0.47
K ₂ HPO ₄	0.28
MgSO ₄	0.08

Fungus

Micro-screened selected cultures of *Geotrichum* sp. were used for all experiments which were conducted at 35 ± 1°C.

Analysis and control

Chemical Oxygen Demand (COD) and Suspended Solids (SS) were analysed according to Standard Methods (1989).

pH-Control was done by a pH-stat (E and H Conducta, Stuttgart), airflow with a rotameter (Fischer and Porter FP-1/2-17-10/80, Gottingen) and temperature by a thermostat/heater (Sicce, model RTR 25/300 PD, Italy).

Oxygen transfer rates

Oxygen transfer rates were calculated from COD mass balances made on COD on solution and the COD of fungal cells.

Bioreactors

Two types of bioreactors were used, namely:

- a micro-screen selector reactor with cell recycling; and

b. a reactor, usually in series with (a) without cell recycle.

a. Micro-screen selector reactor with cell recycle

A rectangular, 0.1 x 0.175 x 1.83 m high aeration column with micro-screen was constructed from Perspex. A membrane (Elastox-T, Biwater) diffuser with two identical halves, was mounted at the bottom of the aeration column. A 11.5 cm by 5.57 cm, 200 μ mesh with overflow collecting chamber was mounted, 1.45 m from the base of the reactor. The effective volume of the reactor was 24.2 l.

b. Reactor without cell recycle

The reactor without cell recycle consists of a square 0.4 m x 0.4 m base with draw-off overflows at 0.4, 0.6, 0.8, 1.0 and 1.2 m from the base. The working volume of the reactor varied and depends on which draw-off level was used. This reactor was fitted with a membrane diffuser at the bottom as described above. Airflow to the reactor was measured with a rotameter (Perflow FR6S, London). The airflow to reactor could be varied between 0 and 4 air volumes per reactor volume per minute.

In addition to the fine bubble diffuser a Rushton-rotor, with a rotor diameter to reactor diameter ratio of 0.3 (Oldshue, 1983) was installed. Figure 2 shows the dimensions of the Rushton-rotor.

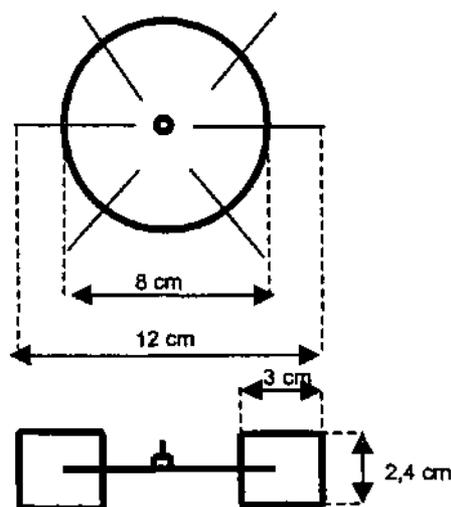


Fig. 2. Rushton-rotor.

The number of rotors (on the same shaft) could be varied from 0 to 4, and the rotation speed from 0 to 600 rpm. A temperature and pH controller were provided as before.

Flow configuration and pH control

Selector micro-screen reactor with cell recycle

a. Manual feedrate control

In the manual feed mode, feedstock was fed by a manual adjusted peristaltic feedpump, set at any predetermined rate. In this case the pH in the reactor was maintained automatically at a constant value (pH = 5) by the pH stat, using 6N – NaOH as neutralising agent.

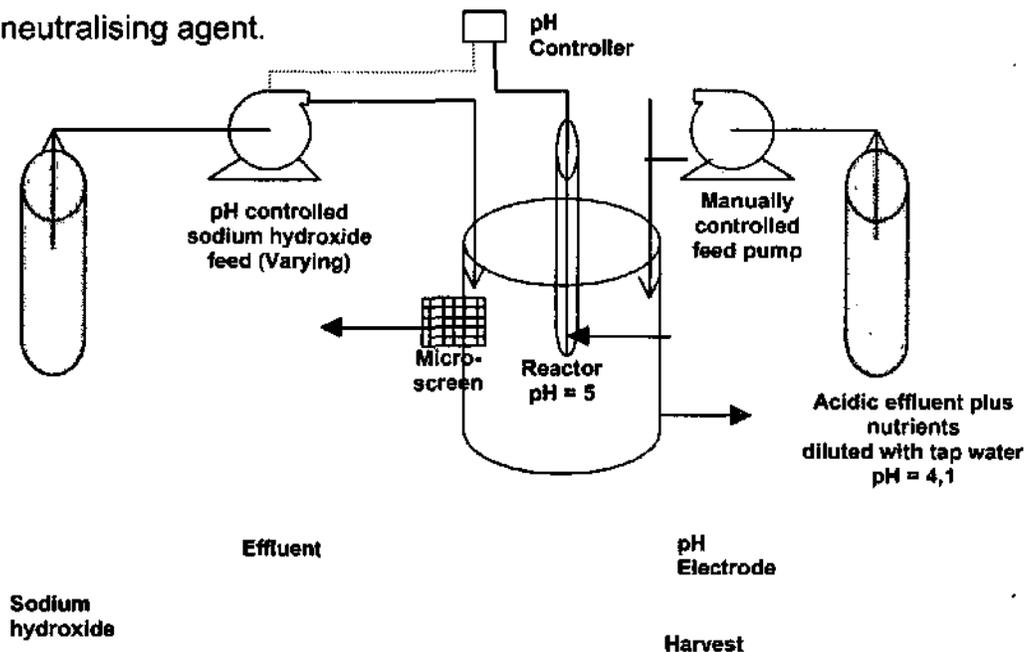


Fig. 3. Reactor with manual feed control.

Figure 3 shown schematically this feed and pH control.

b. Automatic pH controlled feed on demand

In the pH controlled feed on demand mode, the pH controller is set at pH 5. When the pH exceeds pH 5, the pH controller activates the feed pump. As the feed has a pH < 5, the pH in the reactor starts to drop. When the pH reach a value of 4.8 (0.2 pH units is the sensitivity of the pH controller), the feed is stopped until a pH of

about 5 is reached, when the feedpump is again activated. The pH controlled feed on demand mode is shown in Figure 4.

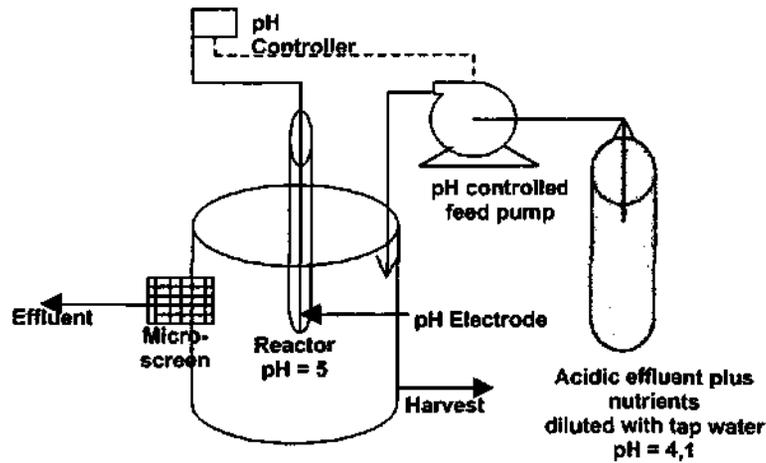


Fig. 4: pH controlled feed on demand.

Reactor without selector and without recycle

This reactor was the second reactor in a series of two, where the first reactor was a selector reactor with cell recycle. This second reactor was equipped with its own temperature and pH controller. The flow configuration for this two-reactor in series composition is shown in Figure 5.

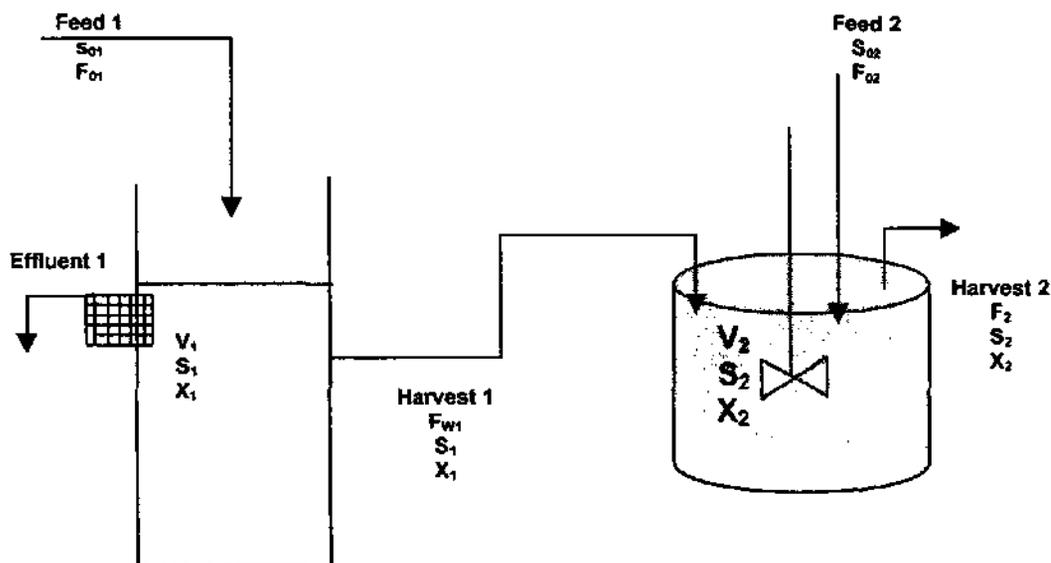


Fig. 5: Two-reactor in series combination.

In this case, tests were generally performed on the second reactor.

BACKGROUND, DESIGN, OPERATION AND RESULTS OF SPECIFIC EXPERIMENTS

Experiment 1: Chemostat vs auxostat operation

Background

Up till now, all research experiments where the selective micro-screen technique were used, the bioreactors were operated manually (Figure 3) like a chemostat (Bailey and Ollis, 1986). To maintain a cell concentration (X , mg/l) of between 4000 and 6000 mg/l, at a cell age (θ_c ,h) less than 18 h (maximum θ_c which still maintains reasonable selection pressure, it was necessary to dilute the feedstock to a COD concentration of about 700 mg/l with tap water.

Sowers *et al.* (1984) described an auxostat where, in contrast to a chemostat, the feed rate to the reactor is determined by the substrate utilisation rate of the culture. Because of the similarity between their substrate (acetic acid) and our feedstock (mixture of short chain fatty acids), this pH method of feed rate control was tried on our system.

Operation

A selective micro-screen reactor was operated on diluted feedstock on the manual feed mode and then changed to the pH controlled feed mode (Figure 4). The operational conditions, i.e. sludge age, hydraulic residence time (τ ,h) was approximately similar for the two modes, namely $\theta_c = 14$ h, $\tau = 3.5$ h.

Results and discussion

The feed and effluent concentrations for the two modes of operation are shown in Table 4.

Table 4: Feed and effluent concentration of a selective micro-screen operated as chemo and auxostat respectively.

Feed: Acid	Chemostat:			Auxostat:		
	Acid (mg/l)	COD (mg/l)	As Acetic (mg/l)	Acid (mg/l)	COD (mg/l)	As Acetic (mg/l)
Acetic	3796	4045	3796	3743	3988	3796
Propionic	1103	1668	1360	1107	1673	1360
i-Butyric	141	255	206	137	248	206
n-Butyric	404	733	592	441	800	592
i-Valeric	92	186	156	92	186	156
n-Valeric	120	244	204	124	252	204
Total		7132	6314		7147	6314

Effluent: Acid	Chemostat:			Auxostat:		
	Acid (mg/l)	COD (mg/l)	As Acetic (mg/l)	Acid (mg/l)	COD (mg/l)	As Acetic (mg/l)
Acetic	752	801	752	124	132	124
Propionic	409	618	504	37	55	45
i-Butyric	108	196	158	6	10	8
n-Butyric	59	106	86	11	19	15
i-Valeric	81	164	137	4	7	6
n-Valeric	15	30	25	16	33	27
Total		1916	1662		256	225

From Table 4 it is clear that lower effluent concentrations were obtained by the auxostat method of operation, than by the chemostat method of operation. With the auxostat operation it was not necessary to partially neutralise the feedstock. Although better substrate removal were obtained, the steady state feed rate was slower than the chemostat method, i.e. τ chemostat = 3.5 h; τ auxostat = 5.75 h.

The probable reason for this slower feedrate is that with the auxostat method of operation, the substrate concentration in the reactor become growth limiting. The auxostat method of feed control has many advantages and should be further investigated.

Experiment 2: Partial neutralisation of feedstock

Background

By operating the selector micro-screen reactor as an auxostat, it was observed that the feed rate was slower than than when the reactor was operated as a chemostat. It was

decided to determine the effect of partial neutralisation of the feedstock on the oxygen transfer rate.

Operation

The fraction of feedstock neutralised was stepwise increased from zero to 2, 3 and 5% respectively. Neutralisation was done for example by removing 2% of the volume of feedstock. The pH of this 2% feedstock is then increased to pH 7 by the addition of 6 N NaOH solution. This neutralised 2% volume is then admixed with the original 98% volume to give a final 2% neutralised feedstock.

The second reactor of a series of reactor was then also run in an auxostat mode and the oxygen transfer rate (r_o) calculated. The process was repeated for higher fractions of neutralisation.

Results and discussion

The results are given in Appendix 2 and shown graphically in Figure 6.

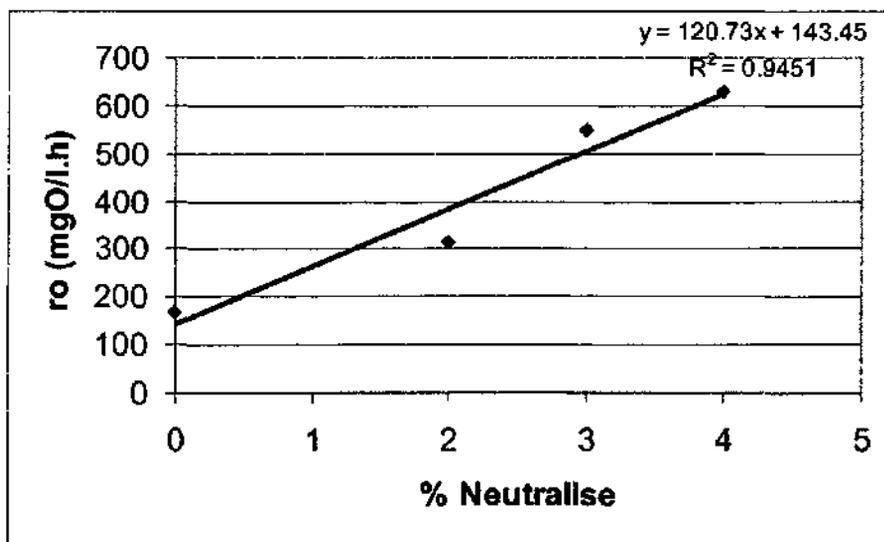


Fig. 6: Effect of partial neutralisation on oxygen transfer.

Figure 6 shows a linear increase in oxygen uptake rate with an increase in partial neutralisation within the fractional neutralisation range tested.

The possible explanation for this observation is two fold. Firstly, as the auxostat method of feed to the reactor is based on changes (increase) in pH due to metabolism, partial neutralisation of the fatty acids increases the pH of the feedstock. The fungi thus had to metabolise less fatty acids from the feedstock to effect an increase in pH, compared to the unneutralised feed. The feed rate thus increases. Secondly by having to metabolise a smaller fraction of fatty acids to effect the required pH change, the unutilised fatty acid concentration in the reactor (as well as in the effluent) increases. This higher concentration of fatty acids do not only increase the growth rate of the fungi (Monod equation), but also allows the fungi to selectively metabolise the more readily utilisable fatty acid species - thus an increase in metabolism and increase in oxygen requirement.

By the auxostat method of feed control and partial neutralisation the metabolic rate of the fungi can be controlled, as well as the fraction of unmetabolised fatty acids in the effluent.

COMPARATIVE STUDIES ON OXYGEN TRANSFER RATE

Background

Once the selective cultivation technique for fungi in a single reactor and in two reactors in series has been established, it was possible to do a series of comparative experiments on oxygen transfer. The purpose of these experiments was to determine the effect of specific variables and combinations of variables which will give the best oxygen transfer. Experiments were done to determine the effect of:

- number of rotors on a shaft;
- rate of rotation of rotor;
- air supply rate;
- depth of air diffuser submergence; and
- biomass concentration on the oxygen transfer rate.

All these experiments were conducted in the second reactor of a chain of two reactors, the first reactor being the selector reactor.

Experiment 3: Effect of the number of rotors on a shaft

Operation

The following experimental conditions were chosen:

Reactor depth = 0,8 m; rotating speed = 375 r.p.m. and air supply rate at 1.0 m³ air / (m³ liquid.min).

The number of rotors on the shaft were increased from 0 to 4. The spacing of the rotors on the shaft was as shown in Table 5.

Table 5: Spacing of rotors on shaft.

No. Rotors	Height above diffusor
1	100 mm
2	100 and 450 mm
3	100, 330 and 560
4	100, 275, 450 and 625 mm

After each change in the number of rotors, the reactors were run until steady state was achieved. COD analysis were then made to determine the oxygen transfer rate.

Results and discussion

The effect that the number of rotors on a shaft have on oxygen transfer is shown in Figure 7.

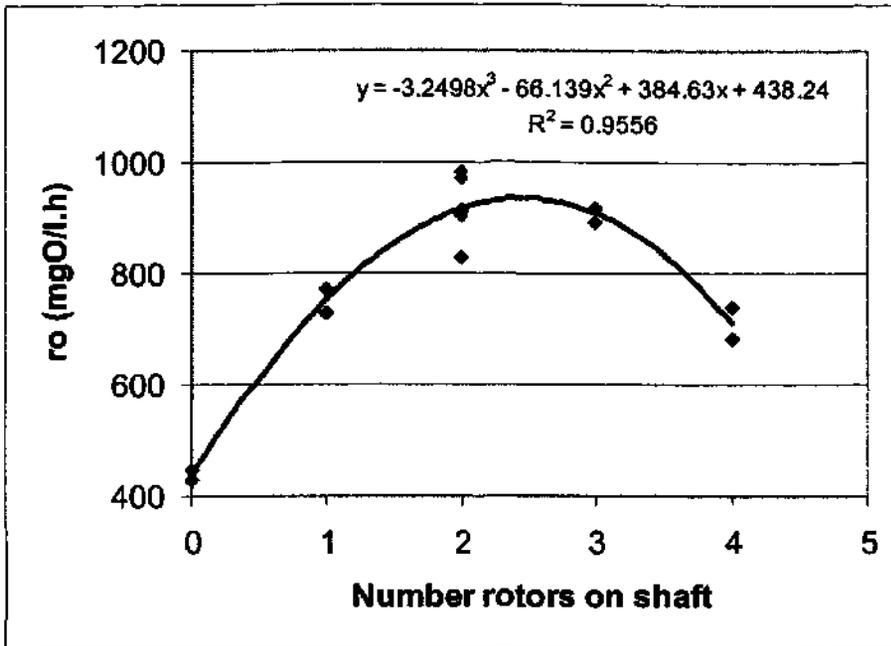


Fig. 7: Effect of number of rotors on the oxygen transfer rate.

Figure 7 shows that the oxygen transfer rate more than doubles (from 400 to > 900) mg O/(l.h) by the addition of rotors to the shaft. The optimum number of rotors seems to be between 2 and 3 rotors per shaft. As it is not possible to have fractional rotors, 2 rotors per shaft seems to be the optimum. Most of subsequent experiments were conducted with 2 rotors on the shaft.

Experiment 4: Effect of rotating speed of rotor on oxygen transfer rate

Operation

The operating conditions for this experiment was very similar to Experiment 3, except that only two rotors per shaft were used, while the rotating speed was stepwise increased from 100 to 650 r.p.m. Oxygen transfer rates were measured for each step increase in r.p.m.

Results and discussion

The results of the rotating speed of the rotor is shown in Figure 8.

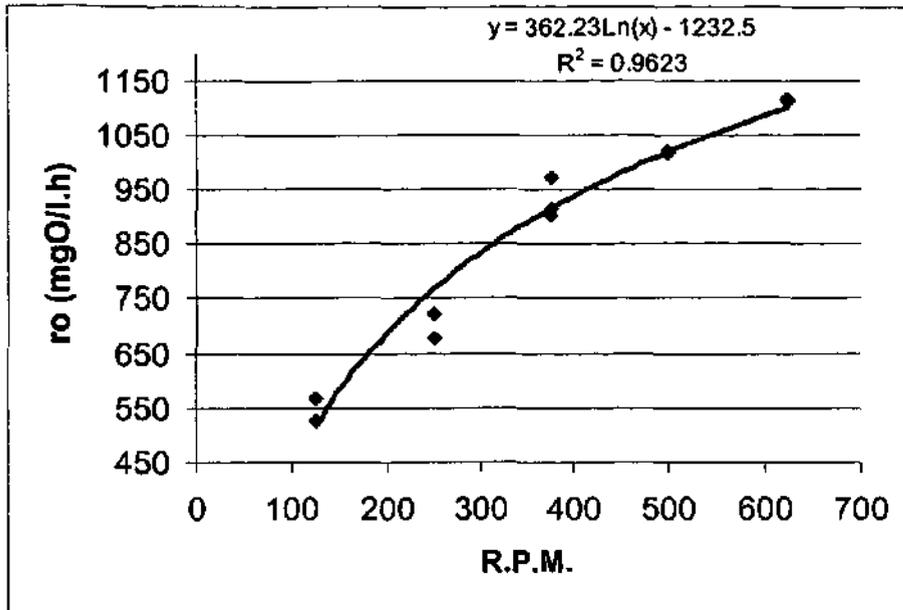


Fig. 8: The effect of the rotating speed of two rotors on oxygen transfer rate.

Figure 8 shows that there is an logarithmic relationship between the rotating speed and the corresponding oxygen transfer rate. Because of the sheer forces induced by the rotor, the rotating speed should be limited to about 450 r.p.m. to prevent filament break-up.

Experiment 5: Effect of airflow rate on the oxygen transfer rate

Operation

The "standardised" experimental conditions of 0.8 m depth, two rotors per shaft and 175 r.p.m. were used, with increasing the airflow rate stepwise from 0.1 – 1.5 m³ air / m³ liquid.min. At each step the oxygen transfer rate was measured.

Results and discussions

The effect of airflow rate on the oxygen transfer rate of a fungal culture is shown in Figure 9.

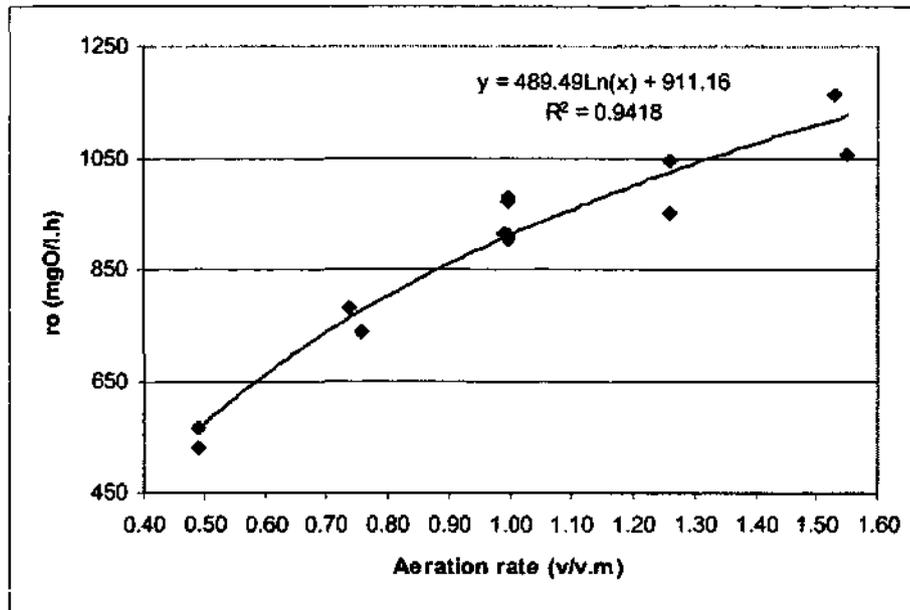


Fig 9: Effect of airflow rate on the oxygen transfer rate.

An increase in airflow rate has an increase in the oxygen transfer rate. At airflow rates of more than 1.0 m³ air / m³ liquid.min, excessive foaming was observed. For practical purposes a maximum airflow rate of 1 m³ air / m³ liquid.min is recommended.

Experiment 6: Effect of reactor depth on the oxygen transfer rate

Operation

Again the standard operating conditions (see Experiment 5) was used. The liquid depth was increased in steps of 0.2 m from 0.4 m to 1.2 m. At each step the oxygen transfer rate was determined.

Results and discussion

The results that show the effect of reactor depth on the oxygen transfer rate of a fungal culture is shown in Figure 10.

Other than for rotating speed and airflow rate (which have a logarithmic relationship), the oxygen transfer rate increases linearly with depth, within the depth range tested. The oxygen transfer rate at a depth of 1.2 m correspond approximately with a rotating speed of

600 r.p.m. and an airflow rate of $1.5 \text{ m}^3 \text{ air} / \text{m}^3 \text{ liquid.min}$, all tested at 0.8 m depth of reactor. This means that reactor depth is an important factor in oxygen transfer efficiency.

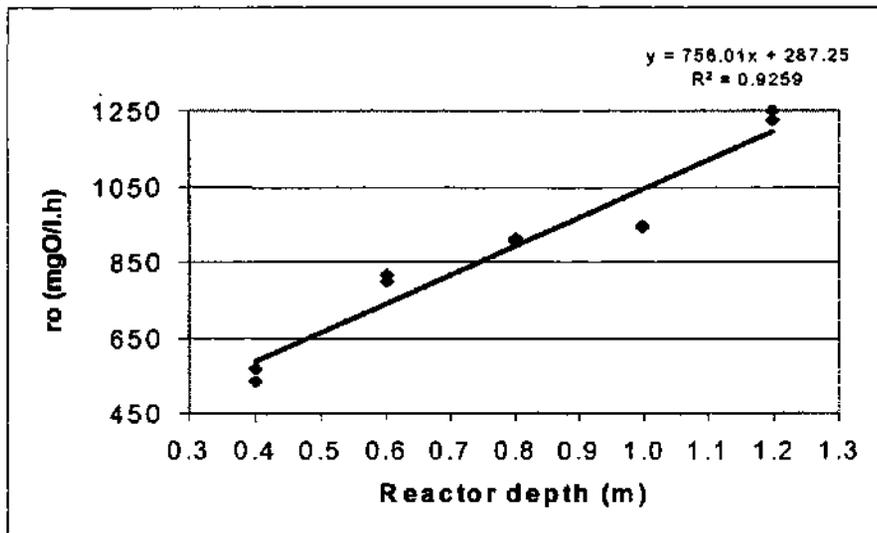


Fig. 10: Effect of reactor depth on oxygen transfer rate.

Experiment 7: Effect of biomass concentration on oxygen transfer and viscosity of the medium

Operation

The standardised operating conditions (depth = 0.8 m, 2 rotors @ 375 r.p.m. and airflow at $1 \text{ m}^3 \text{ air} / \text{m}^3 \text{ liquid.min}$) were chosen and the biomass concentration increased in steps of 1 g/l from 4 to 8 g/l. This increase in biomass concentration was done artificial by harvesting the biomass in the effluent of the test reactor and add it back to the test reactor. For each step increase in biomass, the oxygen transfer rate and viscosity of the reactor contents were determined.

Results and discussion

The results of this experiment is shown in Figure 11.

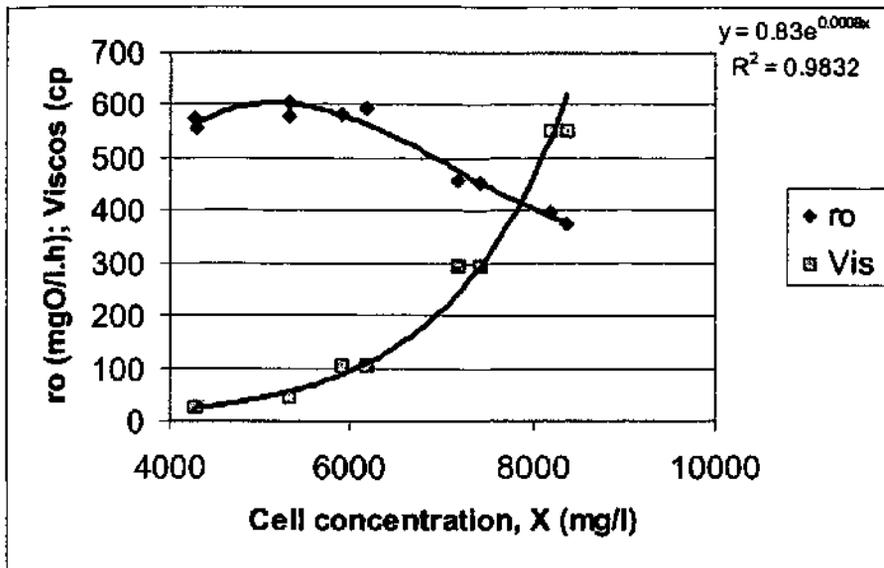


Fig. 11: Effect of biomass concentration on oxygen transfer and viscosity of reactor content.

While the viscosity shown an exponential increase ($y = 0.83e^{0.0008x}$) with a corresponding increase in biomass concentration, it is only after a biomass concentration of about 5 g/l that the oxygen transfer rate is negatively influenced. At a biomass concentration of 5 g/l and more the whole reactor content became visually viscous with the coalescence of air bubbles to form big (20 – 50 mm ϕ) air bubbles.

Operation of this type of fungal system should be limited to a concentration of 5 g biomass / l or less.

SUMMARY AND CONCLUSIONS

1. The screen/selector bioreactor makes the cultivation of filamentous fungi possible. All the experiments were performed on an industrial effluent, illustrating the possible use of fungi for the treatment of specific industrial effluents.
2. Up till now, all experiments with the continuous cultivation of fungi was done by the chemostat-type cultivation method. In this study the auxostat-type cultivation method was introduced. With this method, the micro-organism and the conditions under which it grows, determine the rate at which the culture is fed, i.e. feed-on-demand. With the feed-on-demand method, better substrate removal was achieved

than with the traditional chemostat-type continuous cultivation method. The degree of partial neutralisation of the fatty acid feed affects the growth rate in a pH controlled, feed-on-demand system.

3. A two reactors-in-series set-up, where the second reactor in the series is used as the experimental unit, provides an excellent research tool for comparative studies. With this experimental setup, the following factors that influence the rate of oxygen transfer were studied:
 - a. Number of Rushton rotors to break up air bubbles;
 - b. The rate of rotation of Rushton rotors;
 - c. The rate of air supply;
 - d. The depth of diffuser submergence; and
 - e. The biomass concentration on the oxygen transfer rate.
- 4a. Optimum oxygen transfer occurred with two Rushton rotors per shaft. The oxygen transfer rate increased from ± 420 mg O//.h with no breakup of bubbles to more than 900 mg O//.h with two Rushton rotors on a shaft, rotating at a speed of 375 r.p.m.
- b. By increasing the rotation speed from 375 r.p.m. to 650 r.p.m., the oxygen increases with a diminishing increasing rate to about 1100 mg O//.h. This was about the highest oxygen transfer rate achieved in the filamentous fungi culture.
- c. With an air flow rate of $1.55 \text{ m}^3 \text{ air/m}^3 \text{ liquid.min.}$, the maximum oxygen transfer rate of about 1100 mg O//.h could be achieved. This air flow rate is excessive and caused heavy foaming to occur. A practical maximum air flow rate of $1 \text{ m}^3 \text{ air/m}^3 \text{ liquid.min.}$ is recommended.
- d. A linear relationship between depth of diffuser submergence and oxygen transfer rate exists. Diffuser depth is an important parameter to consider in increasing oxygen transfer in filamentous cultures.

- e. As there is an exponential increase in viscosity with an increase in fungal biomass concentration, the biomass concentration should be limited to a maximum of about 8000 mg/l.
- f. A simplified spreadsheet programme for the calculation of K_{La} and C^*_{∞} from experimental data, is included.

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Appendix 1

Oxygen transfer by non-linear curve fitting

What to do?

Step 1

Use exponential oxygen transfer model:

- $C = C_{inf} - (C_{inf} - C_0) \cdot \exp(-KLa \cdot t)$ where,
- C = Oxygen concentration measured at time t (mgO/l)
- C_{inf} = Maximum oxygen concentration measured at infinite time (mgO/l)
- C₀ = Oxygen concentration at t = 0 (mgO/l)
- KLa = Apparent volumetric mass transfer coefficient (1/s)

Step 2

Create a Spreadsheet with the following variables: C_{inf}, C₀ and KLa respectively. Choose realistic values for each of these variables, eg KLa = 0.15 1/s, C_{inf} = 12 mgO/l and C₀ = 1.0 mgO/l.

Step 3

Make a table of the Time and the corresponding measured dissolved (DO) values. Add a column by using the measured time as a variable and the exponential model with the chosen KLa, C_{inf} and C₀ values to calculate the corresponding C values. Column headings: Time, DO_Mes, and DO_Pred respectively. Add columns Delta and AbsDelta, which are the difference and absolute difference between DO_Mes and DO_Pred values. NB. Not necessary to add the latter columns. The sums of the differences can be calculated by means of Array formulae as shown next to the specific values. The {} of array formulae are obtained by typing the appropriate formula and then "Shift + Ctrl + Enter" to enter the formula as an array formula.

Step 4

Use built-in "Solver" to calculate best-fit values for KLa, C_{inf} and C₀ as follow:
 For KLa: Choose "RSQ" as "target cell" and make it "Max" by changing "KLa" and "Solve"
 For C_{inf} and C₀: Choose "SabsDelta" as "target cell" and make it "Min" by changing "C_{inf} and C₀". Add constraint by setting "SumDelta" = 0 and "Solve". Read also "Stderr" to see error from residual mean square.

Step 5

Plot measured and calculated values to visually see goodness of fit

Example (See "Development of Standard Procedures for Evaluating Oxygen Transfer Devices, American Society of Civil Engineers, New York, PB84-147438, 1983. For data used in this example)

Chosen values:

Apparent volumetric mass transfer coefficient	KLa	0.0669 1/s
Average DO saturation concentration at infinite time	C_{inf}	11.425 mgO/l
DO conc at t = 0, estimated from model	C₀	1.127 mgO/l

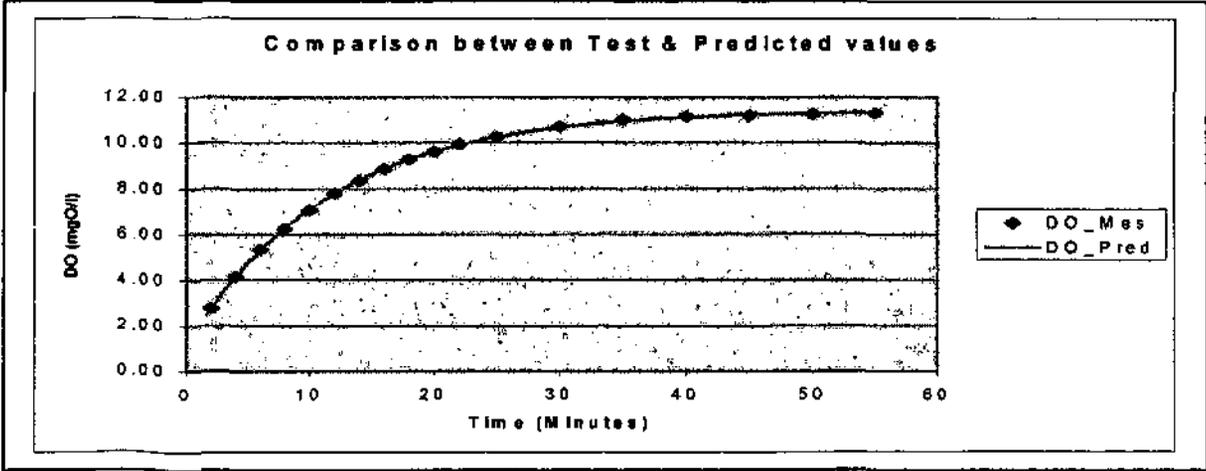
Formulae used for "best-fit" calculations:

Coefficient of determination to measure degree of fit between measured and predicted values	RSQ	0.99988	=RSQ(DO_Pred,DO_Mes)
Sum of Absolute difference of DO measured and model	SabsDelta	0.44127 mgO/l	{=SUM(ABS((DO_Mes)-(DO_Pred)))}
Sum of difference of DO measured and model	SumDelta	9.812E-07 mgO/l	{=SUM(DO_Mes-DO_Pred)}
Estimate of error from residual mean square	Stderr	0.0318 mgO/l	=STEYX(DO_Pred,DO_Mes)

NB For convenience all "Named" cells and cell ranges required for calculations have been printed in Bold

Test data: Measured: Predicted:

Time	DO_Mes	DO_Pred	Delta	AbsDelta
2	2.77	2.77	0.000	3.7E-06
4	4.15	4.15	-0.001	9.6E-04
6	5.35	5.31	0.038	3.8E-02
8	6.25	6.29	-0.037	3.7E-02
10	7.08	7.11	-0.027	2.7E-02
12	7.80	7.80	0.004	4.3E-03
14	8.34	8.37	-0.035	3.5E-02
16	8.85	8.86	-0.011	1.1E-02
18	9.28	9.27	0.010	9.6E-03
20	9.62	9.61	0.006	5.9E-03
22	9.93	9.90	0.027	2.7E-02
25	10.24	10.25	-0.012	1.2E-02
30	10.70	10.67	0.035	3.5E-02
35	11.00	10.83	0.067	6.7E-02
40	11.14	11.11	0.034	3.4E-02
45	11.20	11.22	-0.018	1.8E-02
50	11.25	11.29	-0.041	4.1E-02
55	11.30	11.34	-0.038	3.8E-02
	SumDelta		9.812E-07	
		SabsDelta		0.441268



Appendix2

Partial acid neutralisation with NaOH

Reactor: Second reactor in chain with rotor and bubble aeration.

First reactor in chain = Feed-on-demand with micro-screen

COD of cells Beta 1.195 gO/gCells

%Neutral	So (mg/l)	S1 CODout1 (mg/l)	X1 Cell X1 (mg/l)	F1 Harvst F1 (l/h)	F02 Feed02 (l/h)	F2 Feed2 (l/h)	S2 CODout2 (mg/l)	X2 Cell X2 (mg/l)	Vol2 (l)	ro (mgO/l.h)
0	20805	153	3574	1.108	0.632	1.740	1427	3969	43.610	168
2	21377	489	3167	1.110	0.847	1.957	963	3662	39.410	315
3	21184	451	4060	1.270	1.530	2.800	1001	4720	37.430	549
5	21239	354	2878	1.200	2.180	3.380	2268	4510	39.700	629

Appendix3

Effect of aeration rate on ro

Run No	Depth (m)	Air rate (v/v.min)	Rot speed R.P.Min	No rotors	CODin1&2 (mg/l)	CODout1 (mg/l)	Cell X1 (mg/l)	Harvst F1 (l/h)	Feed02 (l/h)	Feed2 (l/h)	CODout2 (mg/l)	Cell X2 (mg/l)	Vol2 (l)	ro (mgO ₂ /h)	Air Q (l/h)	Air O ₂ (gO ₂ /h)	% O trans
19	0.80	0.49	375	2	16248	725	4068	1.062	8.88	9.96	6089	2574	111.3	529	3272	754	7.82
20	0.80	0.49	375	2	16759	1237	4431	1.18	9.615	11.00	5291	3848	112.2	566	3299	760	8.35
21	0.80	0.74	375	2	16741	1599	4521	0.944	12.951	13.90	6395	2868	111.1	783	4933	1136	7.65
22	0.80	0.76	375	2	16622	1346	4939	1.192	11.331	12.52	5442	3210	109.2	740	4980	1147	7.05
7	0.80	0.99	375	2	15347	812	3977	1.064	17.866	18.93	6465	2498	110.8	914	6582	1516	6.68
5	0.80	1.00	375	2	15797	1277	1127	1.066	13.772	14.84	4614	2934	110.5	904	6630	1527	6.54
6	0.80	1.00	375	2	15140	2260	2619	1.23	18.032	19.26	5355	3044	109.5	972	6570	1513	7.03
8	0.80	1.00	375	2	17540	1794	4330	1.312	14.508	15.82	6164	3493	109.9	910	6594	1518	6.59
9	0.80	1.00	375	2	14842	1428	5187	1.058	12.954	14.01	2002	3828	110.3	981	6618	1524	7.10
23	0.80	1.26	375	2	16056	1348	1061	1.256	16.407	19.66	5253	3488	108.9	1043	6233	1696	5.99
24	0.80	1.26	375	2	16120	1718	3953	1.164	17.285	18.45	5926	3330	108.9	949	6233	1896	5.45
26	0.80	1.53	375	2	16762	1166	4297	1.184	20.77	21.95	6632	3218	108.0	1163	9914	2283	5.50
25	0.80	1.55	375	2	16453	1785	3913	1.132	17.5	18.63	6800	3346	106.7	1056	9923	2285	4.93

Appendix4

Effect of Depth on ro

Run No	Depth (m)	Air rate (v/v.min)	Rot speed R.P.Min	No rotors	CODin1&2 (mg/l)	CODout1 (mg/l)	Cell X1 (mg/l)	Harvst F1 (l/h)	Feed02 (l/h)	Feed2 (l/h)	CODout2 (mg/l)	Cell X2 (mg/l)	Vol2 (l)	ro (mgO ₂ /h)	Air Q (l/h)	Air O ₂ (gO ₂ /h)	% O trans
37	0.40	0.98	375	2	16159	1792	2974	1.126	6.115	7.24	6706	2844	55.9	566	3287	757	4.18
38	0.40	0.98	375	2	16841	1798	3390	1.072	6.282	7.35	6452	3936	56.0	536	3293	758	3.96
35	0.60	0.99	375	2	16681	1483	3122	1.414	10.844	12.26	6199	3101	83.6	800	4966	1144	5.85
36	0.60	0.98	375	2	17087	1596	3790	1.236	9.997	11.23	6444	2784	84.2	815	4951	1140	6.02
5	0.80	1.00	375	2	15797	1277	1127	1.066	13.772	14.84	4614	2934	110.5	904	6630	1527	6.54
7	0.80	0.99	375	2	15347	812	3977	1.064	17.866	18.93	6465	2498	110.8	914	6582	1516	6.68
8	0.80	1.00	375	2	17540	1794	4330	1.312	14.508	15.82	6164	3493	109.9	910	6594	1518	6.59
3	1.00	0.92	375	2	15733	1450	4418	1.348	20.186	21.53	5637	3062	134.2	942	7408	1706	7.41
4	1.00	0.92	375	2	15489	1440	3430	1.064	24.377	25.44	6756	2830	132.4	948	7308	1683	7.46
1	1.20	1.10	375	2	15648	1019	4326	0.942	21.568	22.51	3997	2555	150.5	1227	8933	2288	6.07
2	1.20	1.10	375	2	15916	1782	4160	1.058	27.576	28.63	5766	2734	149.9	1250	9893	2278	6.22

Appendix5

Effect of rotation speed

Run No	Depth (m)	Air rate (v/v.min)	Rot speed R.P.Min	No rotors	CODin1&2 (mg/l)	CODout1 (mg/l)	Cell X1 (mg/l)	Harvst F1 (l/h)	Feed02 (l/h)	Feed2 (l/h)	CODout2 (mg/l)	Cell X2 (mg/l)	Vol2 (l)	ro (mgO ₂ /h)	Air Q (l/h)	Air O ₂ (gO ₂ /h)	% O trans
11	0.80	1.00	125	2	14250	1167	1483	1.052	10.45	11.50	4401	3164	109.6	528	6576	1514	3.82
12	0.80	0.99	125	2	16324	3071	2609	1.36	10.219	11.58	5799	3275	110.6	588	6570	1513	4.15
13	0.80	1.00	250	2	15403	1767	3059	1.222	14.248	15.47	6385	2841	110.2	679	6612	1523	4.91
14	0.80	1.00	250	2	16596	1313	4977	1.172	9.921	11.09	4075	3752	108.8	720	6516	1501	5.21
5	0.80	1.00	375	2	15797	1277	1127	1.066	13.772	14.84	4614	2934	110.5	904	6630	1527	6.54
6	0.80	1.00	375	2	15140	2260	2619	1.23	18.032	19.26	5355	3044	109.5	972	6570	1513	7.03
7	0.80	0.99	375	2	15347	812	3977	1.064	17.866	18.93	6465	2498	110.8	914	6582	1516	6.68
15	0.80	1.00	500	2	17404	1839	4512	1.206	15.414	16.62	5642	3584	109.6	1020	6576	1514	7.38
16	0.80	1.02	500	2	17257	1726	5050	1.076	16.161	17.24	6019	3566	108.2	1017	6622	1525	7.22
17	0.80	1.02	625	2	17859	1532	4162	1.158	16.785	17.94	6306	3495	107.4	1110	6573	1514	7.88
18	0.80	0.99	625	2	17554	1829	3355	1.434	17.186	18.62	6312	3104	110.6	1116	6570	1513	6.16

Appendix 6

Effect of number of rotors

Run No	Depth (m)	Air rate (v/v.min)	Rot speed R.P.Min	No rotors	CODin1&2 (mg/l)	CODout1 (mg/l)	Cell X1 (mg/l)	Harvst F1 (l/h)	Feed02 (l/h)	Feed2 (l/h)	CODout2 (mg/l)	Cell X2 (mg/l)	Vol2 (l)	ro (mgO/l.h)	Air Q (l/h)	Air O2 (gO/h)	% O trans
27	0.80	1.01	375	0	16574	1280	5044	1.126	7.43	8.56	5954	3272	109.2	430	6618	1524	3.08
28	0.80	1.00	375	0	16656	1864	3938	1.144	8.91	10.05	7328	2898	109.9	448	6594	1519	3.24
29	0.80	1.00	375	1	16086	1448	4848	1.066	13.927	14.99	6044	3122	110.3	772	6818	1524	5.59
30	0.80	1.00	375	1	16864	1907	3740	1.224	12.765	13.99	6388	3204	110.0	729	6600	1520	5.27
5	0.80	1.00	375	2	15797	1277	1127	1.066	13.772	14.84	4614	2934	110.5	904	6630	1527	6.54
6	0.80	1.00	375	2	15140	2260	2619	1.23	18.032	19.26	5355	3044	109.5	972	6570	1513	7.03
7	0.80	0.99	375	2	15347	812	3977	1.064	17.866	18.93	6465	2498	110.8	914	6582	1516	6.68
8	0.80	1.00	375	2	17540	1794	4330	1.312	14.508	15.82	6164	3493	109.9	910	6594	1519	6.59
9	0.80	1.00	375	2	14842	1428	5187	1.058	12.954	14.01	2002	3829	110.3	981	6618	1524	7.10
40	0.80	1.00	375	2	15642	1312	5611	0.998	11.995	12.99	2212	4867	110.0	830	6600	1520	8.01
31	0.80	1.00	375	3	14564	870	3116	1.054	14.42	15.47	3943	2885	109.5	918	6570	1513	6.64
32	0.80	1.00	375	3	15868	998	3125	1.052	12.658	13.71	4369	2923	108.6	895	6576	1514	6.47
33	0.80	0.99	375	4	16526	1475	3441	1.082	13.257	14.34	6355	3028	110.9	741	6587	1517	5.42
34	0.80	0.98	375	4	16960	1583	3367	1.472	12.643	14.12	6605	3176	111.3	682	6544	1507	5.03

Appendix 7

Effect of Cell concentration on ro and viscosity

Run No	Depth (m)	Air rate (v/v.min)	Rot speed R.P.Min	No Impellor	CODin1&2 (mg/l)	CODout1 (mg/l)	Cell X1 (mg/l)	Harvst F1 (l/h)	Feed02 (l/h)	Feed2 (l/h)	CODout2 (mg/l)	Cell X2 (mg/l)	Vol2 (l)	ro (mgO/l.h)	Air Q (l/h)	Air O2 (gO/h)	% O trans	Viscosity Centipoises
45	0.40	0.97	375	2	16845	1040	5435	1.188	4.94	6.13	1571	8368	56.5	376	3288	757	2.81	552
46	0.40	0.97	375	2	16845	1040	5435	1.188	5.01	6.20	1670	8187	56.5	396	3288	757	2.95	552
47	0.40	0.97	375	2	18080	1223	5421	1.236	4.292	5.53	2455	7186	57.0	457	3317	764	3.41	296
48	0.40	0.97	375	2	18080	1223	5421	1.236	4.38	5.62	2334	7416	57.0	453	3317	764	3.38	296
49	0.40	0.97	375	2	16956	1069	5238	1.064	5.185	6.25	2978	5899	56.7	583	3300	760	4.35	106
50	0.40	0.97	375	2	16965	1069	5238	1.064	5.304	6.37	2688	6174	56.7	594	3300	760	4.43	106
51	0.40	0.97	375	2	17188	1185	5762	1.07	4.81	5.88	3338	5310	56.7	606	3300	760	4.52	44
52	0.40	0.97	375	2	17188	1185	5762	1.07	4.684	5.75	3438	5320	56.7	578	3300	760	4.31	44
53	0.40	0.97	375	2	16688	1225	5501	1.146	4.408	5.55	3847	4273	56.9	576	3312	763	4.30	27
54	0.40	0.97	375	2	16688	1225	5501	1.146	4.162	5.31	3697	4289	56.9	555	3312	763	4.14	27

Appendix 8

All data obtained on second reactor of series

Calculating mass of oxygen in air:

Elevation of Pretoria 1400 m
 Temperature 30 C
 % Density of O in air 23.2 %O
 Convert H to cm Hg (vgl 6.30 p 186 Bams et al 1 64.770 cmHg
 Density of dry air at given H and T (Rubber Bible 0.993 kg/m³
 Mass of O in 1 liter of air 0.230 gO/l air

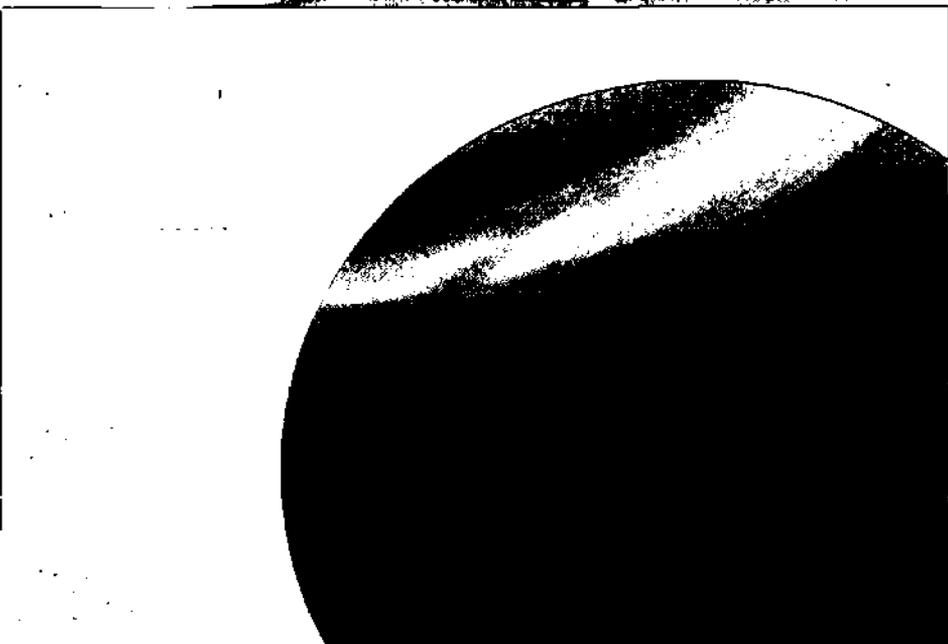
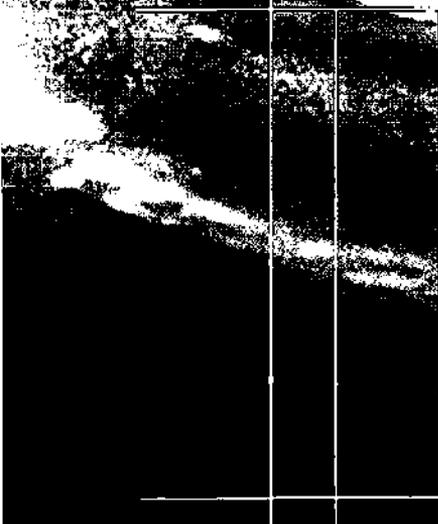
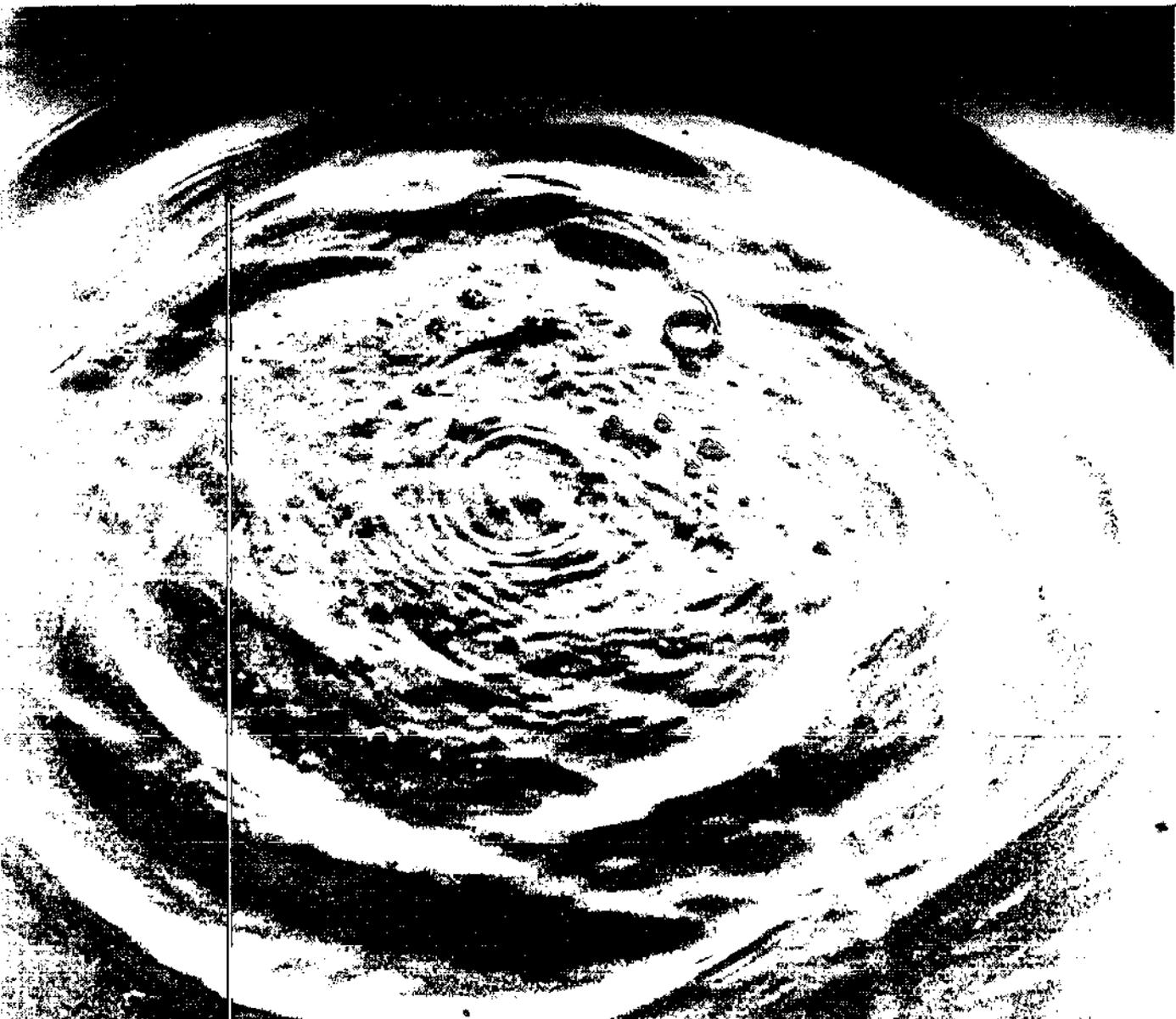
Oxygen transfer with mechanical aerator

Reactor: Second reactor in chain with rotor and bubble aeration.

First reactor in chain = Feed-on-demand with micro-screen

COD of cells Beta 1.195 gO/gCells

Run No	Depth (m)	Air rate (v/v.min)	Rot speed R.P.Min	No impel	So (mg/l)	S1 (mg/l)	X1 (mg/l)	F1 Harvest (l/h)	F02 Feed02 (l/h)	F2 Feed2 (l/h)	S2 CODout2 (mg/l)	X2 Cell X2 (mg/l)	Vol2 (l)	ro (mgO ₂ /h)	Air Q (l/h)	Air O ₂ (gO/h)	% O trans
1	1.20	1.10	375	2	15648	1019	4326	0.942	21.968	22.51	3997	2555	150.5	1227	9933	2288	8.07
2	1.20	1.10	375	2	15916	1782	4160	1.058	27.576	28.63	5768	2734	149.9	1250	9893	2278	8.22
3	1.00	0.92	375	2	15733	1450	4418	1.348	20.186	21.53	5637	3062	134.2	942	7408	1706	7.41
4	1.00	0.92	375	2	15489	1440	3430	1.064	24.377	25.44	6756	2830	132.4	948	7308	1683	7.46
5	0.80	1.00	375	2	15797	1277	1127	1.066	13.772	14.84	4614	2934	110.5	904	6630	1527	6.84
6	0.80	1.00	375	2	15140	2260	2619	1.23	18.032	19.26	5355	3044	109.5	972	6570	1513	7.03
7	0.80	0.99	375	2	15347	812	3977	1.064	17.866	18.93	6465	2498	110.8	914	6582	1516	6.68
8	0.80	1.00	375	2	17540	1794	4330	1.312	14.508	15.82	6164	3493	109.9	910	6594	1519	6.59
9	0.80	1.00	375	2	14842	1428	5187	1.058	12.954	14.01	2002	3829	110.3	961	6618	1524	7.10
10	0.80	1.00	375	2	17184	1371	4399	1.168	13.664	14.85	4485	3515	109.5	1038	6570	1513	7.51
11	0.80	1.00	125	2	14250	1167	1483	1.052	10.45	11.50	4401	3164	109.6	528	6576	1514	3.82
12	0.80	0.99	125	2	16324	3071	2609	1.36	10.219	11.58	5799	3275	110.6	568	6570	1513	4.15
13	0.80	1.00	250	2	15403	1767	3059	1.222	14.248	15.47	6385	2841	110.2	679	6612	1523	4.91
14	0.80	1.00	250	2	16598	1313	4977	1.172	9.921	11.09	4075	3752	108.6	720	6516	1501	5.21
15	0.80	1.00	500	2	17404	1639	4512	1.206	15.414	16.62	5642	3584	109.6	1020	6576	1514	7.38
16	0.80	1.02	500	2	17257	1726	5050	1.076	16.181	17.24	6019	3566	108.2	1017	6822	1525	7.22
17	0.80	1.02	625	2	17659	1532	4162	1.158	16.785	17.94	6306	3495	107.4	1110	6573	1514	7.88
18	0.80	0.99	625	2	17554	1829	3355	1.434	17.185	18.62	6312	3104	110.8	1118	6570	1513	8.16
19	0.80	0.49	375	2	16248	725	4068	1.082	8.88	9.96	6099	2574	111.3	529	3272	754	7.82
20	0.80	0.49	375	2	16759	1237	4431	1.18	9.815	11.00	5291	3848	112.2	566	3299	760	8.35
21	0.80	0.74	375	2	16741	1599	4521	0.944	12.951	13.90	6395	2858	111.1	783	4933	1136	7.65
22	0.80	0.76	375	2	16622	1346	4309	1.192	11.331	12.52	5442	3210	109.2	740	4980	1147	7.05
23	0.80	1.26	375	2	16056	1348	1061	1.256	18.407	19.66	5253	3488	108.9	1043	8233	1896	5.99
24	0.80	1.26	375	2	16120	1718	3953	1.164	17.285	18.45	5926	3330	108.9	949	8233	1896	5.45
25	0.80	1.55	375	2	16453	1785	3913	1.132	17.5	18.63	5800	3346	106.7	1056	9923	2285	4.93
26	0.80	1.53	375	2	16762	1166	4297	1.184	20.77	21.95	6632	3218	108.0	1163	9914	2283	5.50
27	0.80	1.01	375	0	16574	1280	5044	1.126	7.43	8.56	5954	3272	109.2	430	6618	1524	3.08
28	0.80	1.00	375	0	16856	1864	3938	1.144	8.91	10.05	7328	2898	109.9	448	6594	1519	3.24
29	0.80	1.00	375	1	16096	1448	4848	1.066	13.927	14.99	6044	3122	110.3	772	6618	1524	5.59
30	0.80	1.00	375	1	16864	1907	3740	1.224	12.765	13.99	6388	3204	110.0	729	6600	1520	5.27
31	0.80	1.00	375	3	14564	870	3116	1.054	14.42	15.47	3943	2885	109.5	918	6570	1513	6.64
32	0.80	1.00	375	3	15668	898	3125	1.052	12.658	13.71	4369	2923	109.6	895	6576	1514	6.47
33	0.80	0.99	375	4	16526	1475	3441	1.082	13.257	14.34	6355	3026	110.9	741	6587	1517	5.42
34	0.80	0.98	375	4	16960	1583	3367	1.472	12.643	14.12	6605	3176	111.3	682	6544	1507	5.03
35	0.60	0.99	375	2	16681	1483	3122	1.414	10.844	12.26	6199	3101	83.6	800	4966	1144	5.85
36	0.60	0.98	375	2	17087	1596	3790	1.236	9.997	11.23	6444	2784	84.2	815	4951	1140	6.02
37	0.40	0.98	375	2	16159	1792	2974	1.126	6.115	7.24	6706	2844	56.9	566	3287	757	4.18
38	0.40	0.98	375	2	16841	1798	3390	1.072	6.262	7.35	6452	3936	56.0	536	3293	758	3.96
39	0.60	1.00	375	2	15642	1312	5611	0.998	11.038	12.04	2212	4582	110.0	788	6600	1520	5.70
40	0.80	1.00	375	2	15642	1312	5611	0.998	11.985	12.99	2212	4867	110.0	830	6600	1520	6.01
41	1.00	1.27	475	2	16246	1500	5396	1.036	20.43	21.47	3829	3854	125.2	1580	9540	2197	9.00
42	1.00	1.27	475	2	16246	1500	5396	1.036	19.909	20.95	4038	3848	125.2	1522	9540	2197	8.68
43	1.20	1.57	544	2	17609	1482	4855	1.14	24.852	25.99	4444	3418	114.5	1959	10766	2484	9.03
44	1.20	1.57	544	2	17609	1482	4855	1.14	24.049	25.19	4580	3478	114.5	1849	10766	2484	8.52
45	0.40	0.97	375	2	16845	1040	5435	1.188	4.94	6.13	1571	8368	56.5	376	3288	757	2.81
46	0.40	0.97	375	2	16845	1040	5435	1.188	5.01	6.20	1670	8167	56.5	396	3288	757	2.95
47	0.40	0.97	375	2	18080	1223	5421	1.236	4.292	5.53	2455	7186	57.0	457	3317	764	3.41
48	0.40	0.97	375	2	18080	1223	5421	1.236	4.38	5.62	2334	7416	57.0	453	3317	764	3.38
49	0.40	0.97	375	2	16956	1069	5238	1.064	5.185	6.25	2976	5899	56.7	583	3300	760	4.35
50	0.40	0.97	375	2	16956	1069	5238	1.064	5.304	6.37	2688	6174	56.7	594	3300	760	4.43
51	0.40	0.97	375	2	17188	1185	5782	1.07	4.81	5.89	3338	5310	56.7	606	3300	760	4.52
52	0.40	0.97	375	2	17188	1185	5782	1.07	4.684	5.75	3438	5320	56.7	578	3300	760	4.31
53	0.40	0.97	375	2	16688	1225	5501	1.146	4.408	5.56	3847	4273	56.9	576	3312	763	4.30
54	0.40	0.97	375	2	16888	1225	5501	1.146	4.162	5.31	3697	4269	56.9	555	3312	763	4.14



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