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The Loop Reactor for Cultivating Yeast on n-Paraffin Substrate

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For economic mass production by a microbial process, e.g., of single cell protein from a petroleum fraction it is important to find a type of reactor that meets the following requirements:

- large mass-transfer rate for oxygen and substrate at low energy input;
- the reactor must be capable of being constructed and operated in large units;
- a simple and robust design, which is characterized by low construction costs, easy to keep sterile, low maintenance costs, and a high on-stream availability.

The loop reactor, especially the jet loop reactor, is examined with regard to its suitability as a bioreactor; in this context the O_2 transfer, the substrate transfer, the necessary energy input and

the flow pattern are dealt with. The optimal operating conditions (minimum energy input) regarding O_2 conversion and jet power input under consideration of a favorable flow pattern are presented. It is shown that the controllability of the reactor meets the requirements of the culture.

As an example of economically optimal SCP production, the cultivation of yeast on n-paraffin substrate is analysed.

1 Introduction

A fermenter is a reactor for the controlled performance of a microbial metabolic reaction. Since different metabolic reactions require different conditions of cultivation it is advisable to design the reactor according to the requirements of the metabolic reaction to be carried out.

The following treatise will concentrate on the design of a bioreactor to be used for the continuous production of biomass (yeast grown on n-paraffin). It will be shown that a loop reactor is very adequate for this purpose.

The loop reactor is a well-known type of reactor in chemical process technology. Of all modifications described[1, 2], the liquid jet loop reactor is of special interest as a bioreactor.

2 Requirements for Layout and Operation

By suitable design and mode of operation of a bioreactor the technical conditions are provided so that a desirable metabolic reaction can be performed within the range given by the biological system.

In order to facilitate the generation of cell mass the following measures are necessary:

a) a nutrient solution containing certain essential components is inoculated with the strain of microorganism to be cultivated;

b) the cells must be supplied with O_2, available nitrogen, a favorable carbon source and nutrient salts;

c) the heat of reaction as well as the CO_2 generated by cell respiration must be removed, optimal environmental conditions (pH and temperature) must be maintained.

Accordingly the essential task of a bioreactor consists in the transport of mass and energy. In chemical reaction engineering it is usual to make a difference between "macrokinetics" and "microkinetics"[3]. Microkinetics usually refers to the chemical kinetics, in a strict sense, i.e., the direct interaction of molecules, whereas macrokinetics refers to the overall resulting kinetics, which includes transport phenomena like diffusion, convection, heat conduction etc. Applied to the conditions in a bioreactor, microkinetics would refer to the maximal specific growth rate, μ_{max}. This is achieved when each individual cell is supplied with everything necessary for its optimal growth according to its maximum assimilation rate. Everything limiting this growth rate, i.e., gaseous

and liquid products resulting from metabolic activity or the heat created, must be taken away instantaneously. These conditions can be approximately achieved by intensive agitation and aeration at low cell density.

Macrokinetics deals as well with the possible mass and energy transfer rates within the reactor. A direct manipulation of the microkinetics can only be achieved by the choice of operation conditions through which effects can be induced in the immediate vicinity of the individual cell. For the layout of the bioreactor and the choice of the mode of operation it is advisable to examine the demands of the single cell first and then to specify the necessary measures to be taken to influence the conditions in the macro-range. The following aspects should be observed:

- the uniform distribution of cells and substrates in the reactor through adequately fixed convective streams as well as turbulent backmixing,
- the transfer of substrates into the liquid phase, through the liquid film about the cell, through the cell wall, into the interior of the cell as well as the backtransport of gaseous and liquid metabolites,
- removal of the heat of reaction,
- generation of a gas-liquid interface,
- breaking of the liquid film about the cell,
- disruption of cell agglomerates or prevention of their formation,
- the micro emulsification of low soluble substrates like n-paraffin.

This is achieved by introducing zones of high turbulence which affect the core of the liquid by creating micro-shear fields. The suitability of the bioreactor on a commercial scale depends on the fact that these unit operations are carried out with the smallest possible energy expenditure. In addition, the apparatus must run reliably and must render a high on-stream availability combined with low repair and maintenance costs. The apparatus ought to be simple and robust, cheap to manufacture, simple to sterilize and simple to keep under sterile conditions.

3 The Loop Reactor for the Performance of Metabolic Reactions

The loop reactor consists of a cylindrical, vertical vessel with a slenderness ratio of 1:5–1:10, and an inner concentric draft tube. The optimal ratio of inner to outer cylinder diameter is 0.5–0.6 according to[11, 12]. The draft tube is fixed inside the reactor in such a position, that there is a flow connection at the bottom and at the top of the reactor. Thus a directed flow through and round about the draft tube is facilitated. The internal liquid circulation within the loop is induced by means of a liquid jet in the type of bioreactor under discussion here[1, 2].

3.1 Energy Input and Energy Conversion

A prior condition for the realization of the described effects within the micro range is that power is brought into the reactor. This is achieved

a) by jet energy

b) by blowing in of air in order to meet the oxygen demand of the culture.

P_L stands for the kinetic power of the liquid jet; it is dependent upon the flow rate $\dot{V}_{L_1}$ which passes through the nozzle and its dynamic pressure p_1 which can easily be influenced by the nozzle diameter D_1 [1)]

$$P_L = \dot{V}_{L_1}\, p_1 = \frac{8}{\pi^2}\rho_L \frac{\dot{V}_{L_1}^3}{D_1^4}. \tag{1}$$

The liquid jet has a double task

- the dispersion of the liquid gas mixture
- the distribution of the mixture over the entire reactor volume.

The liquid jets are injected in the direction of the liquid flow and so induce directly the circulation flow; the loss of energy by impact and friction at the draft tube or the outer wall which can hardly be avoided in stirrer agitated vessels is reduced here to a negligible amount. Losses by internal friction and diversion caused by the circulating flow can be kept low as long as the optimal reactor geometry is observed[11)]. At the point where gas and liquid jet meet, i.e., for a ring nozzle at the mouth, powerful shear fields occur. These are of importance not only for the formation of fine gas bubbles and a resulting large gas/liquid-interfacial area. Moreover the occurrence of a large energy dissipation concentration in the suction region of the jet is important for the micro emulsification of droplets and for the disagglomeration of cell aggregates.

The power introduced by the gas flow to the two-phase mixture is[1)]:

$$P_G = \dot{M}_G \left(R\, T_G \ln \frac{p_{G_a}}{p_{G_\omega}} + \frac{w_{G_a}^2}{2} \right). \tag{2}$$

The first term represents the power released by the rising gas bubbles via isothermal expansion. The second term represents the kinetic power of the entering gas flow. Generally the second term can be neglected. As long as the nozzle layout is advantageous the kinetic term evaluates to as little as 0.5% of the total power P_G in a reactor filled with liquid to a height of 30 m.

The height of the reactor is favorable for the O_2-transfer for two reasons.

1. The taller the reactor, the longer is the residence time of the gas bubbles, because the minimum distance they all are forced to travel is much longer than in a short compact reactor. In addition fine bubbles are swept along by the circulating liquid and perhaps recirculated several times. This brings about a favorable oxygen conversion. On the other hand, the high oxygen concentration of the feed gas and consequently the concentration gradient is lowered, which is a disadvantage (see [1)], Chap. 9.2).

2. The liquid head of the filled reactor leads moreover to an increased O_2 concentration in the liquid film at the gas/liquid interface. Both the longer residence time of the gas bubbles and the favorable driving concentration gradient due to the higher saturation of the film improve O_2 conversion. As a consequence the reactor can be run with a low aeration rate, which leads to saving of compression energy.

Due to its internal fluid recirculation the loop reactor has a favorable mixing efficiency[30, 41]. That is why it is not necessary – as in the case of certain types of bubble column bioreactors – to inject gas for the intermixing of the phases which of course implies an uneconomic conversion of oxygen.

3.2 Constructional Features and Suitability for Operation

The loop reactor is of a simple design and can be manufactured at low costs. All necessary installations can be mounted in vertical position; that is why it can be cleaned and steamed easily. Any piling up of material inside can be avoided, so that the apparatus can be heated up for sterilization in a short time.

No moving parts are necessary inside the vessel. There is no need for an elaborate shaft sealing – as has to be provided in the case of a stirred tank bioreactor – nor for the problematic shaft bearing within the vessel. That is why the loop bioreactor is far less susceptible to troubles than mechanically stirred reactors. It is easy to maintain sterile operating conditions and to take the necessary steps towards sterilization. The superiority over the mechanically stirred vessel is of special importance for units in the hundreds of cubic meters of liquid contents, which are needed for the bulk production of biomass. The maintenance of circulating pumps installed outside the vessel is easier. There is no need for special measures to prevent the penetration of outside microorganisms through the shaft-sealing of the pumps; a double slide ring sealing with a superimposed sterile sealing liquid circulation is sufficient. Even for very large circulation rates, reliable standard pumps are available. This affects the installation costs as well as the maintenance requirements favorably and improves the on-stream availability of the bioreactor.

Due to its slender construction the loop reactor has a favorable ratio of wall area to contents. For the removal of the heat of reaction the wall of the vessel and – if necessary the draft tube or tubes – can be used as heat exchangers (Fig. 1)[7, 8].

3.3 Controllability

For the aeration of liquids which due to their composition prohibit the coalescence of bubbles (noncoalescing systems) only the specific energy input influences the mass-transfer rate[6]. It could be proved[1, 2, 9] that the generation of the gas/liquid interfacial area for the oxygen transfer correlates especially well with the energy input P_L for a jet loop reactor.

The internal circulation rate $\dot{V}_2$ of a two phase jet loop reactor can best be related (as measured up to $w_G \approx 10$ cm s^{-1}) to the Re number Re_1 based on the nozzle diameter D_1[28, 29]. The circulation around the internal draft tube is important for the mixing of the phases and – as will be shown later on – advantageous for achieving a high productivity rate during such metabolic reactions which tend to the formation of cell aggregates, and for the utilization of substrates, which are almost insoluble in water.

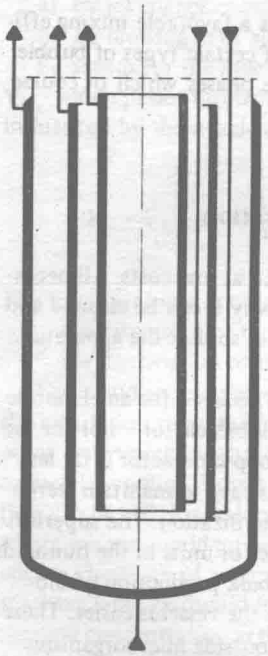


Fig. 1. Arrangement of internal heat exchangers in a loop reactor[4]. Besides the wall of the vessel also the draft tube and if necessary a second tube concentric to the draft tube can be made in the form of coolers with their own inlet- and outlet-connections each for cooling water

Both effects can be adjusted independently according to the respective demand if two-phase nozzles and pure liquid jet nozzles are combined in a certain geometric arrangement (Fig. 2)[10]:

- the two phase nozzles mainly for the primary dispersion of the gas and liquid phase,
- the liquid jet nozzles mainly for the propulsion of the circulation flow and consequently the distribution of the phases and furthermore for the redispersion of all dispersed phases.

In that way the reactor can easily be adjusted to the optimal point of operation, the energy input can be regulated in a flexible manner according to the respective demands of the culture.

3.4 Flow Pattern

A directed internal circulation flow is achieved by means of the concentric draft tube inside the reactor. The hydrodynamics are easy to understand and are predictable[1, 11, 12, 28]. By means of adequate constructional measures the formation of dead zones and the settling of solids can be avoided. Foam generation can be suppressed by strict observation of fixed operating conditions and be supported by the installation of baffles at the flow diversion round the draft tube[13].

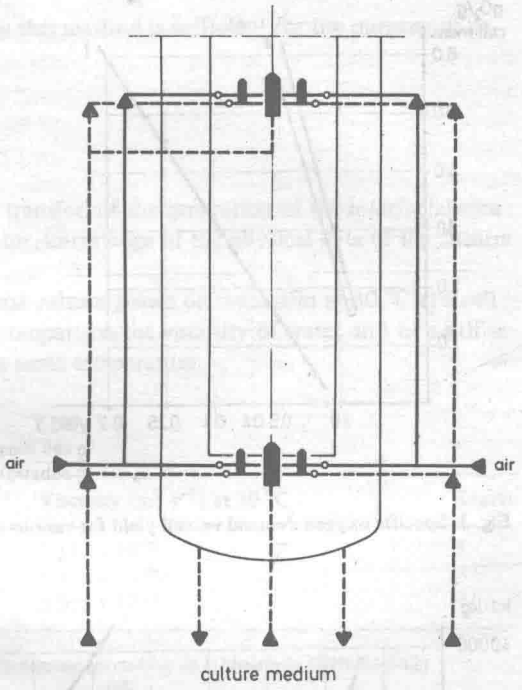


Fig. 2. Combination of pure liquid - nozzles and gas/liquid nozzles in a jet loop reactor[10]. Centrically mounted pure liquid nozzles are surrounded by two phase (gas/liquid) nozzles mounted on a concentrical ring

4 Fundamentals of Design

To enable an adaptation of the construction and the operating conditions to the requirements, some data concerning the metabolic process to be carried out must be given.

4.1 Data Concerning Microbial Growth

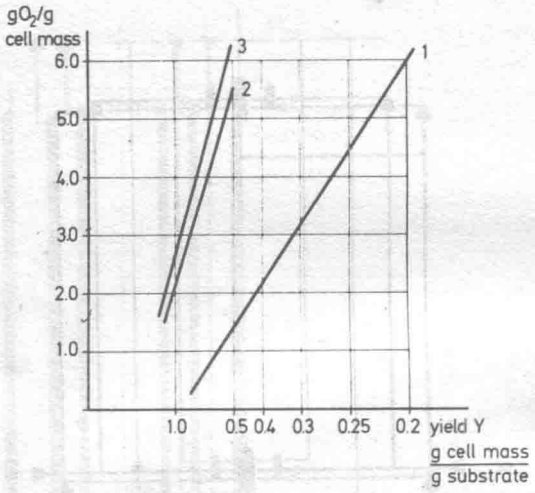
In an unlimited system the biological data of the strain to be cultivated have to be determined; these are

- maximal specific growth rate μ_{max} and
- the cell yield Y.

The specific O_2 demand can be calculated according to[14] from the elementary analysis of cell mass and of the substrate be means of the following equation:

$$f = \frac{32\,C + 8\,H - 16\,O}{Y\,M} + 0.01\,O' - 0.377\,C' + 0.01714\,N' . \qquad (3)$$

It is valid provided that besides cell mass only CO_2 and water are formed and NH_3 is used as the nitrogen source (Fig. 3). From the specific O_2 demand the heat of reaction



Analysis of cell mass (dry basis)

	wt % bacteria	yeast
O	19	30.5
C	53	48
N	12	8.8
H	7.3	6.2
rest ash		

curve 1 bacteria grown on methanol*
curve 2 yeast grown on hexadecane
curve 3 bacteria grown on methane*

* Mateles: Biotechnolog. Bioeng. XIII 1971 S. 581

Fig. 3. Specific oxygen demand vs. cell yield for various substrates[14)]

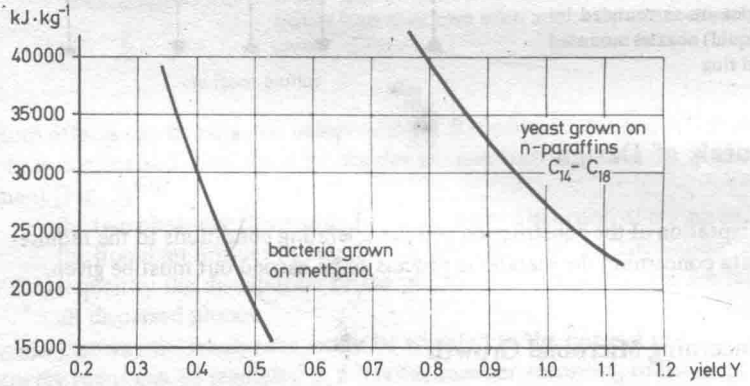


Fig. 4. Heat of reaction vs. cell yield

can be deduced (Fig. 4):

$$q = 14.38\, f \quad \text{kJ kg}^{-1} . \tag{4}$$

Applying Hess' rule to microbial growth, the heat of reaction will be the difference between the heat of combustion of the substrate and of the cell mass generated[16)].

$$q = \frac{H_{o_s} - Y\, H_{o_z}}{Y} . \tag{5}$$

The accuracy of the data evaluated by this method is sufficient for the purpose of designing the bioreactor.

4.2 Physical Data of Media

For prediction of the gas-liquid mass transfer, of the generation of the interfacial area and of the fluid dynamics in the reactor, knowledge of the physical data of the culture medium is important.

Table 1 gives the viscosity of a yeast culture grown on n-paraffin at 30 °C at a cell concentration of x = 20 g l^{-1}. As a comparison the viscosity of water and of a sulfite solution (100 g l^{-1}) are quoted at the same temperature.

Table 1. Viscosity

	Viscosity (m^2 s^{-1}) at 30 °C	Source
Medium, containing 20 g l^{-1} yeast	1.11 x 10^{-6}	a
Distilled water	0.800 x 10^{-6}	17)
Sulfite solution (100 g l^{-1})	1.056 x 10^{-6}	9)

[a] Measurement by means of capillary viscosimeter according to Ubbelohde (DIN 51 562)

The surface tension of culture media containing living yeast or bacteria is given in Table 2. It contains a compilation of measured data. The samples were taken out of the reactor at different times. The respective cell concentration is quoted as well.

Table 2. Surface tension

	Yeast/bacteria	Cellconcentration of culture (g l^{-1})	Temperature (°C)	Surface tension (dyn cm^{-1})
Filtrate[a]	*Methylomonas sp.*	–	18	58[b]
Culture medium containing cells	*Methylomonas sp.*	12	18	57[b]
		12	37	51[b]
Filtrate[b]	*Endomycopsis lipolytica*	–	30	48[b]
Culture medium containing cells	*Endomycopsis lipolytica*	20	30	32[b]
Distilled water	–	–	30	71.2
Sulfite solution (100 g l^{-1})	–	–	30	60.8

[a] Culture medium, cells being removed

[b] Measured by means of a tensiometer according to Lecomte du Noüy (tearing off method)

It is obvious, that the growing cells release metabolic products into the culture medium that reduce surface tension. Surface tension is reduced more in cultures of paraffin metabolising yeast than in cultures of bacteria, grown on methanol. If the cells are removed from the culture medium in which they have grown, a larger surface tension is found than in the original culture medium. The presence of cells at the boundary layer thus reduces surface tension even further.

The density of the culture medium is shown in Table 3. The density is not very different from that of pure water, as could be expected. The cell concentration is small and because of their high water content the density of the cells is almost the same as that of nutrient solution, the density of which is in turn, - because of its low salt concentration - not very different from that of distilled water.

Table 3. Density

	Temperature (°C)	Density (kg m^{-3})
Culture solution with x = 20 g l^{-1} cell concentration (yeast)	30	998
Distilled water	30	995.8 [17]
Sulfite solution (100 g l^{-1})	30	1,095 [9]

Henry's constant He, the distribution coefficient between the gas and liquid phase depends on temperature and salt content of the liquid phase. Table 4 quotes He of oxygen in a yeast culture medium with a cell concentration of x = 20 g l^{-1}. A calculation for another salt content can be carried out on the basis of an equation published by van Krevelen and Hoftyzer[18]

$$\frac{\ln c_s}{\ln c_w} = - k_s I . \tag{6}$$

k_s stands for a sum of independent items, which are characteristic of the positive and negative ions in the solution and for the solved gas.

$$k_s = i_+ + i_- + i_g . \tag{7}$$

The total ionic strength is defined by

$$I = \frac{1}{2} \Sigma c_i z_i^2 . \tag{8}$$

Table 4. Henry's constant for O_2 in different solutions

	Temperature (°C)	
Filtrate from 20 g l^{-1} cell mass containing yeast culture	30	40.0[a] [19]
Distilled water	30	39.3
Sulfite solution (100 g l^{-1}	30	69.0 [9]

[a] O_2 concentration measured according to Winkler[20]

The diffusion coefficient D_L for molecular diffusion of O_2 in a nutrient medium was determined experimentally. The measurements were carried out in a thermostabilized diaphragm cell under quasi stationary diffusion conditions[19].

Table 5. Diffusion coefficient D_L for O_2 in different salt solutions

	Temperature (°C)	Diffusion coefficient D_L ($m^2\ s^{-1}$)
Nutrient medium[a]	30	1.9×10^{-9} [19]
Distilled water	21	2.33×10^{-9}
Sulfite solution	30	2.1×10^{-9} [9]

[a] Composition see Table 6

For other compositions of nutrient salts the diffusion coefficient can be predicted according to Rattcliff and Holderoft's[21] equation:

$$\log \frac{D_w}{D_L} = B \log \frac{\nu_L}{\nu_w} \tag{9}$$

with B = 0.637.

The calculation for another temperature is possible if one applies the Stokes-Einstein equation:

$$\frac{D_L \cdot \nu_L}{T} = \text{const}.$$

Table 6. Composition of nutrient salt medium

H_3PO_4 (85 wt%)	2.0 ml
NH_4OH (25 wt%)	2.8 ml
$MgSO_4 \cdot 7\ H_2O$	0.9 g
K_2SO_4	1.4 g
Na_2SO_4	0.2 g
$Ca(NO_3)_2 \cdot 4\ H_2O$	0.3 g
Trace element solution	1.0 ml
Thiamine solution	1.0 ml
Tap water	1.0 l

If the characteristic data of the media are compared in Tables 1–5 with that of sulfite solution, noteworthy differences are found only in the surface tension and in Henry's constant He.

5 Mass Transfer in a Jet Loop Bioreactor

Maximal specific growth rate μ_{max} can only be realized under ideal conditions. When the layout of a technical reactor is designed in order to achieve optimal macrokinetic conditions the mass transport phenomena must be taken into consideration. The following constructional and operational possibilities can be taken into account in order to influence mass transport phenomena in the bioreactor:

a) choice of reactor geometry, mainly the slenderness ratio $s_L = H_L/D_i$ and the ratio of the draft tube diameter to the inner diameter of the reactor D_E/D_i,
b) power input, i.e., by jet power P_L and by aeration power P_G,
c) positioning the nozzles properly, adjusting their throughput and thereby influencing the flow pattern of the internal circulation.

The following substances have to be transported to the cell membrane:

a) ions of the nutrient salts in the broth, i.e., K^+, Mg^{2+}, Ca^{2+}, Fe^{2+}, NH_4^+, PO_4^{3-}
b) O_2 blown in along with the air
c) C substrate, e.g., n-paraffin.

Calculation of the mass balance for the steady state of a continuous cultivation has shown[22] that the necessary effort for mass transfer increases with the decreasing solubility of the component to be transferred.

$$\bar{s} = s^* - \frac{\mu x}{Y k_{LS} a_s} . \tag{10}$$

From Eq. (10) it can be derived that the mass transfer coefficient k_{LS} for the transport of a substrate into the bulk of the liquid and/or the necessary interfacial area a_S must increase, when the difference between the saturation concentration s^* and the steady state concentration of the substrate $\bar{s}$ decreases. Depression of growth will occur as soon as $\bar{s} < s_{crit}$, where s_{crit} stands for the substrate concentration, which must always be maintained unless growth rate becomes dependent upon concentration.

The nutrient salt components in solution must only pass through the liquid film surrounding the cell. Their transfer takes place by molecular diffusion. By choice of the adequate concentration of each salt component supply of the cells can be secured, the reactor is not required to do anything special.

For the O_2 transfer the resistance is located in the liquid film on the gas/liquid interface. The ratio

$$\frac{c^*_{O_2}}{c_{crit}} \approx 7 \text{ at } 30\,^{\circ}\text{C} .$$

Greater efforts must therefore be made in the case of O_2 supply.

According to Eq. (10) the transfer of low-solubility substrates like paraffin is the most difficult problem limiting the productivity of the culture. In this connection it does not matter whether the transport is performed via solution or particle dispersion.