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# Production of β-1,3-Glucan Exopolysaccharide in Low Shear Systems

## The Requirement for High Oxygen Tension

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## ABSTRACT

A patent culture of *Alcaligenes faecalis* var. *myxogenes* (ATCC 31749) synthesizes "curdlan" during the stationary phase of an aerobic batch fermentation following the depletion of assimilable nitrogen. Because this β-1,3-homoglucan exopolymer is water insoluble, the fermentation broth is of a relatively low viscosity, and consequently offers little resistance to oxygen transfer from gas to the liquid. However, the layer of insoluble exopolymer surrounding the cell mass offers a resistance to oxygen transfer from the liquid to the cell, thereby necessitating an unexpectedly high dissolved oxygen concentration for maximal productivity. The shear sensitive nature of this fermentation restricts improving oxygen transfer by increasing agitation intensity. The requirement for high volumetric oxygen transfer can be met by low shear designs with axial-flow impellers, providing gas dispersion is assisted by the use of sparging devices consisting of microporous materials. The specific respiration rates for growing and curdlan-producing stationary-phase culture were determined to be 7 and 2.7 mmol $O_2$/g cell/h, respectively. At a cell density of 3 g cell/L, the maximal rate of curdlan biosynthesis was about 100 mg/g cell/h, with a requirement for dissolved oxygen (*DO*) of 6.5 mg $O_2$/L (86% air saturation at 30°C and 1 atm.). Whereas, at constant impeller speed, the volumetric oxygen transfer was improved both by increasing the air sparging rate and by using $O_2$-enriched air (30% $O_2$), productivity was not consistently improved by operating at a twofold higher cell density and at a $DO > 6.5$ mg $O_2$/L. At higher cell densities, it would

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appear that shear must be increased to minimize culture clumping and assist in oxygen transfer to the cell.

**Index Entries:** Curdlan; exopolysaccharide; shear; oxygen demand; $DO_{crit}$; OTR, aeration.

## NOMENCLATURE

| | |
|---|---|
| $C^*$ | concentration of dissolved $O_2$ at saturation (0.236 m*M* for air and 0.338 m*M* for 30% $O_2$-enriched air, 30°C, 1 atm) |
| $C$ | observed dissolved oxygen (*see also* DO)· |
| D | dilution rate ($h^{-1}$) |
| $D_i$ | diameter of impeller (cm) |
| *DO* | dissolved oxygen concentration |
| DW | dry cell weight (g dry wt/L) |
| EPS | exopolysaccharide (g DW/L) |
| $K_La$ | vol $O_2$ mass transfer coefficient ($h^{-1}$) |
| *OTR* | $O_2$ transfer rate ($K_La \cdot C^*$) (mmol $O_2$/L/h) |
| $q_p$ | sp. rate of EPS production (mg EPS/g cell/h) |
| STR | stirred tank reactor |
| V/V/M | gas sparging rate (vol gas/vol/liquid/min) |
| $\mu$ | sp. growth rate ($h^{-1}$) |

## INTRODUCTION

Polysaccharides are commercially useful polymers by virtue of their capacity to alter the flow characteristics of water (*1*). Traditionally, these natural hydrocolloids have been extracted from plant tissues and seaweed. Well-known examples are agar, algin, carrageenan, locust bean gum, and gum arabic. They are used as viscosifiers, emulsifiers, thickening, suspending, and gelling agents in diverse commercial applications in industries, such as food, beverage, pharmaceutical, agriculture, mining, and forestry (*1,2*). In certain applications, synthetic polymers, which are derived from petroleum chemical feedstocks, are used to extend or replace the natural plant gums. However, both the natural plant hydrocolloids and synthetic polymers suffer from a common serious disadvantage in that their sources are either limited or restricted, which brings into question not only their capacity to supply the projected demand, but also the economics of their production.

Polysaccharides are a structural component of bacterial cell walls, and certain bacteria synthesize extracellular polymers known as microbial exopolysaccharides (EPS) (*3*). Some of these EPS function as hydrophilic colloids and dramatically alter the flow properties of aqueous systems at relatively low concentration (*4*). The growing demand for inexpensive, high-quality viscosifiers has produced a new and burgeoning fermenta-

tion industry (*5*). Through controlled fermentations, the potential exists to produce a variety of different high-performance microbial polysaccharides of consistent composition and quality inexpensively (e.g., dextran, pullulan, xanthan, and gellan gum) (*5,6*). The extreme high viscosity generated at relatively low product concentrations (about 20 g/L) seriously limits heat and mass transfer, and represents a major constraint to increased productivity (*7,8*). Since these fermentations are aerobic, the supply of oxygen to the cell in an environment that limits its transfer both from gas to liquid and throughout the medium becomes of critical importance (*7,8*). The oxygen transfer capacity of the bioreactor can be improved by increasing either the oxygen transfer coefficient ($K_La$) or the partial pressure of oxygen in the sparging gas (*9*). The conventional approach to increasing the $K_La$ includes upgrading the agitation intensity (power input) and/or the superficial gas velocity of the sparging gas (*10*). However, increasing agitation is counterproductive for shear-sensitive systems (*10–12*).

"Curdlan" is the collective name given to a class of water-insoluble, β-1,3-homoglucan exopolymers that exhibit unique thermogelling properties and are synthesized by certain species of two different genera of bacteria, *Alcaligenes* and *Agrobacterium* (*13–18*). The uncharged, linear EPS can be recovered as an insoluble gel upon neutralization of the alkalinized cell-free fermentation broth (*19*). The insoluble nature of curdlan means that the fermentation broth is of a relatively low viscosity compared to other soluble EPS, such as xanthan or gellan gum (*20*). Consequently, the transfer of oxygen from gas to the liquid medium is not a limiting factor in curdlan fermentations; however, as an insoluble layer surrounding the cells, curdlan does present a barrier to oxygen transfer from dissolved gas to the site of oxygen consumption within the cell (*21*). Since the rate of oxygen diffusion across the surrounding boundary layer of EPS is proportional to the dissolved oxygen concentration (*DO*) (*7*), it has been postulated that curdan fermentations might exhibit a requirement for an unusually high *DO* (*21*), despite the fact that curdlan synthesis occurs during the stationary phase, after growth ceases because of the depletion of assimilable nitrogen (*17*). This is consistent with earlier observations with shake flask batch fermentations where it was noted that EPS biosynthesis was oxyen limited, but that growth was not (*12*).

Another feature of curdlan fermentations that had been observed was that shake flask cultures consistently produced a higher mol-wt polymer with superior gelling and rheological properties compared to the product derived from stirred tank reactors of conventional design with Rushton turbine impellers (*21,21*). Although the apparent shear-sensitive nature of the curdan fermentation can be accommodated in low-shear designs (*23*) employing axial-flow impellers for the production of high-quality polymer product (*22*), these systems are much less efficient at oxygen transfer (*12, 24*). *DO* electrodes do not function well in curdlan fermentations in low-shear systems, because the surface of the probe is easily fouled by a layer

of the cell-EPS matrix. Therefore, it has not been possible to correlate the *DO* directly with the rate of curdlan production (*24*). The oxygen transfer rate (OTR) can be estimated by measuring the rate of sulfite oxidation as a function of the agitation intensity and rate of gas sparging (*12,24*). Although the requirement for oxygen has been quantitated in terms of the oxygen transfer capacity of variously designed bioreactors (*12*), unlike the *DO*, the OTR cannot be sensed on line, and it is therefore not a practical indicator for process control. The *DO* is a "net-sensed" parameter, and its value is derived from the difference between the rate of oxygen supply and utilization. Hence, it is possible to estimate the DO indirectly from a knowledge of both the oxygen transfer capacity of the bioreactor and the oxygen demand of the culture. The present study examines the oxygen demand of the culture during both growth and polymer production phases of the batch fermentation and attempts to define quantitatively the relationship between *DO* and the rate of curdlan production by determining the value for $DO_{crit}$, above which the specific rate of polymer biosynthesis ($q_p$) is no longer oxygen limited. Another objective of this work was to attempt to improve productivity through operation at higher cell density (>3 g cell/L), whereby the increased oxygen demand was met either (i) by increased superficial air velocity or (ii) by using $O_2$-enriched air.

## MATERIALS AND METHODS

### Organism and Culture Conditions

*Alcaligenes faecalis* var. *myxogenes* ATCC 31749 is a β-1,3-glucan-producing, patent culture (*25,26*) obtained from the American Type Culture Collection (Rockville, MD). Batch cultures were conducted aerobically in a defined mineral salts medium (*17*) with glucose (50–80 g/L) as the sole carbon source. The bacterial cell density was proportional to the amount of assimilable nitrogen in the medium with 28 m*M* $NH_4Cl$ giving a biomass concentration of 3 g DW cell/L (*17*).

### Analytical Procedures

Exopolymer was recovered from the fermentation broth as described previously (*17*). The $K_La$ was determined by a standard chemical method using sulfite oxidation with cupric ions as catalyst (*27*). The $K_La$ was determined as a function of the rotational speed of the impeller(s) and/or the rate of gas sparging for different fermentor designs with respect to agitation and aeration. The respiration rate was determined by fermentor off-gas analysis of chemostat and batch cultures as previously described (*28*).

## Bioreactors—Agitation and Aeration

Batch fermentations were conducted in bench-top STRs equipped with sensing and control devices for agitation (rpm), air sparging (V/V/M), pH, and temperature. Operating conditions were as described previously (*12*) for different configurations with respect to mixing and aeration. High shear systems employed Rushton turbine radial-flow impellers, whereas low-shear systems used axial-flow impellers.

## Oxygen Supply— The Oxygen Transfer Rate (OTR)

The capacity of a bioreactor to transfer oxygen from gas to liquid depends both on its design (configuration with respect to agitation and aeration) and on the conditions (both physical and chemical) under which it is operated. Oxygen is only sparingly soluble in water. Consequently, in order to satisfy the demand of a microbial culture, it is necessary to renew the dissolved oxygen by transfer of oxygen from gas to the liquid medium. The equilibrium concentration of dissolved oxygen is given by the relationship

$$C^* = H \cdot \mathrm{pP\,O_2} \tag{1}$$

where pP $O_2$ is the oxygen pressure of the gas phase (in equilibrium with air at 1 atm pP $O_2$=0.209 atm) and $H$ is Henry's constant (1.13 m$M$ at 30°C and 1 atm) and where $C^*$=saturation concentration of oxygen (0.236 m$M$ or 7.55 mg $O_2$/L).

The rate of oxygen transfer from an air bubble to the liquid medium is given by the following relationship:

$$\mathrm{OTR} = \mathrm{dC/dt} = K_L a\,(C^* - C) \tag{2}$$

where $K_L a$ is the volumetric mass transfer coefficient. The determination of the $K_L a$ is important for establishing the aeration efficiency of a particular bioreactor design and to quantify the effects of operating variables (agitation speed and gas flow rate) on the capacity to provide oxygen in solution.

## Oxygen Demand or Oxygen Uptake Rate (OUR)

In aerobic fermentations, the respiring culture removes oxygen that is dissolved in the liquid phase. The oxygen uptake rate (OUR) of the aerobic culture is represented by the following relationship:

$$\mathrm{OUR} = q_{O2} \cdot (x) \tag{3}$$

where $q_{O2}$ represents the specific respiration rate of the aerobic culture (U of mass $O_2$/U dry biomass/U time) and $x$ is the culture cell density (U of dry biomass/U vol).

### Estimating the Dissolved Oxygen Concentration (*DO*)

When the supply of oxygen (OTR) is balanced by the oxygen demand of the culture (OUR), then the following relationship holds:

$$\text{OTR} = \text{OUR}$$

$$K_L a\,(C^* - C) = q_{O2}\cdot(x) \tag{4}$$

Solving for $C$ (the observed value for dissolved oxygen)

$$C = C^* - \text{OUR}/K_L a$$

$$C/C^* = 1 - \text{OUR}/K_L a \cdot C^* \tag{5}$$

but since in the measurements of oxygen transfer rate by sulfite oxidation,

$$\text{OTR}_{max} = K_L a \cdot C^* \tag{6}$$

then

$$C/C^* = (1 - \text{OUR}/\text{OTR}_{max}) \tag{7}$$

where $C/C^*$ represents the ratio of DO to $DO_{sat}$ and, when multiplied by 100, represents DO as "% saturation." From the above relationships, it follows that

$$C = C^*(1 - \text{OUR}/\text{OTR}_{max}) \tag{8}$$

where $C^*$ is the value for *DO* at saturation (under specified conditions of temperature, pressure, and chemical composition of the liquid). Thus, if values for both OUR and OTR are known (or assumed), one can calculate a value for *DO* from the relationship given immediately above.

The critical value for *DO* ($DO_{crit}$) is defined as the value below which the respiration rate becomes limited by the rate of oxygen transfer, be it from gas or liquid or from liquid to the cell. Hence, if the $K_L a$ is not adequate, oxygen will be directly limiting for respiration and indirectly limiting for energy-dependent processes, such as growth. Similarly, if the DO —the driving force for diffusion of oxygen across a resistive cell boundary layer—were to be inadequate, then the energy-dependent process of EPS biosynthesis would become limited by oxygen.

## RESULTS AND DISCUSSION

In batch fermentations, the production of curdlan occurs following growth in a nitrogen-deficient medium with excess carbon source (*17,18*). At a cell density of 3 g/L, the rate of exopolymer production was twofold higher in a "standard" STR (fitted with radial-flow Rushton turbines) compared to a shake flask, where the OTR of the respective systems was determined to be about 20 and 80 mmol $O_2$/L/h (*12,24*). The results of

these preliminary experiments had pointed to the importance of adequate oxygen transfer in terms of process productivity. Although growth appears to be indifferent to the shear intensive turbulence created by radial-flow impellers, the demonstrated shear sensitive nature of the curdlan fermentation rules out the use of Rushton turbines, at least not during the stationary phase, when the culture is synthesizing exopolymer (*12*).

From theoretical considerations with respect to oxygen demand associated with growth, maintenance metabolism, and polymer biosynthesis (*28*), it was originally assumed that even agitation systems with relatively low capacity for oxygen transfer would be adequate in terms of meeting the significantly reduced oxygen requirement of a stationary-phase culture that was producing exopolymer (*23*). Since the oxygen demand of a stationary-phase culture was predicted to be relatively low in comparison to that of a rapidly growing culture, in theory at least, the $DO_{crit}$ for the curdlan-producing culture should be easily accommodated by a system characterized by a much lower $K_La$ than exhibited by the "standard" bioreactor equipped with aeration efficient, high shear, Rushton turbines.

## OUR for Growth and Curdlan Production

The culture cell density ($x$) is proportional to the amount of assimilable nitrogen (as $NH_4Cl$) added to the fermentation medium (*17*). From fermentor off-gas analyses, the specific respiration rate associated with exponential growth was determined to be 7 mmol $O_2$/g cell/h (Table 1). The $DO_{crit}$ for growth of most aerobic cultures is generally quite low—around 2.5–5% air saturation (*9,29*). Hence, for a cell density of 3 g/L, oxygen would not be expected to be growth limiting for a system with an OTR > 22 mmol $O_2$/g cell/h.

The specific respiration rate of a curdlan-producing, stationary-phase culture was determined to be 2.7 mmol $O_2$/g cell/h and is comprised of two components: (i) maintenance respiration and (ii) respiration associated with generating the energy (ATP) required for EPS biosynthesis (Table 1). Based on proposed models for curdlan biosynthesis (*3,18*) that are analogous to the energy requirement for glycogen (starch) synthesis, it can be assumed that the energy required for polymer elongation is 2 mol ATP/mol glucose added. Further, it is assumed that the P/O ratio associated with oxidative phosphorylation is 2.0 (equivalent to 27 mol ATP/mol glucose oxidized). Using these assumptions, a respiratory (oxygen) demand of 0.27 mmol $O_2$/g cell/h can be calculated in terms of the energy required solely for EPS production (Table 1). Surprisingly, this represents only 10% of the total respiration rate of the polymer-producing, stationary-phase culture, the remaining 90% being attributable to the measured maintenance respiration rate of 2.4 mmol $O_2$/g cell/h (Table 1). Since the oxygen demand related to exopolymer biosynthesis represents only a very small fraction of the total oxygen demand, the assumption regarding the efficiency of energy conservation (i.e., P/O = 2) becomes relatively insignificant.

Table 1
Determining the Oxygen Demand
Associated with Growth and Exopolymer Production

| Growth phase | Condition | Specific Respiration Rate, mmol $O_2$/g cell/h[a] Component | | Total |
|---|---|---|---|---|
| Growth | | | | |
| Exponential phase (near $\mu_{max}$) | C or *N*-limited chemostat culture (at $D_{crit}=0.25$/h) | | | 7.0 |
| EPS production | *N*-limited | | | |
| Stationary phase ($\mu=0$) | chemostat culture (at D=0) | Maintenance respiration | 2.40 | |
| | | Calculated respiratory demand for EPS biosynthesis | | |
| | | if P/O=1 | 0.50 | |
| | | if P/O=2 | 0.27 | |
| | | if P/O=3 | 0.20 | |
| | Stationary-phase batch producing EPS[b] | | | 2.70 |

[a]Respiration rates were determined from off-gas analysis as described previously (ref. *28*) P/O is the ratio of ATP formed per atom of oxygen consumed in respiration and is a measure of the efficiency of energy conservation associated with oxidative phosphorylation.
[b]Measured specific rate of $CO_2$ production and assumed RQ=1.

Funahashi et al. (*30*) have shown that the specific rate of xanthan production is not limited with respect to oxygen supply at $K_La>60$/h. This is in direct contrast to work conducted in our lab that demonstrated a requirement for unexpectedly high volumetric oxygen transfer (>200/h) in curdlan fermentations (*12,24*). Furthermore, we have shown that this requirement can be met by low shear bioreactor designs employing axial-flow impellers where gas dispersion is assisted through the use of sparging devices consisting of microporous materials (Fig. 1). Unfortunately, the OTR is not an operating parameter that can be sensed directly "on line," and it is therefore not a practical indicator for process control. By contrast, the *DO* can be determined "on line," but unfortunately, DO electrodes do not function well in curdlan fermentations (particularly in low-shear systems), because the surface of the probe is easily fouled by a layer of the cell-EPS matrix. The DO is a "net-sensed" parameter that represents the difference between the rate of oxygen supply and utilization. Therefore, despite the lack of direct read-out from a functional *DO* probe, having determined the value for the oxygen demand of the curdlan-producing culture, it is now possible to represent the data of Fig. 1 in

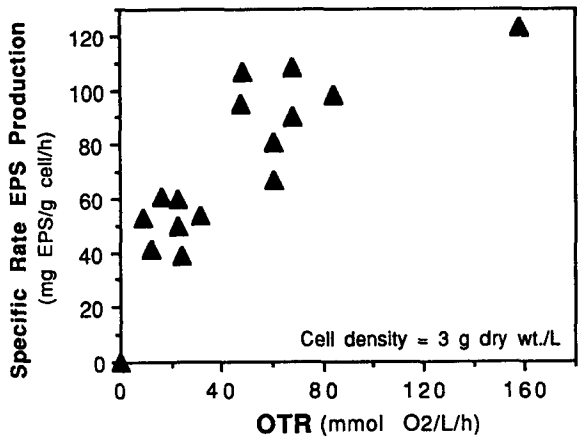


Fig. 1. The relationship between the OTR and the specific rate of EPS production. Bioreactors of various designs with respect to agitation and aeration were used as described previously (*12*). In all cases, air was used to ventilate the cultures, and the stationary-phase cell density was 3 g DW cell/L.

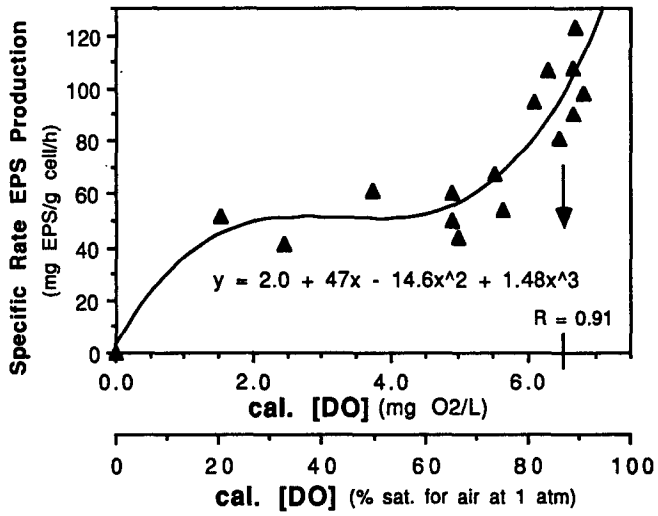


Fig. 2. The specific rate of EPS production as a function of the dissolved oxygen concentration. The value of *DO* was calculated from the relationship between oxygen supply (OTR) and demand (OUR). The data are from Fig. 1. The arrow shows the value for $DO_{crit}$ at 6.5 mg $O_2$/L (equivalent to 86% sat. with air at 1 bar).

terms of $q_p$ as a function of *DO* (Fig. 2). Under the fermentation conditions conditions examined, the $DO_{crit}$ for curdlan production appears to be about 86% air saturation (at 1 atm) or 6.5 mg $O_2$/L (Fig. 2).

The *DO* electrode was found to perform much better in high shear systems that employed turbine impellers (Fig. 3). Although such systems produced polymer product of inferior "quality," they were useful with regard to providing direct evidence relating to the effect of *DO* on $q_p$ in

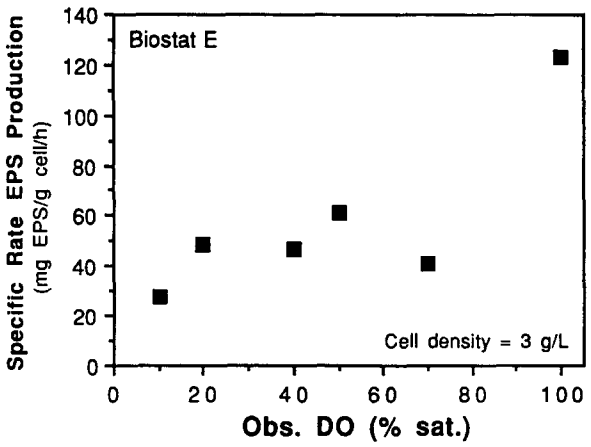


Fig. 3. The specific rate of EPS production as a function of *DO*. The *DO* was measured with a polarographic electrode. The STR was a Biostat™ 10E (B. Braun) with *DO* maintained at a set point by automatic variation in rate of air sparging. Agitation was accomplished by three radial-flow flat-blade turbines ($D_i$ 8.9 cm) with baffling provided by a tube heat exchanger.

confirmation of the results illustrated in Fig. 2. Although these results point to the importance of maintaining a high *DO* for maximal rates of curdlan production, very different situation has been observed in pullulan fermentations where productivity was improved at low *DO* and low shear (*31,32*). McNiel and Kristiansen (*33*) found that pullulan production increased with increased stirrer speed, but did not distinguish between the effect of shear owing to the impeller and the *DO*.

For the most part, our observations on curdlan fermentations have been at a constant cell density of 3 g DW cell/L. Another objective of this study was to attempt to improve productivity through operation at two-fold higher cell density. The oxygen demand will increase in direct proportion to the cell density, and the shear sensitive nature of the curdlan fermentation means that increasing the rotational speed of the agitator is not the preferred approach to increasing the $K_La$. However, two alternative approaches are compatible with shear sensitive systems: (i) increasing the superficial gas velocity and (ii) increasing the partial pressure of oxygen in the sparging gas. Membrane oxygenators offer an efficient means of increasing the OTR capacity of a bioreactor (*34*). Oxygen-enriched air in the concentration range of 25–35% $O_2$ can be produced in a single pass using a membrane separator system (*35*).

If, for example, oxygen-enriched air at 30% $O_2$ is used to sparge the bioreactor, the value for $DO_{sat}$ at 30°C would increase from 7.55 mg $O_2$/L to 10.83 mg $O_2$/L. Assuming the $DO_{crit}$ remains relatively constant over the range of cell densities being tested, then the OTR requirement will be lessened in proportion to the degree of oxygen enrichment. Assuming the specific respiration rate is constant (2.7 mmol $O_2$/g cell/h), then the

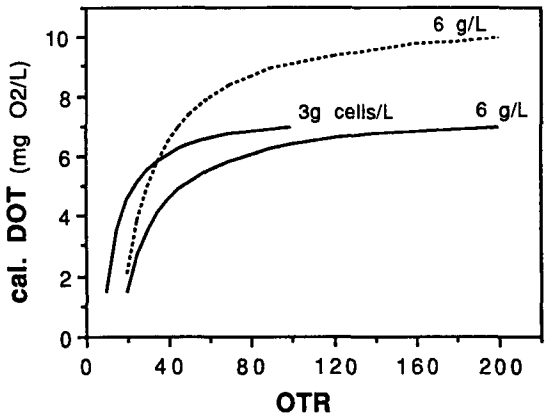


Fig. 4. Theoretical relationship between OTR and *DO* for different operating conditions. The specific respiration rate was assumed to be constant at 2.7 mmol $O_2$/g cell/h. The solid lines are for air sparging at cell densities of 3 and 6 g/L. The dashed line is for a sparging gas that is oxygen-enriched (30% $O_2$ with balance $N_2$) at a cell density of 6 g/L.

relationship between OTR and *DO* is predictable (Fig. 4). Furthermore, assuming that the $DO_{crit}$ remains the same for cell densities > 3 g/L, the OTR required to exceed the $DO_{crit}$ can also be determined (Fig. 4).

Because of the shear sensitive nature of the curdlan fermentation, we chose to conduct our experiments with an agitation/aeration configuration that was known to yield high-quality polymer product—this being a round bottom vessel fitted with an overhead drive and a 7-cm marine-type propeller (no baffles). The gas was sparged through a sintered glass disk located under the propeller. The gas flow rate was monitored with a mass flow meter. The rotational speed was kept constant at 500 rpm. The results are presented in the Fig. 5, in which OTR (as $K_La \cdot C^*$ in mmol $O_2$/L/h) is plotted against the gas sparging rate where the gas used was either air or $O_2$-enriched air (30% $O_2$). Since in theory the $K_La$ is not affected by the pP $O_2$, the OTR should be directly proportional to the pP $O_2$. However, in the case of the $O_2$-enriched air, the standard chemical method (sulfite oxidation) appears to yield $K_La$ values that are not porportional to the pP $O_2$ (Fig. 5). The accuracy of the sulfite oxidation method is not known in experiments where the pP $O_2 > 0.209$ atm.

The results of batch fermentations conducted at a twofold higher cell density (about 6 g/L) with variously configured bioreactors are summarized in Table 2. The OTR values are those determined by the sulfite oxidation method, and the values in parentheses represent the minimal value for OTR that would be required to satisfy the assumed $DO_{crit}$ of 6.5 mg $O_2$/L (Table 2). Figure 6 represents the superimposition of the data from Table 2 (at cell densities of about 6 g/L) on the plot previously shown in Fig. 2. From Fig. 6, it is obvious that, at the higher cell density of 6 g/L, in

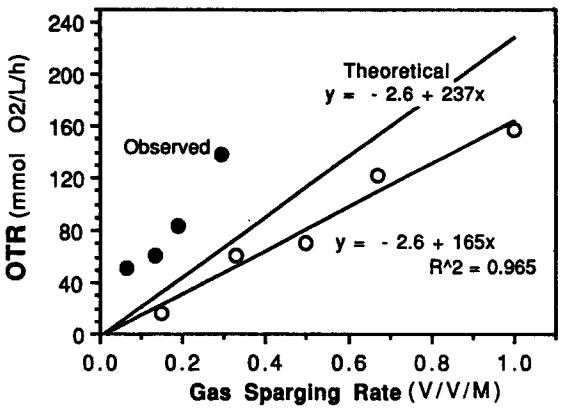


Fig. 5. Observed and theoretical determination of OTR as a function of the rate of gas sparging. The bioreactor was a modified NBS MultiGen™ F2000 fitted for top drive and a single axial-flow marine-type propeller ($D_i$ 7 cm) over a sintered-glass disk gas sparger. The $K_La$ was determined by the chemical, sulfite oxidation method (27). The line (without data points) represents the predicted OTR with $O_2$-enriched air (30%) based on the observations with air sparging (open circles). ○ Air (21%), ● 30% oxygen.

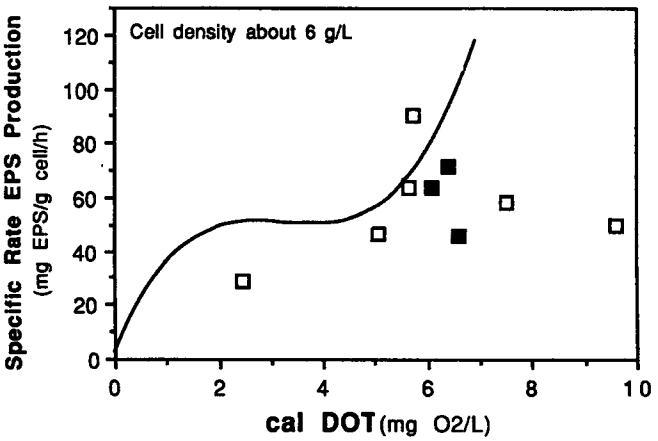


Fig. 6. The specific rate of EPS production as a function of the estimated *DO* for a twofold increase in cell density. Further details are given in Table 2. ■ Air (21%), □ 30% oxygen.

at least three instances the assumed $DO_{crit}$ was surpassed without the expected attending high specific rate of curdlan production. In one case, the sparging gas was air, but in the other two instances, it was the $O_2$-enriched air. Too much oxygen can be toxic (*9*), but it has not been reported to what extent curdlan-producing cultures are sensitive to inhibition by excess oxygen.

The assumption being made here is that the threshold *DO* ($DO_{crit}$) remains constant over a broad range of cell densities, but the experimental results do not offer conclusive evidence to support this hypothesis. A

Table 2
Effect of Oxygen on the Specific Rate of Exopolysaccharide Production at Twofold Higher Cell Density

| Fermentor design and configuration | Biomass g/L | Baffles | Operational parameters: Agitation Speed rpm | Agitation Tip velocity cm/s | Gas sparging Gas | Gas sparging Flow V/V/M | Gas sparging OTR[e] mmol $O_2$/L/h | Gas sparging Cal DOT mg $O_2$/L | Productivity $q_p$[d] mg/g cell/h |
|---|---|---|---|---|---|---|---|---|---|
| High shear system | | | | | | | | | |
| radial-flow "Rushton turbines" | | | | | | | | | |
| Air sparged through holes at bottom of central shaft | | | | | | | | | |
| NBS F2000[a] | | | | | | | | | |
| 3 turbine imps. (4.9 cm) | 6.0 | + | 850 | 218 | air | 0.33 | 84 (116) | 6.10 | 64 |
| Low shear system | | | | | | | | | |
| axial-flow impeller systems | | | | | | | | | |
| Air sparged through sintered glass disk (3 cm) under propeller | | | | | | | | | |
| Modified NBS F2000[b] | | | | | | | | | |
| Round bottom tank | 6.0 | – | 500 | 220 | air | 0.67 | 108 (116) | 6.42 | 72 |
| with top drive and | 6.0 | – | 500 | 220 | air | 0.80 | 130 (116) | 6.60 | 46 |
| with single marine | | | | | | | | | |
| propeller (7 cm) | 6.0 | – | 500 | 220 | +$O_2$[f] | 0.15 | 21 (40.7) | 2.27 | 29 |
| | 6.0 | – | 500 | 220 | +$O_2$[f] | 0.33 | 146 (40.7) | 9.60 | 50 |
| Air sparged through sintered glass disk (3 cm) under propeller | | | | | | | | | |
| Mod. NBS F2000 with | 5.9 | – | 500 | 144 | +$O_2$[f] | 0.10 | 34 (40.0) | 5.74 | 90 |
| helical prop (5.5 cm) | 6.0 | – | 500 | 144 | +$O_2$[f] | 0.10 | 34 (40.7) | 5.65 | 64 |
| | 6.7 | – | 500 | 144 | +$O_2$[f] | 0.10 | 34 (45.5) | 5.05 | 47 |
| Air sparged through porous SS disk (5 cm) under propeller | | | | | | | | | |
| NBS BioFlo II[c] with two marine | | | | | | | | | |
| propellers (7 cm) | 5.2 | + | 500 | 220 | +$O_2$[f] | 0.625 | 46 (35.3) | 7.50 | 58 |

[a]The NBS MultiGen™ F2000 fermentor is a magnetically coupled bottom drive unit (vol = 1.5 L).
[b]The modified F2000 (round bottom) was fitted with a variable-speed, top-drive motor.
[c]The NBS BioFlo II™ is a top-drive unit (vol = 4 L).
[d]$q_p$ = specific production rate.
[e]OTR = oxygen transfer rate. (The OTR values in parentheses indicate the minimum OTR required to satisfy the assumed $DO_{crit}$ of 6.5 mg $O_2$/L (it is a function of both the cell density and the pP $O_2$ in the sparging gas).)
[f]+$O_2$ = oxygen-enriched air (30% $O_2$ balance $N_2$).

culture with a nominal biomass concentration of 3 g/L (from 28 m*M* ammonium as sole nitrogen source) with a specific rate of EPS production of 100 mg/g cell/h would be expected to produce a polymer concentration of about 14.4 g/L by 48 h of elapsed fermentation time (EFT—as stationary phase of a batch culture). It has been under these conditions that most of the data relating to the relationship between $q_p$ (as initial rate) and OTR (as $K_L a \cdot C^*$ determined by the sulfite oxidation method) (*27*) have been generated.

Since the layer of EPS surrounding the cell is proposed to represent the barrier to oxygen transfer to the cell thereby limiting the cell's capacity to respire at a rate sufficient to meet the energy demand of polymer biosynthesis, it is reasonable to assume a relationship between the thickness of the boundary layer of EPS and the driving force (as *DO*) required to maintain a maximal rate of polymer biosynthesis. The concentration of recoverable EPS in the culture broth could be taken as an estimate of the thickness of this boundary layer. It is conceivable, therefore, that the $DO_{crit}$ required for $q_p^{max}$ might depend on the amount of oxygen-limiting EPS surrounding the cell (estimated from the concentration of recoverable EPS in the broth) as well as the conditions of agitation (shear) and mixing during the fermentation. One of the roles of the agitation device is mixing so as to homogenize the culture broth properly. There must be sufficient circulation flow (pumping) induced by the agitator to avoid "dead zones" (areas where the culture is not well mixed) in the bioreactor (*10*). In this type of EPS fermentation, where the product is relatively water insoluble, it will remain associated with the cell, unless there is sufficient shear stress provided by the agitator to cause it to be removed from the cell surface. However, too much shear stress might be detrimental to the cell-polymer complex resulting in cell damage (lysis), whereby the cell wall-membrane complex becomes ruptured and vital elements are lost resulting in cell death (e.g., electronmicroscopy has revealed a large proportion cell ghosts under conditions of high shear stress where radial flow turbine impellers were used to provide mixing) (unpublished observations.)

In experiments designed to increase productivity, where the cell density was doubled (increased from 3 to 6 g/L by increasing the concentration of sole nitrogen source from 28 to 56 m*M*), the concentration of EPS would be the same as a fermentation conducted at 3 g/L, but in only half the EFT (assuming constant $q_p$). Therefore, after about 24 h of stationary phase, the concentration of EPS would be expected to be in excess of 14.4 g/L, and it is conceivable that for $q_p$ to remain relatively constant in "double biomass" fermentations, the value for *DO* must increase beyond 6.5 mg $O_2$/L. This would be especially critical in situations where the mixing was not sufficient to keep the cells from aggregating and forming clumps. In such clumps, the inner most cells could easily become nutrient starved (oxygen, glucose, and so forth) (*36*) and stop producing EPS. Therefore, under certain mixing conditions, the culture may become self-limiting with

respect to the specific rate of polymer production ($q_p$) such the $q_p$ is maximal at a cell density of about 3 g/L. To correct this situation would require attention to both adequate *DO* and mixing (shear), with the constraint that too intense mixing might cause both cell damage and an inferior "quality" product as reflected by the decrease in the rheological and gelling (gel strength) characteristics of the recovered (processed) polymer.

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