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Influence of Operating Conditions and Vessel Size on Oxygen Transfer During Cellulase Production

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Abstract

The production of low-cost cellulase enzyme is a key step in the development of an enzymatic-based process for conversion of lignocellulosic biomass to ethanol. Although abundant information is available on cellulase production, little of this work has examined oxygen transfer. We investigated oxygen transfer during the growth of *Trichoderma reesei*, a cellulase-producing microorganism, on soluble and insoluble substrates in vessel sizes from 7 to 9000 L. Oxygen uptake rates and volumetric mass transfer coefficients (k_La) were determined using mass spectroscopy to measure off gas composition. Experimentally measured k_La values were found to compare favorably with a k_La correlation available in the literature for a non-Newtonian fermentation broth during the period of heavy cell growth.

Index Entries: Oxygen transfer; mass transfer coefficient; cellulase; cellulose; ethanol.

Introduction

A key step in the production of ethanol from lignocellulosic biomass is converting cellulose to sugars prior to fermentation of these sugars to ethanol. One promising technology being considered for this process is based on using cellulase. Cellulase is a multicomponent enzyme system that can effectively hydrolyze cellulose to glucose. Cellulase is typically produced by submerged cultivation using the fungus *Trichoderma reesei*. This aerobic microorganism requires good oxygen transfer to achieve good growth and subsequent production of cellulase. Although some information is available on the influence of operating conditions (e.g., agitation and

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aeration rates) on cellulase production *(1–3)*, little of this work has specifically examined oxygen transfer rates (OTRs). The usual approach is to maintain the dissolved oxygen (DO) concentration above 20% air saturation by manipulating agitation and aeration rates or by increasing the oxygen concentration in the sparge air.

Cellulase cultivations are non-Newtonian because of the hyphal-fungal morphology and the high concentrations of solid substrates sometimes used in these cultivations. Researchers have investigated oxygen transfer in non-Newtonian fungal cultivations *(4–6)*, during production of highly viscous polysaccharides (e.g., xanthan gum) *(7,8)* or in the presence of solids *(9)*. Schugerl *(10)* reviewed the many mechanisms by which high viscosity unfavorably influences mixing and oxygen transfer. With increasing viscosity higher power inputs are required to achieve similar levels of mixing, gas/liquid interfacial area is reduced because of increasing bubble size, gas/liquid interfacial area is reduced by bubble coalescence, stirring efficiency is reduced because the impeller is more easily flooded, the residence time of large bubbles decreases, and the mass transfer coefficient k_L decreases.

OTRs or oxygen uptake rates (OURs) have been measured and reported by many researchers. OTRs are determined by vessel-operating conditions (e.g., agitation and aeration rates, viscosity), and OURs are a measure of the oxygen utilization rate of the microorganisms. OTR equals OUR at steady state. Atkinson and Mavituna *(11)* summarized OTR data obtained in both small and large vessels using a variety of different methods (e.g., sulfite oxidation, oxygen balance, or biologic outgassing). In general, values reported using the sulfite method are higher than values reported by other methods *(11)*; that is, the sulfite method overpredicts OTR for biologic systems. In small bioreactors (<100 L), the reported sulfite values range from 50 to 500 mol/(L·h). For measurements performed in small bioreactors on active biologic systems (i.e., using oxygen balance technique or biologic outgassing), OURs as high as 140 mmol/(L·h) are reported. OURs up to 22 mmol/(L·h) are reported for 20,000- to 42,000-L bioreactors, also determined using biologic systems.

In more recent studies, an OUR of 30–35 mmol/(L·h) was measured in a 2-L vessel (1.3-L working volume, 730 rpm, 0.31 vessel volumes/min [vvm]) for a bacterial fermentation *(12)*. In a vessel configuration very similar to the 1500-L fermentor used in the present study (see Materials and Methods), Junker et al. *(13)* measured OURs of 28–50 mmol/(L·h) depending on impeller type in an 800-L vessel (600-L working volume, 200 and 275 rpm, 0.37–0.83 vvm) during a *Streptomyces* cultivation. The best performance (50 mmol/[L·h]) was obtained with downward-pumping, Lightnin A315 impellers (tank diameter to impeller diameter ratio [T/D] of 2.0), and the worst performance (28 mmol/[L·h]) was obtained with the Prochem Maxflo T impellers (T/D = 2.3). Rushton impellers (T/D = 2.7) achieved 41 mmol/(L·h) and Prochem Maxflo T impellers (T/D = 2.0) achieved 40 mmol/(L·h). Amanullah et al. *(14)* studied oxygen transfer during a

xanthan gum fermentation in a 6-L bioreactor equipped with three Rushton impellers (T/D = 2.0). Agitation and aeration rates were held at 1000 rpm and 0.5 vvm, respectively, and DO concentration was maintained at 20% of air saturation by supplementation with oxygen. Typical OURs ranged from 5 to 14 mmol/(L·h). In another study, Amanullah et al. *(15)* used a 150-L bioreactor to investigate the effect of impeller type on oxygen transfer during a xanthan gum fermentation. Aeration rate was fixed at 0.5 vvm and agitation speed was varied to maintain DO above 15% of air saturation. OUR values up to approx 10 mmol/(L·h) were measured and the best performance was obtained using a Prochem Maxflo T impeller.

In a fungal cultivation to produce xylanase from 2% birch wood xylan (similar to cellulase production using a solid substrate), Hog et al. *(16)* measured OURs up to 25 mmol/(L·h) in a 15-L vessel (10-L working volume, 200 rpm, 1.0 vvm). Lejeune and Baron *(2)* performed cellulase production using *T. reesei* QM 9414 in a 20-L vessel (15-L working volume, 130–400 rpm, 0.2 vvm) on a 1% (w/v) Avicel medium. Although OURs were not directly measured, carbon dioxide evolution rates (CERs) were measured and should be nearly equal to OURs during aerobic metabolism. A maximum CER of 8 mmol/(L·h) was measured at an agitation rate of 300 rpm. Marten et al. *(3)* performed cellulase production using *T. reesei* RUT-C30 grown on 5% (w/v) Solka-Floc in a 16-L vessel (10-L working volume, 250 rpm). They measured volumetric mass transfer coefficients (k_La) of 500–600 h^{-1} at the beginning of the cultivation, and then k_La decreased to near 300 h^{-1} by 24 h and remained at this value for the rest of the cultivation.

While conducting numerous studies to investigate cellulase production using cellulosic substrates, we had the opportunity to measure OURs at a variety of vessel sizes. In particular, we conducted runs in 7-L bench-scale vessels and in pilot-scale vessels ranging from 160 to 9000 L using both soluble (glucose) and insoluble (Solka-Floc) substrates. Our objectives were to determine whether existing k_La correlations could be used to predict OURs during cellulase production and to examine how oxygen transfer is affected by vessel size. This information can ultimately be used to estimate capital and operating costs for the cellulase production section of a biomass-to-ethanol conversion facility.

Materials and Methods

Microorganism

The microorganism used was *T. reesei* L27 *(17)* grown on potato dextrose agar plates until sporulation occurred. Spores were suspended in 15% (w/v) glycerol and stored in vials at –70°C.

Vessels

Bench-scale cultivations were performed using New Brunswick Bioflo 3000 fermentation systems (Edison, NJ) in 7-L vessels. Airflow to the vessels was controlled using external MKS Instruments (Andover, MA) type

Table 1
Vessel and Impeller Dimensions

	Vessel size (L)			
	7	160	1500	9000
T (m)[a]	0.165	0.51	1.07	1.83
H (m)[a]	0.038	0.17	0.38	0.89
*H*1 (m)[a]	0.076	0.32	0.61	0.97
Baffle width (m)[b]	0.016	0.044	0.089	0.20
Bottom impeller				
Type	Rushton	Rushton	Rushton	CBI[c]
Diameter (m)	0.076	0.20	0.46	0.97
Width (m)	0.016	0.05	0.10	0.20
Top impeller				
Type	Rushton	CBI[c]	CBI[c]	CBI[c]
Diameter (m)	0.076	0.30	0.56	0.91
Width (m)	0.016	0.05	0.13	0.20

[a]See Fig. 1 for definitions.
[b]All vessels contain four equally spaced baffles.
[c]Curved Blade Impeller (Prochem Maxflo T).

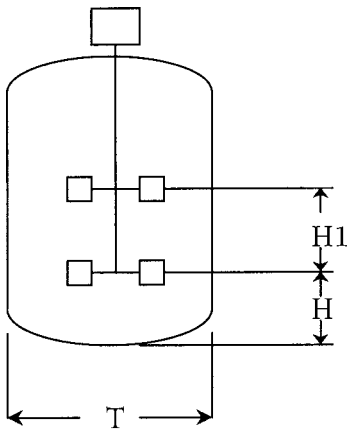

Fig. 1. Dimensions of vessels used in this study.

1159B mass flow controllers. Data for all cultivations in the bench- or pilot-scale vessels were collected and logged by computers at 5-min intervals.

Pilot-scale cultivations were performed in vessels ranging from 160 to 9000 L using fully instrumented, packaged fermentation systems manufactured by Associated Bio-Engineers and Consultants (Allentown, PA). Table 1 and Fig. 1 give dimensions for vessels used in this work. All vessels were equipped with Rushton turbines, Prochem (Robbins and Myers, Dayton, OH) Maxflo T hydrofoils, or a combination of the two impellers. The Prochem hydrofoil is designed to enhance bulk mixing but has also been shown to be more efficient for oxygen transfer in viscous mycelial cultiva-

tions *(18)*. Seed growth for pilot-scale cultivations was performed in a New Brunwick Bioflo IV 20-L fermentation system.

Bench-Scale Cultivations

The medium for inoculum growth and cellulase production was a modified Mandel medium as previously reported *(19)*, but sodium citrate was only used for shake-flask cultivation and not in the larger vessels. A first-stage seed culture was grown by inoculating a 250-mL shake flask (50-mL working volume) containing 2% (w/v) glucose (only the concentration of the carbon source is reported from now on because the other medium components remained constant) with a single vial of frozen stock culture. The flask was incubated at 28°C in a temperature-controlled shaking incubator operating at 200 rpm for 36 h.

A second stage of seed growth is required to condition the microorganism to enzyme production. Fifteen milliliters of first-stage culture (5% [v/v] inoculum) was transferred to a 1-L shake flask (300-mL working volume) containing 1% (w/v) Solka-Floc (a purified cellulose from Fiber Sales and Development, Urbana, OH) and incubated at 28°C and 200 rpm for 48 h. The 7-L vessel (4-L working volume) containing 5% (w/v) Solka-Floc was inoculated with 200 mL of second-stage culture (5% [v/v] inoculum). When more than one vessel was used during a run (up to four vessels could be operated simultaneously), second-stage culture from separate shake flasks was combined before inoculation of the multiple vessels. The temperature in the 7-L vessels was controlled at 28°C and pH was maintained at 4.8 by the addition of either 4 *N* NH_4OH or 2 *N* H_3PO_4. The airflow rate was set at 5 L/min (1.25 vvm), and agitation was allowed to vary between 450 and 600 rpm to maintain the DO concentration above 30% of air saturation. Sterile diluted (1:10) antifoam (Ucon Lubricant LB-625; Union Carbide, Danbury, CT) was added manually as needed to control foaming. These runs lasted 7 d.

There was a slight modification to the aforementioned procedures during one experiment (consisting of two vessels) when glucose was used as the carbon source instead of Solka-Floc. In this case, the second-stage culture was inoculated with a 10% (v/v) first-stage culture, the medium for second-stage seed growth contained 3% (w/v) glucose, and the second stage was incubated for only 24 h. The medium for the 7-L vessels contained 5% (w/v) glucose. All other conditions were as reported except that the run was terminated at 70 h after all the glucose was consumed.

Pilot-Scale Cultivations

Two experiments were conducted in the pilot-scale equipment using conditions listed in Table 2. The pilot-scale cultivations used most of the procedures described for the bench-scale cultivations, except that more stages for seed production were required. In the first experiment, *T. reesei* L27 was grown in the 1500-L vessel on 5% (w/w) Solka-Floc. Four stages

Table 2
Operating Conditions for Pilot-Scale Cultivations

	Vessel size (L)			
	20	160	1500	9000
Common operating conditions				
Temperature (°C)	28	28	28	28
pH	Uncontrolled	4.8	4.8	4.8
Absolute pressure (kPa)	80	180	180	180
Dissolved oxygen (%)[a]	Uncontrolled	>20	>20	>20
Acid-H_3PO_4	—	—	70% (w/w)	70% (w/w)
Base-NH_4OH	—	4 *N*	30% (w/w)	30% (w/w)
First experiment				
Working volume (L)	10	110	1100	
Solka-Floc concentration (% [w/w])	1.0	1.0	5.0	
Culture time (h)	28	124	168	
Inoculum (% [v/v])	6.0	9.0	10.0	
Agitation (rpm)	150–300	150–200	70–130	
Aeration (L/min)[b]	5	40–50	300–1000	
Second experiment				
Working volume (L)	15		600	6000
Glucose concentration (% [w/w])	2.0		2.0	5.0
Culture time (h)	24		28	50
Inoculum (% [v/v])	4.0		2.5	10.0
Agitation (rpm)	300		100	45–105
Aeration (L/min)[b]	7.5		300	1900–2100

[a]Agitation and aeration were adjusted to maintain target dissolved oxygen level.
[b]Flow at standard conditions of 21°C and 1.0 atm pressure.

of seed production were necessary to produce the required volume of inoculum. Two stages were done in shake flasks using procedures discussed above for bench-scale cultivations, followed by growth in the 20 and 160-L vessels at conditions summarized in Table 2.

In the second experiment, *T. reesei* L27 was grown on 5% (w/w) glucose in the 9000-L vessel. Again, four stages of seed production were used, but in this case only the first stage was done in shake flasks. Two 1000-mL shake flasks (300-mL working volume) were inoculated with spores from a frozen vial. This culture was grown for 48 h and then used to inoculate the 20-L vessel that was subsequently used to inoculate the 1500-L vessel. The conditions listed in Table 2 were used.

Measurement of OUR

OURs (mmol/[L·h]) were calculated from measurements of inlet and outlet gas flow rate and gas composition using a VG Prima 600 mass spectrometer (Fisons, Middlewich, UK), according to Eq. 1:

$$\text{OUR} = \frac{1}{V}(q_i Y_i^{02} - q_o Y_o^{02}) \tag{1}$$

in which V is the fermentor working volume (L), q_i is the inlet airflow rate (mmol/h), q_o is the outlet flow rate (mmol/h), Y_i^{O2} is the oxygen concentration in the inlet air (mol%), and Y_o^{O2} is the oxygen concentration in the outlet gas (mol%). The outlet flow rate is not measured but can be readily calculated from a nitrogen mass balance. Since nitrogen is not consumed, a mass balance reduces to the following simple expression:

$$q_o = q_i\left(\frac{Y_i^{N2}}{Y_o^{N2}}\right) \tag{2}$$

in which Y_i^{N2} and Y_o^{N2} are the nitrogen concentration in the inlet air and outlet gas (mol%), respectively. Substituting Eq. 2 into Eq. 1 yields the following final expression for OUR:

$$\text{OUR} = \frac{q_i}{V}\left[Y_i^{02} - Y_o^{02}\left(\frac{Y_i^{N2}}{Y_o^{N2}}\right)\right] \tag{3}$$

The airflow rate q_i is calculated from the volumetric airflow rate F_i (L/min) measured at standard conditions using the ideal gas law:

$$q_i = (P_s F_i / RT_s) \tag{4}$$

in which P_s (1.0 atm) and T (21°C for the pilot-scale fermentor systems and 0°C for the MKS mass flow controllers) are standard pressure and temperature, respectively, for the particular mass flowmeter used; and R is the ideal gas constant (0.08203 [atm·L]/[mol·K]).

Estimation of OTR for Pilot-Scale Vessels

The $k_L a$ (h^{-1}) and the driving force for oxygen transfer ($c^* - c$) govern the rate of oxygen transfer according to Eq. 5:

$$OTR = k_L a\,(c^* - c) \tag{5}$$

in which c^* is the saturated oxygen concentration or solubility (mmol/L) determined from Henry's Law:

$$p_i = Hc^* \tag{6}$$

and c is the actual DO concentration (mmol/L), p_i is the partial pressure of oxygen in the sparge gas (atm), and H is Henry's coefficient (1.0 atm/[mmol·L]) *(20)*. The $k_L a$ is usually given by a correlation of the following form:

$$k_L a \propto \left(\frac{P_g}{V}\right)^n u_s^m \tag{7}$$

in which (P_g/V) is gassed power input per unit volume and (u_s) is the gas superficial velocity. The exponents n and m and a proportionality constant are determined by fitting experimental data.

Power Input

McCabe and Smith *(21)* give an ungassed power correlation for a non-Newtonian (pseudoplastic) liquid in a vessel equipped with a single six-bladed turbine (Rushton) and four baffles. The correlation relates the power number (N_p) to the modified Reynolds number (Re_m). These terms are defined as follows:

$$Re_m = \frac{ND^2\rho}{\mu_a} \tag{8}$$

in which N is the agitation rate (s^{-1}), D is the impeller diameter (m), ρ is the fluid density (kg/m^3, assumed to be 1.02 g/cm^3 in all calculations), and μ_a is the apparent viscosity (kg/[m·s]). The power number is

$$N_p = \frac{P_o g_c}{N^3 D^5 \rho} \tag{9}$$

in which P_o is the ungassed power for a single impeller (W); and g_c is the Newton's Law proportionality factor, which is 32.174 lb-ft/s^2-lb_f in English units and is unity and dimensionless in SI units. Since power correlations depend on impeller type, tank and impeller dimensions, and impeller placement, any correlation will only give approximate results. The total ungassed power input (P_T) is approximated as the single impeller power times the number of impellers.

On aeration, the power drawn by an impeller decreases because of an effective decrease in fluid density caused by the holdup of air under highly aerated conditions. This effect can reduce the gassed power input to as little as 35% of the ungassed power input. Gassed power input was estimated from published experimental data for a non-Newtonian fermentation broth *(22)*.

k_La Correlations

Correlations for k_La are available for both Newtonian liquids and a non-Newtonian fermentation broth.

Bailey and Ollis *(23)* give the following correlation for coalescing water, a highly Newtonian liquid:

$$k_La\ (s^{-1}) = 0.0026\left(\frac{P_g}{V}\right)^{0.4} u_s^{0.5} \tag{10}$$

in which the power input per unit volume is expressed in W/m^3 and u_s is the superficial velocity (m/s). This equation is valid for V <2600 L and $500 < P_g/V < 10{,}000$ W/m^3 (~5–10 hp/1000 gal).

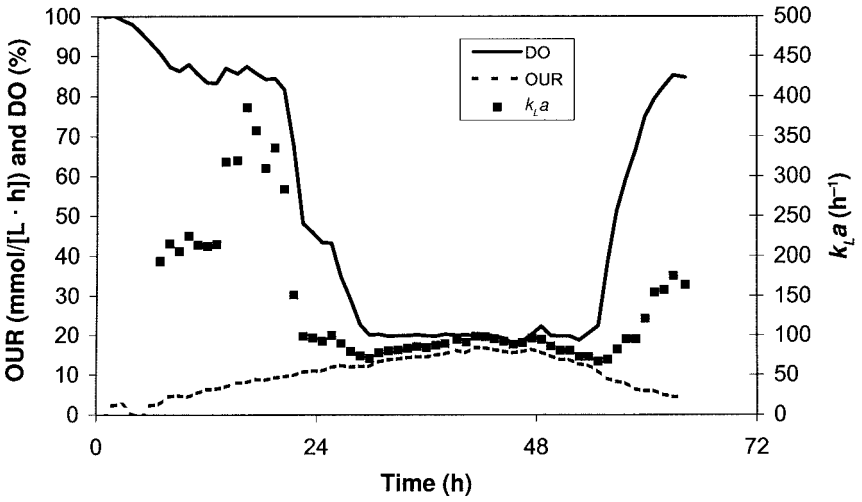

Fig. 2. DO, OUR, and measured k_La during growth on glucose in a 7-L vessel.

Wang et al. *(22)* present the following k_La correlation for a non-Newtonian fermentation broth for vessel sizes from 20 to 30,000 L during a fungal cultivation:

$$k_La\ (h^{-1}) = 8.42\left(\frac{P_g}{V}\right)^{0.33} u_s^{0.56} \tag{11}$$

in which power input per unit volume is expressed in hp/1000 L and the superficial gas velocity in cm/min.

Results

Bench-Scale Results

Figure 2 shows profiles for DO, OUR, and measured k_La values for one of the glucose cultivations (one of two vessels). During the first 12 h of the cultivation, k_La was about 200 h^{-1} but increased to 300–400 h^{-1} for the next 12 h even though both aeration and agitation rates remained constant. After the start of rapid cell growth, k_La dropped to much lower values between 60 and 90 h^{-1}. During the period of high oxygen uptake from 30 and 54 h, DO concentration was maintained at 20% by increasing the agitation rate. This is reflected by the slight increase in measured k_La; we suspect that a larger increase was not seen because the rapidly increasing cell concentration (data not shown) was also causing broth viscosity to increase. The DO increases only after glucose is nearly consumed when the culture begins to sporulate and lyse, producing a noticeable drop in viscosity. This is also reflected by an increase in k_La after 54 h.

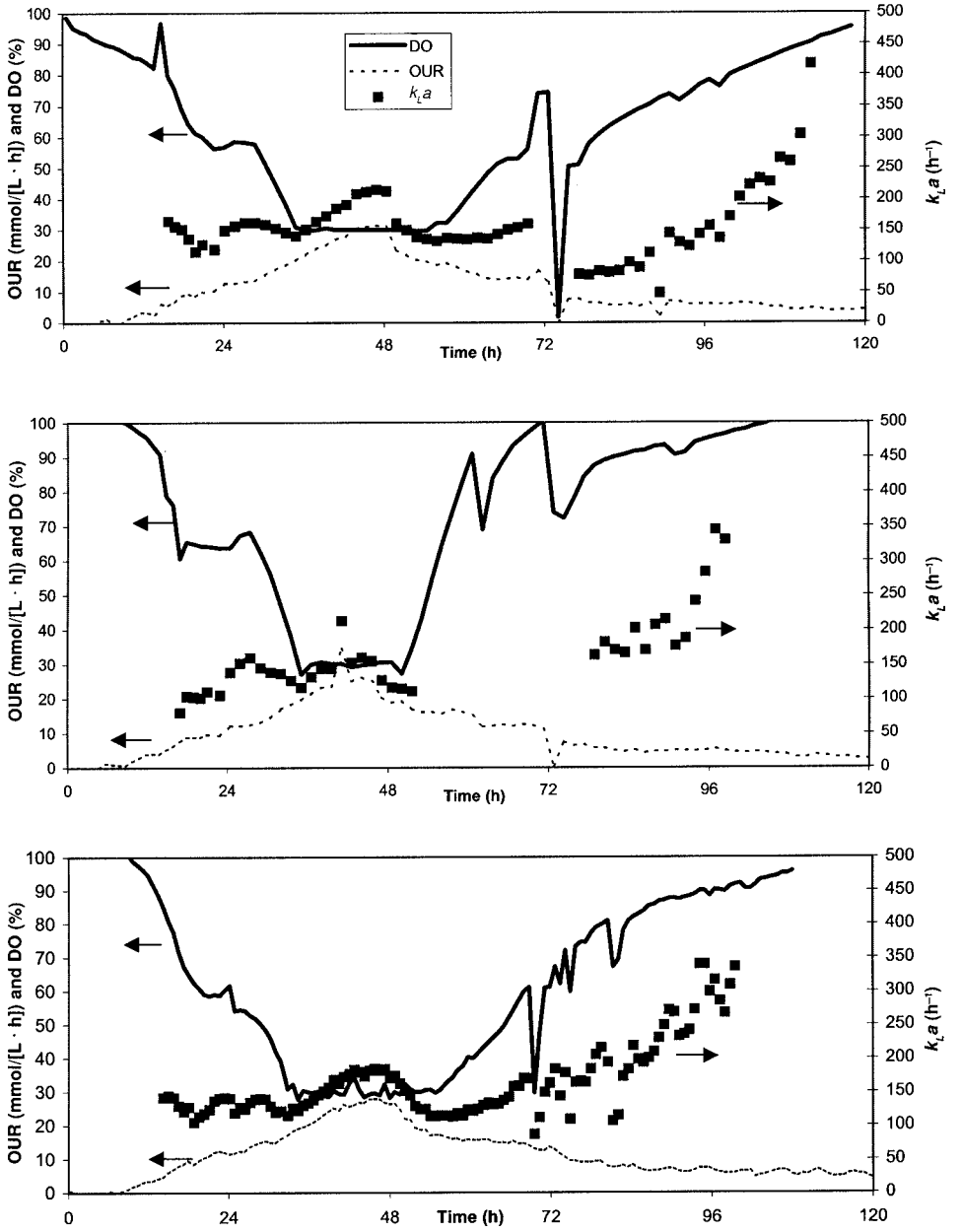

Fig. 3. DO, OUR, and measured k_La during growth on Solka-Floc for three runs in 7-L vessels.

Figure 3 presents typical results for the bench-scale Solka-Floc runs (three of six cultivations). Some k_La data are missing because operating problems (foaming that caused loss of airflow and erratic pressure fluctuations) did not allow calculation of good values. The DO, OUR, and k_La profiles for all of the runs are remarkably similar and generally display the

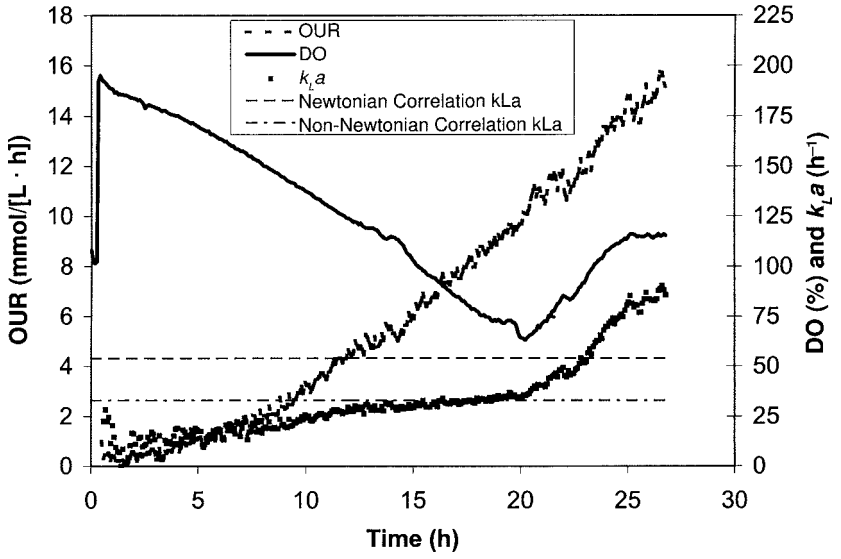

Fig. 4. DO, OUR, and measured k_La during a seed growth on glucose in the 1500-L vessel and values of k_La calculated from Eq. 10 (Newtonian) and Eq. 11 (non-Newtonian).

same trends seen during glucose cultivations. However, during the period when agitation rate was increased to control DO concentration at 30% (typically between 36 and 54 h), there was a noticeable increase in k_La values. As expected, because the thick Solka-Floc slurry hinders mass transfer, the k_La values during the initial stages of these cultivations were lower than values measured for the glucose cultivation. The k_La values were between 100 and 200 h^{-1} during the first 3 d of cultivation and, similar to what was observed in the glucose cultivations, began to rise after the carbon source was consumed and cell lysis began to thin the broth. The maximum OUR for these cultivations ranged from 30 to 35 mmol/(L·h), which is quite similar to OURs reported by other researchers for these vessel sizes.

Pilot-Scale Results

Data from pilot-scale cultivations were obtained during inoculum growth and in the final production vessels (1500- or 9000-L vessels). Figure 4 shows DO, OUR, and measured k_La profiles during inoculum growth on 2% (w/w) glucose in the 1500-L vessel. The inoculum was harvested after 27 h with the broth still containing 10 g/L of glucose. Figure 4 also shows the k_La values calculated from Eqs. 10 and 11 for Newtonian and non-Newtonian broth rheology assuming an apparent viscosity of 1500 cP. Junker et al. *(13)* measured apparent viscosities ranging from 500 to 2200 cP for a *Streptomyces* cultivation depending on impeller type. Viscosity values from 600 to 1000 cP were obtained for Maxflo T impellers and values of 2200 for Rushton impellers, so an intermediate value of 1500 cP was used

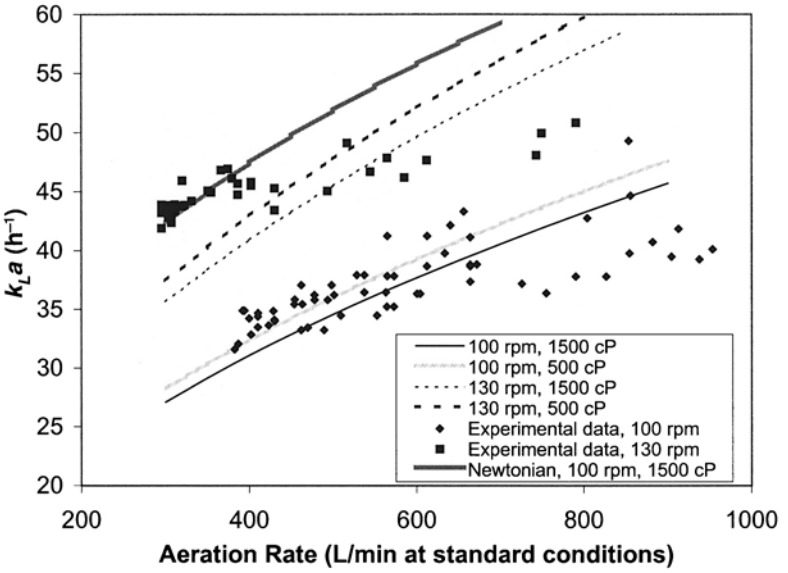

Fig. 5. Measured k_La values as a function of aeration rate during growth on Solka-Floc in the 1500-L vessels. k_La values are calculated from Eq. 11 and shown for the two agitation rates utilized during this run at two different viscosities. k_La values are also calculated from Eq. 10 at one agitation rate and viscosity.

in our calculations; but as shown later (see Fig. 5), calculated k_La values are not extremely sensitive to viscosity.

Since both agitation rate (100 rpm) and aeration rate (300 L/min at standard conditions) were held constant for the entire 27-h seed cultivation, Eqs. 10 and 11 predict constant k_La. The trend of increasing k_La values during this run suggests that during the early stages of this cultivation, the broth rheology is changing to enhance mass transfer. Although unexplained, there are indications of this same type of behavior during the early stages of the bench-scale glucose cultivation (Fig. 2). The trend appears to reverse after approx 25 h, but the cultivation was not run long enough to confirm this trend. Although the non-Newtonian correlation gives lower values for k_La, neither correlation does well at predicting the experimental results during the early part of the cultivation. The k_La correlations were not used to model bench-scale data because the agitation power correlations are based on data obtained from larger vessels.

Figure 5 presents measured and predicted k_La values for the 5% (w/w) Solka-Floc cultivation in the 1500-L vessel. Predicted k_La values are shown for apparent viscosities of 500 and 1500 cP using the non-Newtonian correlation and for an apparent viscosity of 1500 cP using the Newtonian correlation. The experimental data are for the period from 48 to 66 h, during which reliable data acquisition and control occurred. DO was controlled at 30% by adjusting the aeration rate using one of two different agitation rates (130 rpm was lowered to 100 rpm at 54 h). As seen by comparing Fig. 5 to 3, these results were collected during a period of heavy cell growth and

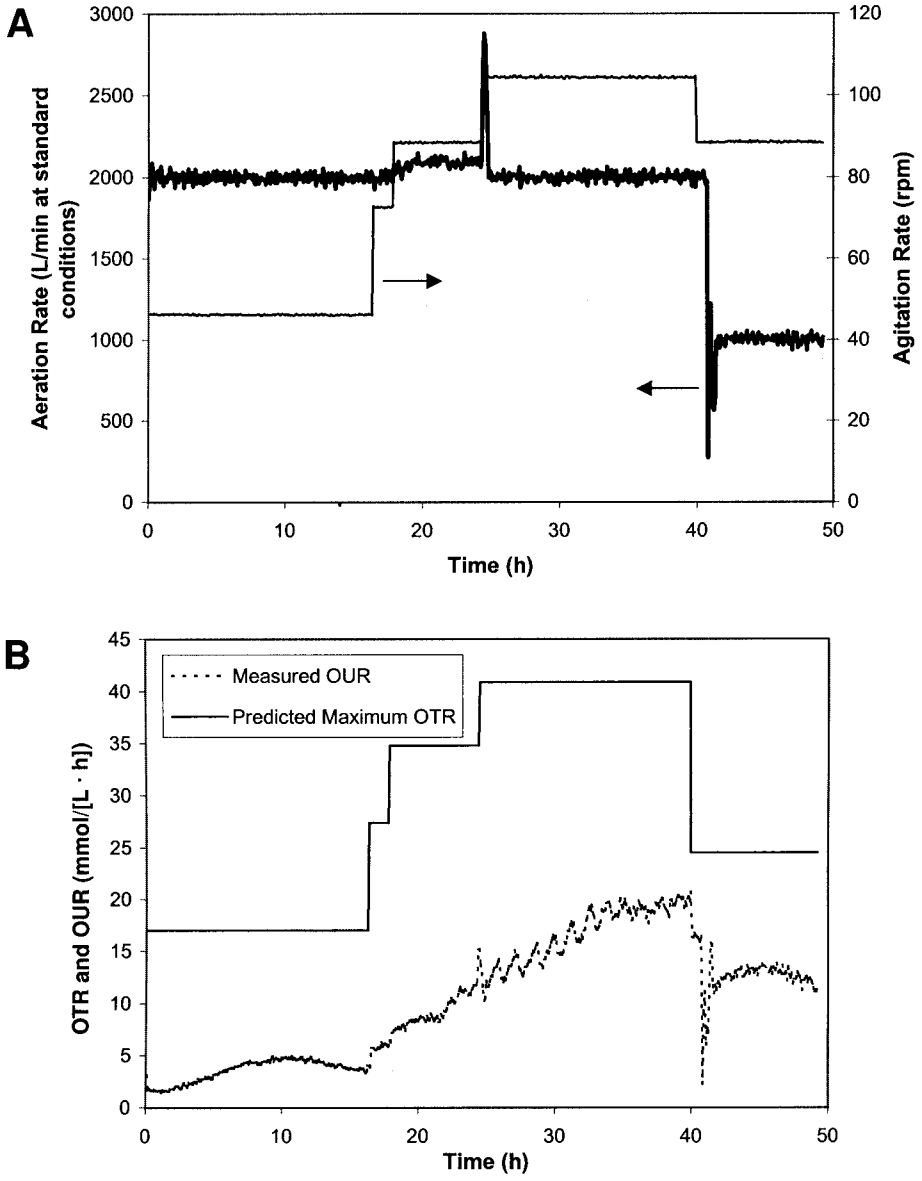

Fig. 6. **(A)** Aeration and agitation rates during growth on glucose in the 9000-L vessel and **(B)** measured OUR compared to the predicted maximum OTR calculated from Eq. 11 for this cultivation.

substrate utilization, before substrate depletion and cell lysis significantly decrease broth viscosity.

Although the trend of measured k_La values is not well predicted by the correlations, the measured values, even at the extremes are within ±25% of the predicted values from the non-Newtonian correlation at 1500 cP over a broad range of aeration rates. This is certainly within the expected accu-

racy of these types of correlations. However, as seen from previous results, this correlation only appears to work reasonably well during the period of heaviest cell growth and oxygen utilization and not as well during the early and late stages of cultivation. As expected, the Newtonian correlation did not fit the data. Significant changes in apparent viscosity have little effect on the predicted k_La values because changes in viscosity have only a minor effect in the power calculation. However, the equations do not account for the other ways in which viscosity can significantly influence oxygen transfer, as previously discussed, e.g., by influencing both the mass transfer coefficient (k_L) and interfacial area (a).

Because of mechanical failure of a DO probe during the 5% (w/w) glucose cultivation in the 9000-L vessel, the k_La values were not measured during this run. However, Fig. 6A presents the agitation and aeration rate profiles during this run. Using this information, the maximum OTRs were calculated using the non-Newtonian correlation assuming a DO concentration of 0. The maximum OTRs are compared to measured OURs in Fig. 6B. We believe that the DO was actually never near 0. Thus, we expected that measured OUR would be lower than calculated OTR if the correlation predicted reasonable OTR. Since the measured OURs are less than the predicted maximum OTR, we have some confidence that the correlation is effective and will work for even larger vessels.

Discussion

This study shows that a non-Newtonian correlation for k_La can be used to estimate OTR in cellulase production runs during the period of heavy cell growth and oxygen utilization for the vessel sizes used (160–9000 L). For an ethanol production facility, the cellulase production tanks are likely to be at least 500,000 L. It is unlikely that the correlation will work at this large scale, since it has been suggested that the exponents in k_La correlations are functions of scale *(24)*. Nevertheless, the non-Newtonian k_La correlation was utilized to explore the effect of vessel size on oxygen transfer using two commonly used scale-up criteria: constant shear or impeller tip speed (4.04 m/s) and constant power (gassed) input per unit volume (500 W/m^3). Figure 7 presents the results of these calculations for 9000-, 100,000-, and 500,000-L vessels. These calculations assume a DO concentration of 0, an average pressure of 2.0 atm, and a working volume of 75% of the total vessel volume. These calculations also assume that the vessels have similar geometries (i.e., vessel height/vessel diameter = 2.0, vessel diameter/impeller diameter = 1.9).

The results show that regardless of the scale-up criteria used, it is possible to achieve better oxygen transfer in the larger vessels. Oxygen transfer is enhanced at larger scales because higher superficial velocities are achieved when the dimensional ratios of the vessels are held constant. As size increases, however, it may be impractical to construct a vessel with a 2:1 height-to-diameter ratio. In addition, the agitation rates required to

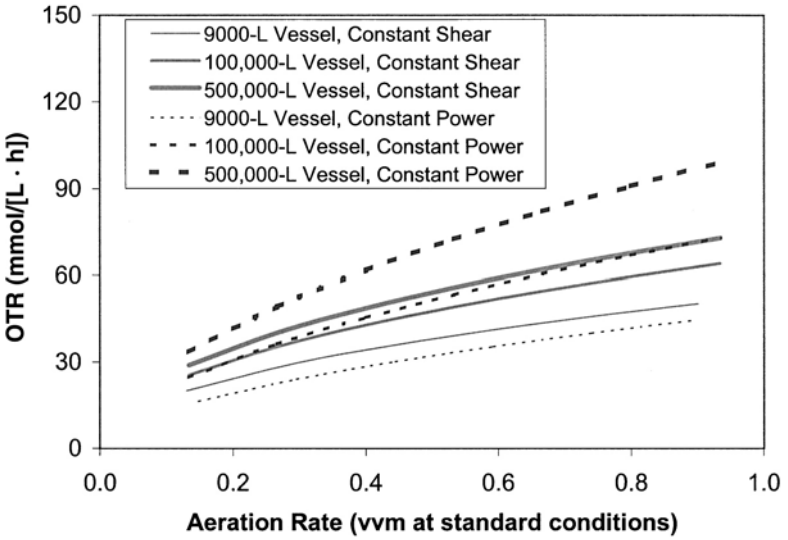

Fig. 7. OUR as a function of vessel size using either constant shear or constant power as the scale-up criterion.

achieved these power inputs may not be practical at very large scale or may produce shear rates that are too high. Oxygen transfer is lower in larger vessels when using shear rate as the scale-up criteria because less power input per unit volume is needed in larger vessels to achieve the same impeller tip speed. Scale-up based on shear rate may be more appropriate for cellulase production because of the reported shear sensitivity of cellulase *(25)*.

Unfortunately, it is probably not possible to simplify scale-up of cellulase production using the correlation presented herein. Accurate predictions of oxygen transfer at large scales are difficult because of the large changes that occur in broth rheology as well as the mounting problems with bulk mixing, efficient bubble breakup, and gas distribution. One researcher has suggested a modification to k_La correlations to include a viscosity term *(26)*. Additionally, impeller flooding is known to occur at much lower aeration rates in larger vessels, particularly in viscous cultivations *(24)*. In practice, it is probably not possible to achieve higher OTRs in larger vessels than can be achieved in smaller vessels. Our study nevertheless provides some useful oxygen transfer rate data and insight into some of the scale-up issues for cellulase production. This information supports efforts to improve economic analysis of enzyme-based ethanol production processes.

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