0273-2289/91/2829—0667$03.60

Bioreactor Design Considerations in the Production of High-Quality Microbial Exopolysaccharide

HUGH G. LAWFORD* AND JOYCE D. ROUSSEAU

Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada M5S 1A8

ABSTRACT

An examination into the effect of bioreactor design on the production of β,1,3-glucan exopolysaccharide ("curdlan") by selected patent cultures of *Alcaligenes faecalis* and *Agrobacterium radiobacter* revealed that low shear mixing achieved through the replacement of the radial-flow flat-blade impellers that are commonly supplied in "standard" commercial bioreactors, by low shear (high-pumping) axial-flow impellers, leads to an increase in the *quality* of the exopolymer recovered during the stationary-phase of batch fermentations. Whereas "Rushton turbine" impellers were effective in providing high rates of oxygen transfer necessary for high cell density fermentations, the high shear-to-flow ratio characteristic of this design produced a product of inferior quality, but with characteristics very similar to that of the commercially available "curdlan standard." Curdlan is water insoluble, and consequently, the fermentation broth is of a relative low viscosity compared to other soluble microbial polysaccharides. Whereas curdlan does not constrain mass transfer from gas to liquid, it nevertheless offers a resistance to oxygen transfer from the liquid to the cell by virtue of the layer of insoluble exopolymer surrounding the cell mass, thereby necessitating an unexpectedly high dissolved oxygen concentration for maximal productivity. 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.

Index Entries: Curdlan; exopolysaccharide; shear-sensitive mixing; bioreactor design; aeration.

*Author to whom all correspondence and reprint requests should be addressed.

Abbreviations: C^*, conc'n of dissolved O_2 at saturation (0.236 m*M* in water, 30°C, 1 atm); D_i, diameter of impeller (cm); DW, dry cell weight (g dry wt/L); EFT, elapsed fermentation time (stationary-phase) (h); EPS, exopolysaccharide (g DW/L); K_La, vol O_2 mass transfer coeffic. (h^{-1}); N, rotational speed of impeller (rev/s); OTR, O_2 transfer rate ($K_La \cdot C^*$) (mmol O_2/L/h); q_p, p. rate of EPS production (*see* SPR); SPR, sp. production rate (mg EPS/g cell/h); STR, stirred tank reactor; D_t or T, diameter of fermentor tank (cm); V/V/M, gas (air) sparging rate (vol gas/vol liquid/min).

INTRODUCTION

Biotechnology has responded to the increasing demand for inexpensive, high-quality, viscosifiers and biodegradable polymers, and today the large-scale production of microbial exopolysaccharides (EPS) exemplifies a new fermentation industry with global markets representing many hundreds of millions of dollars annually. Through controlled fermentations, the potential exists to produce inexpensively a variety of different high-quality microbial polysaccharides of consistent composition and quality. In contrast to the extensive literature relating to the chemistry of these fermentation biopolymers (EPS), it would appear that much less attention has been paid to the influence of physical parameters on biological response and the quality of the recovered biopolymer.

The transformation of a microbial ("fermentation") process from the laboratory to full-scale commercial operation is not easy, and requires careful consideration of bioreactor design, scale-up, and operating parameters because this piece of equipment is central to determining the technical feasibility of the entire process, as well as the potential for commercial success of the product. The choice of a particular bioreactor design depends on a number of interactive, but sometimes opposing factors, such as the requirement for mixing and oxygen transfer in a viscous medium where restrictions exist with respect to the amount of "shear" that can be tolerated.

One of the first steps in developing a commercial fermentation product involves the transition from shake flask to a more economic and convenient production vessel—the so-called "stirred tank reactor" (STR). The use of an STR offers greater potential for controlling the physicochemical environment and the oxygen limitation often associated with shake flask cultures can be addressed through standard engineering strategies relating to increasing oxygen mass transfer for the purpose of increasing specific productivity. In the case of β-1,3-glucan ("curdlan") produced by selected strains of *Alcaligenes faecalis* and *Agrobacterium radiobacter* (*1–5*), the exopolymer represents a barrier to oxygen transfer to the culture, thereby necessitating an unusually high dissolved oxygen tension (DOT) for maximal rates of polymer biosynthesis (*6*). Shake flask cultures have been observed to produce a higher mol wt polymer with superior gelling and rheological characteristics (*6*). Therefore, our approach (a kind of reverse engineering) has attempted to simulate the low shear mixing environment of the shake flask in an STR.

In this study, we have examined various bioreactor configurations designed to effect a reduction in the shear stress on the cell/polymer complex. We have tested the aeration capacity and the specific rate of polymer production, as well as the "quality" of the isolated exopolymer according to previously established criteria relating to specific proprietary applications (*7,8*). The objective was to provide sufficiently high rates of oxygen transfer for maximal rates of polymer biosynthesis without creating a detrimental shear environment that would compromise the quality of the recovered polymer product.

MATERIALS AND METHODS

Organisms and Culture Conditions

Alcaligenes faecalis var. *myxogenes* ATCC 31749 (*9,10*) and ATCC 21680 and *Agobacterium radiobacter* ATCC 21679 (*11*) are β-1,3-glucan-producing, patent cultures, and were obtained from the American Type Culture Collection (Rockville, MD). Growth was done aerobically in a defined mineral salts medium (*5*) with glucose (50 g/L) as the sole carbon source. The bacterial cell density was proportional to the amount of assimilable nitrogen in the medium (28 m*M* NH_4Cl) giving a biomass concentration of about 3 g DW/L.

Analytical Procedures

Procedures for exopolymer recovery from the fermentation broth were as described previously (*5*). Intrinsic viscosity measurements were performed on dilute solutions of salt-free samples of EPS in the range 0.02–0.05% (dry W/V) in 0.3*M* NaOH at 25°C. The microprocessor controlled autoviscometer (Ecoplastics Ltd., Willowdale, Can.) was designed by J. Guillet at the University of Toronto (*12*). The K_La (determined by a standard chemical method using sulfite oxidation with cupric ions as catalyst [*13*]) of different fermentor designs with respect to agitation and aeration was measured as a function of the rotational speed of the impeller(s) and/or the rate of air sparging (data not shown).

Bioreactors—Agitation and Aeration

Batch fermentations were conducted in 500 mL Erlenmyer flasks (50 mL media) and variously configured bench-top STRs equipped with sensing/

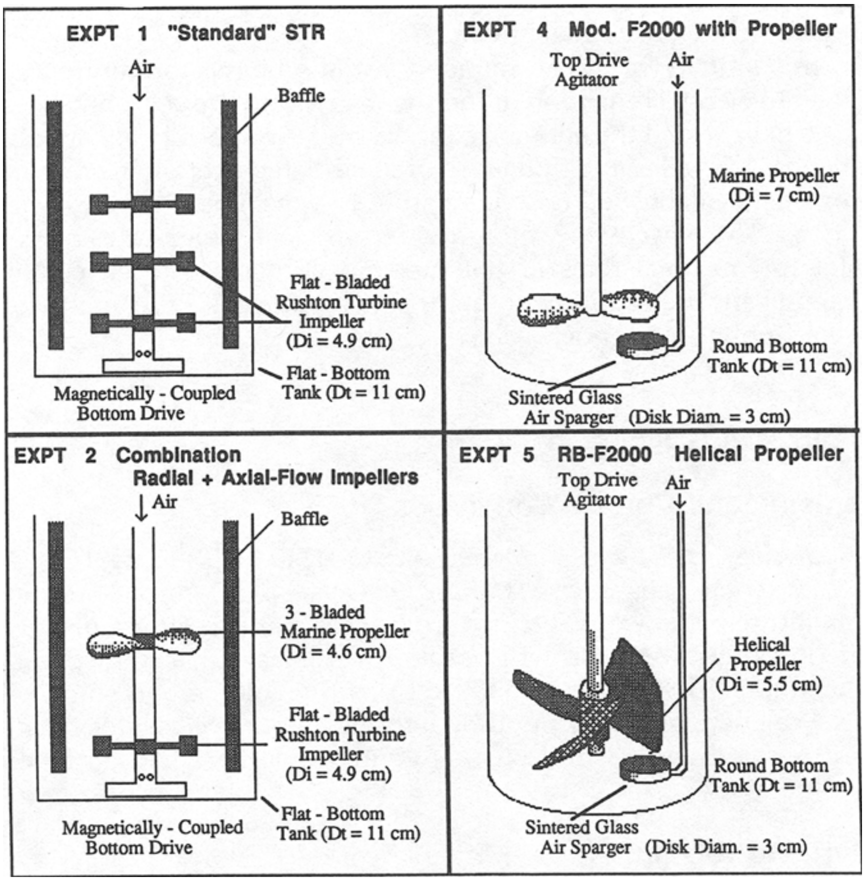

Fig. 1. Experimental design and fermentor configuration with respect to aeration and agitation. Experiments are detailed in Tables 2 and 3.

control devices for agitation (rpm), air sparging (V/V/M), pH, and temperature. The MultiGen (F2000), BioFlo II (5 L), and Microferm (MF114) were from New Brunswick Scientific Co. (Edison, NJ). The Biostat E, with either baffle or draught tube heat exchangers, was from B. Braun (Allentown, PA). The marine (7 cm) and helical (5.5 cm) propellers were obtained from Cole-Parmer (Chicago) and BioLafitte (Princeton, NJ), respectively; the sintered-glass and porous stainless-steel air spargers were from Canlab Scientific (Toronto) and Pall (Canada) Inc. (Mississauga), respectively. Operating conditions were as described previously (*6*) with configurations with respect to mixing and aeration shown in Figs. 1 and 2.

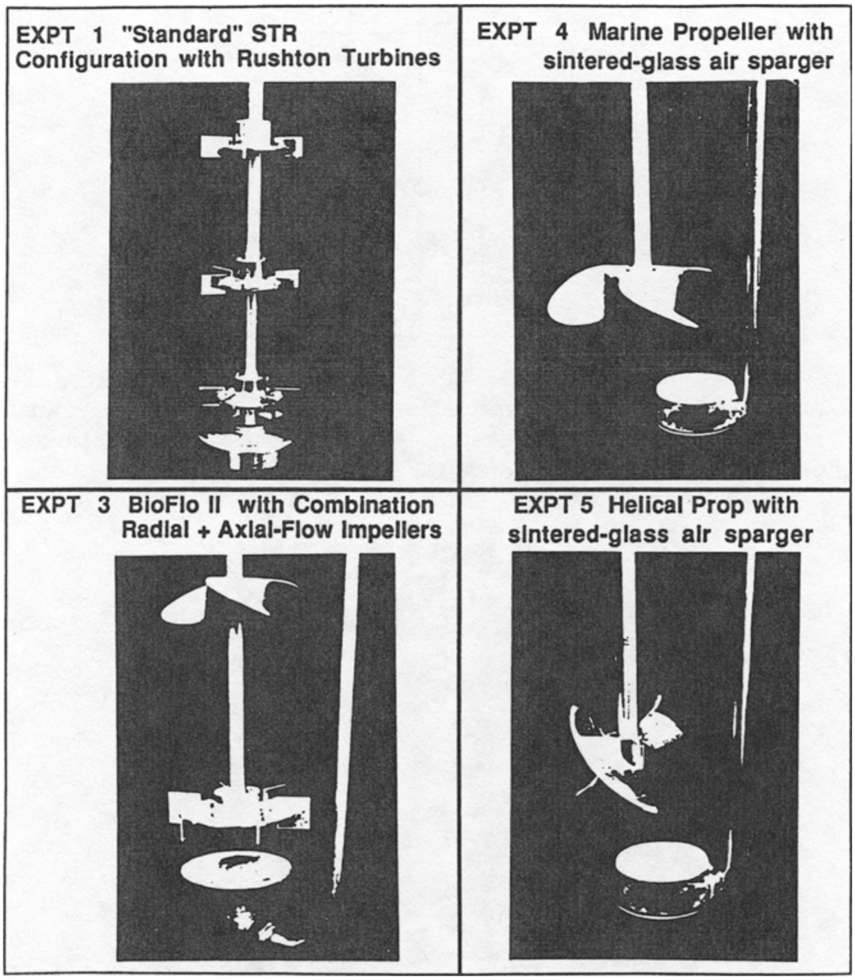

Fig. 2. Photographs of the various aeration and agitation configurations. Experiments are detailed in Tables 2 and 3.

MIXING DEVICES AND FLUID FLOW DYNAMICS

Radial- and Axial-Flow Impellers

As fluid flow inducers, impellers can be classified in two general types: axial flow and radial flow. An axial-flow impeller is one in which the principal locus of flow occurs along the axis of the impeller (parallel to the impeller shaft). A radial-flow impeller discharges flow along the impeller radius in distinct patterns (*14*) (Fig. 3).

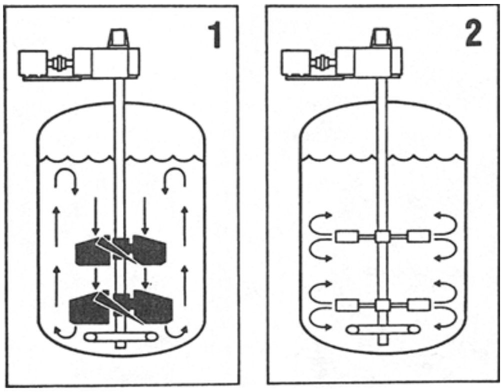

Fig. 3. Fluid flow patterns induced by impellers of different design. 1. Axial-flow impellers—low shear and good pumping (circulation). 2. Radial-flow impellers—high shear with less pumping. (Reproduced with permission of Mixing Equipment Company, Rochester, NY.)

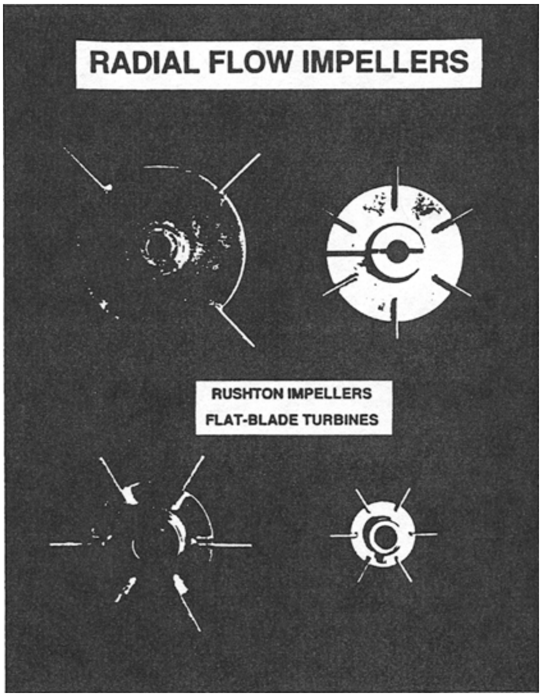

Fig. 4. Radial-flow impellers.

The flat-bladed radial-flow impeller or "Rushton turbine" is a high shear, low pumping impeller and is commonly employed in fermentors because of its efficiency in gas-liquid contacting. For an STR, the term "standard" configuration refers to the use of "Rushton turbines," since most fermentation equipment manufacturers supply this type of impeller as standard fitting (Fig. 4).

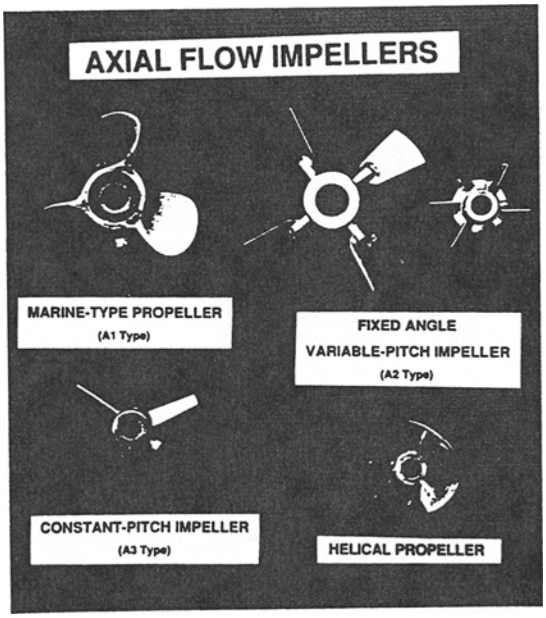

Fig. 5. Axial-flow impellers.

Axial-flow impellers typically produce less shear and more pumping. They include the propeller (type A1) that draws less power than most other impellers of the same diameter. Two other types are the constant angle (45°) blade impellers (type A2) and the newer hydrofoils (type A3—variable angle and blade width for constant pitch) that were developed to produce more flow and less shear (Fig. 5). Other very important variables include (*14*):

1. The amount of power per unit volume
2. Impeller location—including spacing on the shaft
3. D_i/D_t ratio—typically there is an optimal value for each type of impeller
4. Use of baffles.

Impeller Turbulent Shear Stress, Flow Intensity, and Impeller Pumping

An important role of the impeller is to provide good mixing so that a uniform or homogeneous environment is present throughout the entire volume of the culture broth. There are two types of mixing: shear flow and circulation flow. Table 1 defines these mixing parameters in terms of the rotational speed of the impeller(s) and the impeller diameter (D_i) (*15*). In an STR, three different types of flow regions can exist:

1. The "micromixing region" around the impeller
2. The "macromixing region," which is dominated by the circulating flow (pumping) and
3. A stagnant ("dead") zone where there is little or no mixing.

Table 1
Mixing and Fluid Dynamics

TURBULENT SHEAR STRESS
is proportional to $N^2D_i^2$

IMPELLER PUMPING POWER
is proportional to ND_i^3

Ratio of Impeller Turbulent Stress to Impeller Pumping
Shear : Flow = N/D_i (cm.sec)$^{-1}$

SHEAR FLOW INTENSITY
is proportional to ND_i

where N is the impeller rotational speed (rps)
and D_i is the impeller diameter (cm)

The geometric design of the tank and the circulation flow (pumping) induced by the agitator(s) must be sufficient to avoid "dead zones."

The cells (together with associated exopolymer) exist in the micromixing zone in a dynamic fashion, because they are moved away from the intensive shear zone by virtue of the pumping action of the impeller. It has been suggested by Wang and Fewkes (*15*) that the shear-to-flow ratio (determined as N/D_i) is a quantitative indicator of this dynamic equilibrium. Interzone mixing ("blend times") is especially important in viscous fluids.

The Role of Agitation in Aerobic Fermentations

Agitation affects mass transfer from gas to liquid in three basic ways:

1. It disperses the gas into smaller bubbles and thus increases the interfacial area (a)
2. It increases the gas–liquid contact time because circulation of gas bubbles in eddies cause gas "hold up," and
3. The increased turbulence caused by agitation will decrease the thickness of the stationary liquid film and, consequently, increase the value of K_L.

Radial flow (turbine) impellers are efficient at effecting oxygen transfer by virtue of their ability to increase turbulence. However, by forcing the ventilating gas through porous materials, such as sintered-glass, microporous ceramic, or stainless steel, gas dispersion can be effected, thereby avoiding the shear stress of high-speed, radial-flow turbines.

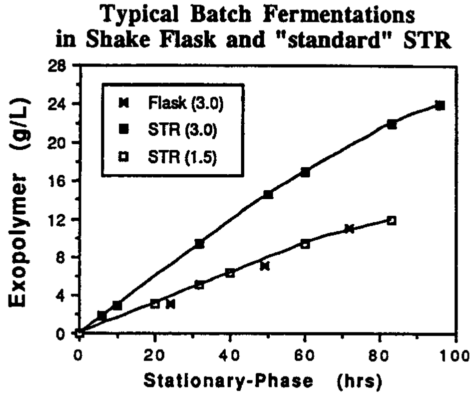

Fig. 6. Typical batch fermentations for production of β-1,3-glucan EPS. *A. faecalis* ATCC 31749 grown aerobically in mineral salts medium (*5*) in either 500 mL Erlenmyer flask (50 mL media) or NBS MultiGen F2000 with Rushton turbines operating at 850 rpm with 0.33 V/V/M air. The numbers in brackets refer to the cell density (g DW/L). Time zero is at the onset of stationary-phase when growth stops because of depletion of assimilable nitrogen.

RESULTS

In batch fermentations, the production of β-1,3-glucan EPS occurs following growth in a nitrogen-deficient medium with excess carbon source (*4,5,16*). The product production profiles for batch fermentations conducted in both shake flask and a "standard" STR fitted with three Rushton turbines (tip velocity of 220 cm/s) are illustrated in Fig. 6. The specific rate of production (q_P) in the flask fermentations was only 50% of that in the STR. Since in shake flask fermentations, the q_P decreased as the volume of media was increased, it was concluded that these cultures were limited by the relatively low oxygen transfer capacity (K_La measured as 76 h^{-1}) of the system (A. Kligerman, personal communication). These initial experiments pointed to the importance of adequate oxygen transfer in terms of process productivity.

However, the efficiency of the radial-flow Rushton turbines in effecting the requisite oxygen requirement of the polymer-producing culture was counteracted by the negative effect of this shear intensive system on the "quality" of the isolated exopolymer. Product quality is a relative term that can only be properly defined in terms of end-use application (*7*). Although previously we have described "quality" in terms of such physical characteristics as mol wt and gel strength (*7,17*), in this study we have measured the intrinsic viscosity of the purified exopolymer (in 0.3*M* NaOH [*18*]), since this is directly proportional to the mol wt (*19*). Whereas EPS produced in the "standard" STR exhibited properties consistent with our product standard ("curdlan" obtained from Wako Pure Chemical Industries Ltd.—a subsidiary of Takeda Chemical Industries Ltd., Osaka, Japan), the EPS isolated from the shake flask cultures was consistently of much higher "quality" (Fig. 7). Figure 7 illustrates the potential for process improvement with respect to product quality and focuses attention on the influence of the bioreactor design with respect to mixing. The results of experiments with variously designed bioreactors using radial-flow and axial-flow impellers are summarized in Tables 2 and 3, respectively.

The data from these experiments are presented graphically to illustrate the effect of different mixing parameters on product quality (intrinsic viscosity)—the effect of impeller shear flow intensity (Fig. 8) and impeller shear-to-flow ratio (Fig. 9). Alterations in design with respect to mixing and aeration can be expected to affect the oxygen transfer capacity of the bioreactor. The relationship between the K_La and the specific rate of exopolymer production (data from Tables 2 and 3) is shown in Fig. 10.

Additional experiments were conducted with radial-flow impeller systems to investigate the possible effect of shear flow intensity on productivity, under conditions where the K_La was not a rate-limiting factor, and these results are presented in Fig. 11. For comparative purposes, Fig. 11 also shows data from a study of similar design conducted by Funahashi et al. (*20,21*) on xanthan production by *Xanthamonas campestris.*

DISCUSSION

Batch processes for EPS production often last several days, and it has been observed that the viscosity of the fermentation broth (as in the case of soluble polymers, such as pullulan and xanthan) or the intrinsic viscosity (proportional to the mol wt) of the recovered exopolymer (as with the insoluble β-1,3-glucan) often decreases in the later stages of the fermentation. Quite early on in our research on curdlan, we had observed that the viscosity of the broth exhibited a characteristic profile with a peak at about 24 h (stationary-phase fermentation time), but the reason for the decline thereafter was a mystery (*4*).

Measurements of the intrinsic viscosity of the isolated exopolymer as a function of time suggested that, in STRs of conventional design (with Rushton turbines), the polymer was rapidly degraded. The degree of polymerization (mol wt) has a marked influence on properties of commercial importance, such as the tensile strength of the thermally induced gel (*17*). Saito et al. (22) had observed that the degree of polymerization of curdlan varied considerably for four different batch fermentations, and it is interesting to note in the context of our investigation that the explanation offered for the wide variation in mol wt was that it was somehow dependent upon an "unidentified fermentation condition" (22). Nevertheless, the value of the intrinsic viscosity that we isolated from the "standard" STR corresponded to that of our curdlan "standard" obtained from Wako Pure

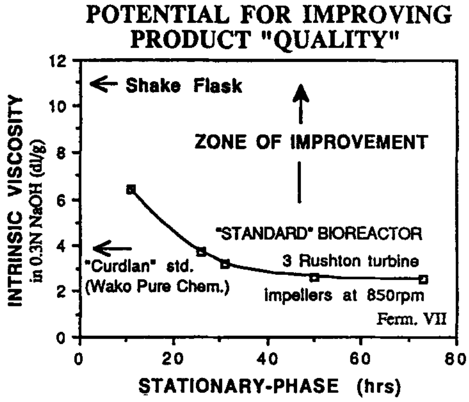

Fig. 7. The potential for improving product quality. Intrinsic viscosity of product as a function of elapsed fermentation time for a typical batch fermentation conducted in a "standard" bioreactor with radial impellers. The values typically obtained from shake flask cultures over the entire time course of the fermentation are shown as well as the value for the curdlan "standard" material from Wako Pure Chem. Ind. Ltd. (Japan).

Table 2
Effect of Bioreactor Design With Respect To Aeration and Agitation on Product Quality and Specific Rate of Exopolymer Production
Experiments with radial-flow impellers and combinations of radial and axial-flow impellers

FERMENTOR DESIGN and CONFIGURATION	OPERATIONAL PARAMETERS								Quantity	Quality
	Aeration					Agitation				
Expt. No.	Liquid Vol. (L)	Baffles	D_i/T	Air Flow Rate V/V/M	K_{la} 1/hr	Speed RPM	ND_i cm/s	N/D_i 1/cm.s	SPR mg/g/hr	Visc. (48hr) dl/g
Alcaligenes faecalis ATCC 31749										
RADIAL-FLOW "Rushton turbines"										
Air sparged through holes at bottom of central shaft										
1. NBS F2000 3 turbine imps. (4.9cm)	1.5	+	0.45	0.33	339	850	69.4	2.89	98	3.5
"	1.5	+	0.45	0.33		600	49.0	2.04	110	3.7
as in Expt. 1	1.5	+	0.45	1.00	200	500	40.8	1.70	107	2.9
+ sintered-glass gas sparger(s)	1.5	+	0.45	1.00	160	400	32.7	1.36	52	3.0
COMBINATIONS - RADIAL + AXIAL-FLOW IMPELLERS										
Air sparged through holes at bottom of central shaft										
2. NBS F2000 1 prop (4.6 cm) top + 1 imp (4.9 cm)	1.5	+	0.45	0.33	136	700	57.2	2.38	54	4.3
Air sparged through microporous SS sparger under turbine impeller										
3. NBS BioFlo II Marine prop. (7 cm) top Flat-blade turbine (8.25cm) over sparger	4.0	+	0.50	0.38		500	68.7	1.01	45	-

The NBS MultiGen F2000 glass fermentor is a magnetically-coupled bottom drive unit. The modified F2000 (round bottom) was fitted with a variable speed, top-drive motor. The NBS BioFlo II is a top drive unit (different configurations are illustrated Materials & Methods - Figs. 1 & 2). SPR = specific production rate (q_p); K_{la} = vol. oxygen transfer coefficient; Visc. = intrinsic viscosity of isolated exopolymer at 48hrs stationary-phase as measured in 0.3N NaOH.
The "shear flow intensity" is proportional to ND_i whereas N/D_i represents the ratio of "impeller turbulent shear stress" to "impeller pumping power".

Table 3
Effect of Bioreactor Design With Respect To Aeration and Agitation on Product Quality and Specific Rate of Exopolymer Production
Experiments with different types of axial-flow impellers

FERMENTOR DESIGN and CONFIGURATION	OPERATIONAL PARAMETERS								Quantity	Quality
	Aeration					Agitation				
	Liquid Vol. (L)	Baffles	D_i/T	Air Flow Rate	K_{la}	Speed	ND_i	N/D_i	SPR	Visc. (48hr)
Expt. No.				V/V/M	1/hr	RPM	cm/s	1/cm.s	mg/g/hr	dl/g
AXIAL-FLOW IMPELLER SYSTEMS										
Alcaligenes faecalis ATCC 31749										
Air sparged through sintered glass disk (3 cm) under propeller										
4. Modified NBS F2000	1.5	-	0.64	0.08	47	500	58.3	1.19	53	5.8
Round bottom tank with top drive and	1.5	-	0.64	0.15	93	500	58.3	1.19	56	6.3
with single marine propeller (7cm)	1.5	-	0.64	0.33	220	500	58.3	1.19	95	9.0
	1.5	-	0.64	0.33	271	600	70.0	1.43	108	7.3
Alcaligenes faecalis ATCC 21680										
4a. (as in Expt. 4)	1.5	-	0.64	0.33	220	500	58.3	1.19	102	7.7
Agrobacterium radiobacter ATCC 21679										
4b. (as in Expt. 4)	1.5	-	0.64	0.33	220	500	58.3	1.19	89	8.0
Alcaligenes faecalis ATCC 31749										
Air sparged through sintered glass disk (3 cm) under propeller										
5. Mod. F2000 with Helical prop (5.5 cm)	1.5	-	0.50	0.33	288	500	45.8	1.52	90	9.7
Air through ring sparger under bottom propeller										
6. B.Braun (model E) draught tube; 3x 8.9 cm var.-angle props.(45°)	10	d/t	0.45	0.35	51	500	74.1	0.94	41	7.9
7. B. Braun baffled by tube heat exchanger	10	h/e	0.45	1.00	174	500	74.1	0.94	81	5.8

The B. Braun (Biostat E) is a bottom-drive unit; d/t = draught tube and h/e = tubular heat exchanger (further details are given in Legend to Table 2).

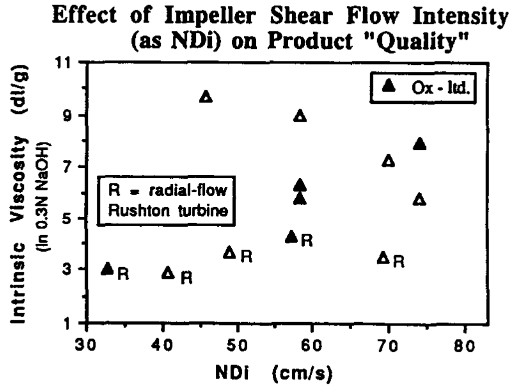

Fig. 8. Effect of impeller shear flow intensity (ND_i) on product quality. Closed triangles are data from fermentations where the q_p was less than maximal. Data taken from Table 2 and 3.

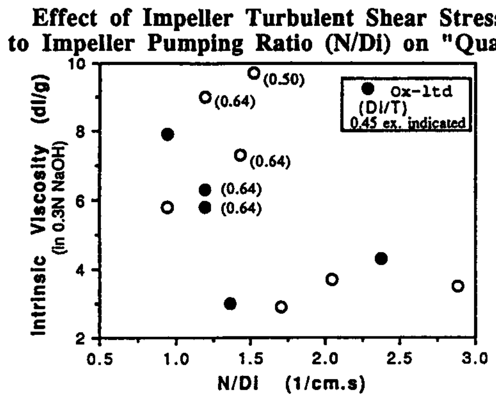

Fig. 9. Effect of impeller shear-to-flow ratio (N/D_i) on product quality. Closed circles are data from fermentations where the q_p was less than maximal. Numbers in the brackets refer to the ratio of impeller diameter to tank diameter, which is 0.45 unless otherwise indicated. Data taken from Tables 2 and 3.

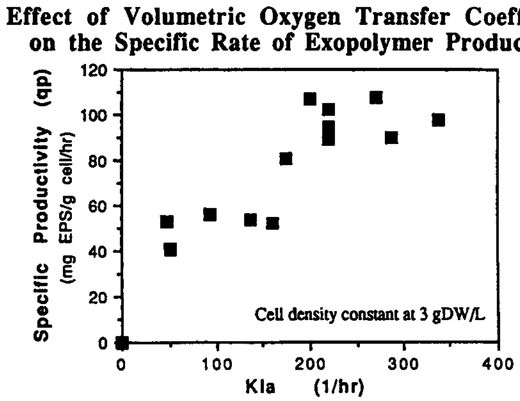

Fig. 10. Relationship between K_La and the specific rate of EPS production. In all cases, the cell density was the same (3 g DW/L). Data taken from Tables 2 and 3.

Chemical Industries Ltd. The decrease in the intrinsic viscosity that we observed for curdlan fermentations is similar to the decline in broth viscosity observed by others for pullulan (*23,24*) and xanthan (*25*). The unexpected observation that the product of shake flask cultures was superior and stable over the entire 4 d of the fermentation prompted us to explore engineering strategies for the prevention of polymer degradation through relatively simple design modifications with respect to agitation to effect a reduction in shear and an improvement in mixing (circulation flow).

Largely because most commercially available bioreactors come equipped with Rushton turbines, it was around this design that most of our initial research was performed. Moraine and Rogovin (*26*) had suggested that agitation was an important operational parameter in xanthan fermenta-

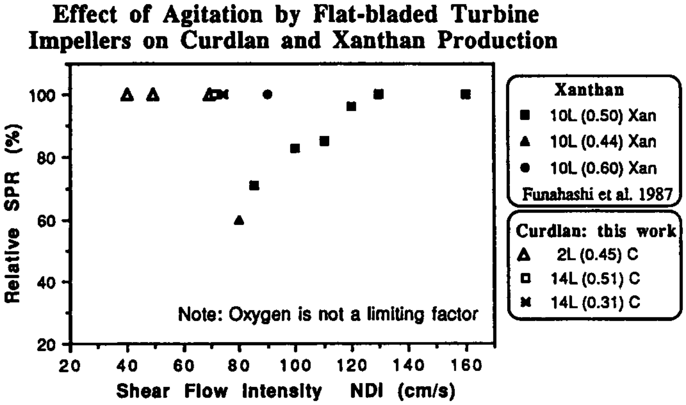

Fig. 11. Effect of agitation by flat-bladed turbine impellers on curdlan and xanthan production. Data for xanthan fermentations were taken from Funahashi et al. 1987 (*20*). In none of these fermentations was oxygen considered to be limiting the rate of EPS production. For xanthan, the K_La was >60/h, and for curdlan, it was >200/h. Numbers in the brackets refer to the ratio of impeller diameter to tank diameter. For curdlan fermentations, either an NBS F2000 or MF114 was used. In all cases, the impellers were Rushton turbines.

tions and speculated that the shear flow caused by impeller rotation removed the xanthan gum layer around the cells, thereby facilitating mass transfer. Earlier we had observed a correlation between impeller tip velocity and q_P for curdlan production, but no account of O_2 transfer rate was made at that time (*27,28*). The results of a quantitative analysis of the effect of Rushton turbines on q_P for xanthan conducted by Funahashi et al. (*20,21*) suggested that the shear flow caused by these radial-flow impellers was an essential factor not only for oxygen transfer, but also "other actions." However, under conditions where oxygen availability was not limiting q_P, our observations on the effect of impeller shear flow intensity with curdlan was quite different from xanthan (Fig. 11), thereby emphasizing the importance of this type of shear intensive mixing in highly viscous systems as in the case of the soluble exopolymers, such as xanthan, pullulan, or gellan.

Good viscosifiers are characterized by their ability to alter drastically the flow properties of the solvent when added in small amounts, but "curdlan" is not water soluble, and in comparing this fermentation to other well-established EPS fermentations, it is worth noting that the β-1,3-glucan exopolymers (at comparable concentrations of about 20 g/L) do not yield an unmanageably viscous fermentation broth (about 310 cps after 4 d [*4*] compared to about 11,000 cps for pullulan [*29*] and 40,000 cps for xanthan [*30*]). The high viscosity generated by relatively low product concentrations represents the singular major constraint to increased productivity in xanthan and pullulan fermentations by virtue of the high power input required for adequate mixing and mass transfer.

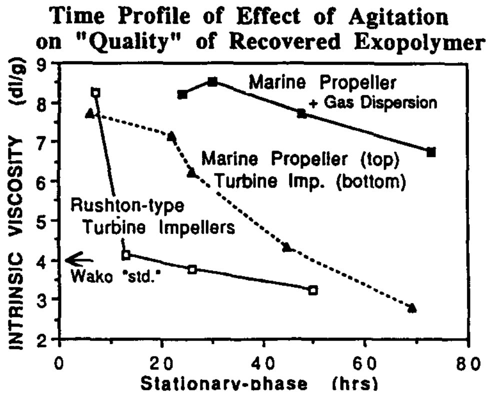

Fig. 12. The beneficial effect on product quality produced by axial-flow impellers—a time profile of the effect of agitation. Experimental configurations with respect to agitation and aeration are illustrated in Fig. 1 and 2 and Tables 2 and 3.

The effect of shear produced by radial-flow impellers was consistently detrimental to product quality as determined by intrinsic viscosity of the isolated curdlan (Table 2 and Fig. 8). Even when used in combination with a propeller to assist circulation flow, the marginal initial improvement in quality was not maintained throughout the entire fermentation (Fig. 12). Reducing the rotational speed from 850 to 400 rpm appeared to have no effect other than to compromise productivity (Table 2). By contrast, designs incorporating axial-flow impellers (Table 3), for the most part, yielded product whose quality was superior to that of the Wako standard and rivaled that produced in the low shear environment of the shake flask (Figs. 8 and 12).

The results obtained in this investigation clearly showed that alterations in reactor design with respect to shear and flow, specifically brought about by the replacement of the radial-flow impellers with axial-flow impellers, effected the intended improvement in the fermentation process (Fig. 12). The impeller pumping capacity was postulated by Steel and Maxon (*31*) to be an important parameter in nonNewtonian fermentation broths where viscosity was a constraint to mass transfer. Wang and Fewkes (*15*) suggested that, for nonNewtonian broths, "in order to minimize the transport resistance of nutrient within the mycelial mat or clump, the operating conditions and geometric design of the impeller should attempt to maximize the shear-to-flow ratio" (as N/D_i). They observed an exponential increase in the apparent critical dissolved oxygen concentration for a nonNewtonian novobiocin fermentation when the shear-to-flow ratio was <1.0 (*15*). In this study, we examined the effect of the shear-to-flow ratio on product quality. The results of our limited survey are equivocal, but suggest that a ratio in excess of about 1.5 might be damaging to the exopolymer (Fig. 9). These obesrvations are unfortunately complicated by the superimposed effect of inadequate mass transfer of oxygen under certain conditions, as determined by the less than maximal q_p.

Initial shake flask experiments suggested that productivity could be compromised by inadequate supply of oxygen. However, the apparent shear sensitive nature of the curdlan fermentation rules out the use of Rushton turbines, at least during the stationary phase (after cell proliferation ceases because of the depletion of assimilatory nitrogen) when the culture becomes shear sensitive. By contrast, growth appears indifferent to the shear intensive turbulence created by radial-flow impellers, and we have found no adverse effect in using this type of impeller design to meet the high oxygen demand of high cell density cultures (9 g DW/L—Lawford and Rousseau, unpublished observations).

From theoretical considerations with respect to oxygen demand associated with growth, maintenance, metabolism, and polymer biosynthesis (*32*), it was originally assumed that even 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 (*27*). That is to say that the DOT_{crit} of the stationary-phase (curdlan-producing) culture should, in theory, be easily accommodated by a system characterized by a much lower K_La than exhibited by the "standard" bioreactor design with aeration-efficient Rushton turbines. In this study, we examined the oxygen transfer capacity of each mixing design, and when the K_La was plotted as a function of the q_p (Fig. 10), it revealed the requirement for an unusually high volumetric oxygen transfer coefficient. By contrast, the specific rate of xanthan production is not limited with respect to oxygen availability in systems with a $K_La > 60$ h^{-1} (*20*). Since the oxygen demand of the stationary-phase culture is not high (*32*), it can be concluded that the insoluble β-1,3-glucan exopolymer surrounding the cells (as revealed by electromicroscopy) forms a resistance barrier to oxygen transfer from the liquid to the cell. Consequently to achieve maximal rates of EPS biosynthesis, there is a demand for a high DOT to provide the necessary driving force to overcome the diffusive limitation imposed by the exopolymer. Therefore, unlike the case of the soluble EPS, where there is a constraint with respect to mass transfer of oxygen from gas to liquid, in the case of curdlan, the resistance to mass transfer is from the liquid to the cell. It seems reasonable to suggest that any action made by the mixing device to effect a decrease in the thickness of the oxygen diffusion barrier created by the exopolymer would facilitate mass transfer and improve yield and productivity.

It also seems possible that the shear sensitivity of the EPS-producing culture derives from the association between insoluble polymer fibres and the bacterial cell (as revealed by electronmicroscopy [*28*]). Although the manner in which the exopolymeric fibres and the bacterial cell are associated is not known, it has been speculated that high shear would exacerbate cell rupture. Experiments of a preliminary nature indicate that culture viability (as determined by viable cell counts) remains higher in the low shear reactor (*19*). To what extent cell lysis might also lead to the release of β-glucanase activity into the fermentation broth is not known. Although an "endo-glucanase" activity has been implicated (*19*), the mechanism for the decrease in intrinsic viscosity remains problematic at this time. Although our studies have been restricted to curdlan-type biopolymers, similar observations are now being reported by others working with soluble exopolysaccharides, and we feel that our observations and conclusions regarding important control parameters might be of universal application in the production of commercial microbial exopolysaccharides.

ACKNOWLEDGMENT

This research was supported by a research contract to the University of Toronto from George Weston Ltd. with matching funds provided by a URIF Grant to HGL through the Ontario Ministry of Colleges & Universities. The collaboration and assistance of Dr. Don Potts, Dr. Jared Fein, and Anne Kligerman at the Weston Research Centre is gratefully acknowledged. The authors are also indebted to Hans Preusting, Bill Orts, Karen Hill, and Elizabeth Prosen for technical assistance and Steve Lawford for the computer graphics in Fig. 1.

REFERENCES

1. Harada, T., Misaki, A., and Saito, H. (1968), *Arch. Biochem. Biophys.* **124,** 292–298.
2. Harada, T. (1972), *Fermentation Technology Today.* G. Terui, ed., Proc. IVth Intl. Ferm. Symp., Kyoto, Japan, pp. 603–607.
3. Harada, T. (1977), *Extracellular Microbial Polysaccharides.* Sanford, P. A. and Laskin, A., eds., ACS Series 45, Washington, DC, pp. 265–283.
4. Phillips, K. R. and Lawford, H. G. (1982), *Progress in Industrial Microbiology,* vol. 18, D. E. Bushell, ed., Elsevier Scientific. Publ. Co., Amsterdam, pp. 201–229.
5. Phillips, K. R. and Lawford, H. G. (1983), *Can. J. Microbiol.* **29,** 1270–1276.
6. Lawford, H. G. and Rousseau, J. D. (1989), *Biotechnol. Letts.* **11,** 125–130.
7. Lawford, H. G., Fein, J. E., and Kligerman, A. (1988), *Biotechnology Research Applications,* Gavora, J., Gerson, D. F., Storer, A., and Woodley, J. H., eds., Elsevier Appl. Sciences, Essex, UK, pp. 110–119.
8. Lawford, H. G. (1988), *ACS Polymer Preprints* **29,** 633–634.
9. Lawford, H. G. (1982), US Patent 4,355,106 assigned to George Weston, Ltd.
10. Lawford, H. G., Phillips, K. R., and Lawford, G. R. (1982), *Biotechnol. Letts.* **4,** 689–694.
11. Kimura, H., Sato, S., Nakagawa, T., Nakatani, H., Matsukura, A., Suzuki, T., Asai, M., Kanamaru, T., Shibata, M., and Yamatodani, S. (1973), US Pat 3,754,925 assigned to Takeda Chemical Ind. Ltd. (Japan).

12. Kilp, T., Houvenaghel-Defoort, B., Panning, W., and Guillet, J. E. (1976), *Rev. Sci. Instrum.* **47(12),** 1496–1502.
13. Cooper, C. M., Fernstrom, G. A., and Miller, S. A. (1944), *Ind. Eng. Chem.* **36,** 504–509.
14. Oldshue, J. (1983), *Fluid Mixing Technology,* Chemical Engineering McGraw-Hill Pub. Co., New York.
15. Wang, D. I. C. and Fewkes, R. C. J. (1977), *Lilly Centenial Fermentation Symp.,* pp. 39–56.
16. Railton Phillips, K. (1982), M. S. Thesis, Microbiology, University of Toronto, Ontario, Canada.
17. Lawford, H. G., Fein, J. E., and Kligerman, A. (1989), Eur. Patent Appl'n., pub. no. 0-322-393.
18. Ogawa, K., Watanabe, T., Tsurugi, J., and Ono, S. (1972), *Carbohy. Res.* **23,** 399–405.
19. Orts, W. J. (1987), M. S. Thesis, Biochemistry, University of Toronto, Ontario, Canada.
20. Funahashi, H., Maehara, M., Taguchi, H., and Yoshida, T. (1987), *J. Chem. Eng. Japan* **20,** 16–22.
21. Funahashi, H., Hirai, K-I., Yoshida, T., and Taguchi, H. (1988), *J. Ferment. Technol.* **66,** 355–364.
22. Saito, H., Misaki, A., and Harada, T. (1968), *Agr. Biol. Chem.* **32,** 1261.
23. Catley, B. J. (1970), *Biotechnol. Letts.* **3,** 190.
24. Rho, D., Derdouri, A., Choplin, L., and LeDuy, A. (1987), Proc. 37th Ann. Can. Conf. Eng., 16.
25. Kulicke, W. M. (1985), Abstracts 190th ACS Nat. Meeting, September (Chicago).
26. Moraine, R. A. and Rogovin, P. (1973), *Biotechnol. Bioeng.* **15,** 225.
27. Lawford, H. G., Keenan, J. E., Phillips, K. R., and Orts, W. J. (1986), *Biotechnol. Letts.* **8,** 145–150.
28. Lawford, H. G., Phillips, K. R., Keenan, J. E., and Orts, W. J. (1986), *Proceedings BioExpo '86,* Cahners Publishing, Boston, pp. 305–320.
29. LeDuy, A., Marsan, A., and Coupal, B. (1974), *Biotechnol. Bioeng.* **16,** 61–67.
30. Charles, M. (1976), *Adv. in Biochem. Eng.* **8,** 1–63.
31. Steel, R. and Maxon, W. D. (1962), *Biotechnol. Bioeng.* **4,** 231–240.
32. Phillips, K. R., Pik, J. R., Lawford, H. G., Lavers, B. H., Kligerman, A., and Lawford, G. R. (1983), *Can. J. Microbiol.* **29,** 1331–1338.