Diffusion and reaction in microbial aggregates

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DIFFUSION AND REACTION IN MICROBIAL AGGREGATES

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Direct experimental measurements of mass flux are used to determine diffusivities of glucose, oxygen, KNO_3 and K_2SO_4 in microbial aggregates. Values allow prediction of mass transfer resistance in biological reactors.

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INTRODUCTION

Theoretical work in the biochemical engineering field has been directed toward attempts to model the substrate removal process in fluidized and fixed-film microbial systems in terms of the basic rates involved. The models all consider a succession of rate processes, including gas sorption into a liquid phase, molecular or turbulent diffusion across a liquid boundary layer, molecular diffusion through floc material to the cells themselves, conversion of the substrate to reaction products, and the movement of the products out of the system. Various authors have made different assumptions about the relative magnitude of these rates to simplify the problem by allowing one or more of these steps to be neglected.

Much of the research has been devoted to demonstrating that the rate-limiting step in the substrate removal process is a mass transfer step. Since any mechanistic model incorporating mass transfer through microbial aggregates must be sensitive to variations in the diffusion coefficient, it is important to understand how differences between organisms or growth conditions can affect the diffusion coefficient.

A direct method was developed to measure diffusion coefficients in biological floc and experiments were designed to detect the effects of growth conditions and organism species on

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EXPERIMENTAL APPARATUS

The apparatus used to measure the diffusion coefficients of oxygen, glucose, and potassium nitrate through biological floc was a modified plastic Millipore filtering unit consisting of two well-mixed chambers separated by a membrane-template assembly containing the biological material. One chamber contained a high concentration of substrate; the other chamber a low concentration. The substrate diffused through the floc into the chamber of low concentration. Measurements of substrate concentration were taken periodically in this chamber to monitor changes with time.

The brass template was 13.9 cm^2 in diameter, 391 microns thick, containing 132 holes, each 0.208 cm in diameter. The biological floc was placed into these holes and a membrane was placed on each side of the template to insure immobilization of the floc. The membranes used for the glucose and potassium nitrate experiments were 0.45 micron pore size, 150 microns thick, type HA Millipore membrane filters, all from the same lot to insure uniformity. For the oxygen diffusivity experiments, Silastic silicone, medical grade, membranes, 76 microns thick were used. These membranes had a considerably lower resistance to oxygen diffusion than the Millipore filters. However, glucose and potassium nitrate diffused very slowly through the Silastic membranes. To place the membrane-template assembly in the apparatus, a brass gasket was fitted to each side of the template outside the membranes. This was to prevent wrinkling or movement of the membranes when the upper chamber was being screwed on tightly to the lower chamber. A teflon gasket was placed on top of the brass gasket prior to placement on the apparatus to make a water seal to prevent leakage from the top chamber.

The Millipore filtering unit was modified by the removal of the filter support, which then presented an effective cross-sectional area for the membrane-filter assembly of 13.85 cm^2. The bottom section of the apparatus, with a total capacity of 370 ml, was the high concentration reservoir for glucose and potassium nitrate and was kept well-mixed with a magnetic stirring bar. The top reservoir, with a capacity of 250 ml, was filled with 200 ml deionized water at the beginning of the run and was well mixed by an electric driven paddle mounted overhead.

For the oxygen diffusivity experiment, the Millipore

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apparatus was further modified by the insertion of a dissolved oxygen probe in the lower chamber. The lower chamber was filled with deionized water containing dissolved oxygen (DO) in the range of 8-9 mg/l. The upper chamber was filled with water with DO in the range of 30-40 mg/l. A diffuser was mounted inside the upper chamber, and connected to a pure oxygen source. During the course of a run, pure oxygen was diffused into the upper chamber at a rate of 100 ml/min to maintain the high DO concentration and to provide mixing. Oxygen diffused from the upper chamber through the membrane-template assembly into the lower chamber where the DO level was monitored by an oxygen probe.

RESULTS AND DISCUSSION

Results indicating the diffusion coefficients of glucose and oxygen in microbial floc are presented in Tables I and II.

The theory of diffusion in liquids (1) predicts a ratio of 1.30 for $D^{30^\circ C}$ to $D^{20^\circ C}$. Observed values varied from 1.27 to 1.38 indicating that molecular diffusion was being measured and not convective flow or reaction rate.

Measurements indicate no significant change in diffusion coefficient for glucose with increasing concentration up to 1000 mg/l.

Any change that affects the composition or ratio of extracellular material to viable cells in the biological reactor will affect measurement of molecular diffusion coefficients through the material. Two variables that can influence the characteristics of the floc are carbon to nitrogen ratio (C/N), substrate type and solids retention time (in solids recycle system, particularly).

Diffusion coefficients through biological solids grown on high C/N can be expected to vary due to higher proportions of extracellular polysaccharide produced under such conditions. Results indicate statistically significant differences in D_{GB} with changing C/N although the quantitative effects differ considerably. Microphotographs indicate that C/N has a much greater effect on D_{GB} when the biomass consists of aggregations of single cells into floc. If filamentous organisms are prominent, the effect is reduced. The reaction rates at low C/N ratios were three to eight times larger than those at high C/N ratios. At high C/N ratios the microorganisms have only a limited ability to form new cells because nitrogen, a required growth factor, is limited. At low

Table II. Diffusion Coefficients of Glucose and Oxygen Through Microbial Floc as a Function of Carbon to Nitrogen Ratio During Growth, Solids Retention Time at 20°C. Replicable within ±12% for Glucose and ±14% for Oxygen (4).

Substrate	C/N	SRT	Diffusion Coefficient ($\times 10^5$) D_{GB}	D_{OB}	Glucose Reaction Rate* K_G
Glucose	5	1 days	0.11 cm^2/sec	0.96 cm^2/sec	7.7
	20		0.15	1.64	2.6
	50		0.14	1.95	1.5
	5	5	0.16	0.41	3.2
	20		0.18	1.65	1.5
	50		0.21	1.05	0.4
	5	20	0.17	0.50	1.9
	20		0.06	0.77	0.8
	50		0.21	0.73	0.2
Measured					
D_{GW} and D_{OW} at 20°C			0.63	2.25	
Reported					
D_{GW} and D_{OW} at 20°C (3)			0.60	2.10 ±20%	

* mg glucose/mg biomass/day

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NOTATION

C/N	carbon to nitrogen ratio (by weight)
D	diffusion coefficient (cm^2/sec)
D_{GB}	diffusion coefficient of glucose in microbial aggregates (cm^2/sec)
D_{GW}	diffusion coefficient of glucose in water (cm^2/sec)
D_{OB}	diffusion coefficient of oxygen in microbial aggregates (cm^2/sec)
D_{OW}	diffusion coefficient of oxygen in water (cm^2/sec)
K	zero-order reaction rate constant (mg/liter/day)
K_G	zero-order reaction rate constant for glucose with microbial aggregates (mg/liter/day).
L	thickness of a microbial film on a flat surface (microns)
RF	radius of a spherical microbial aggregate (microns)
SRT	solids retention time (days).

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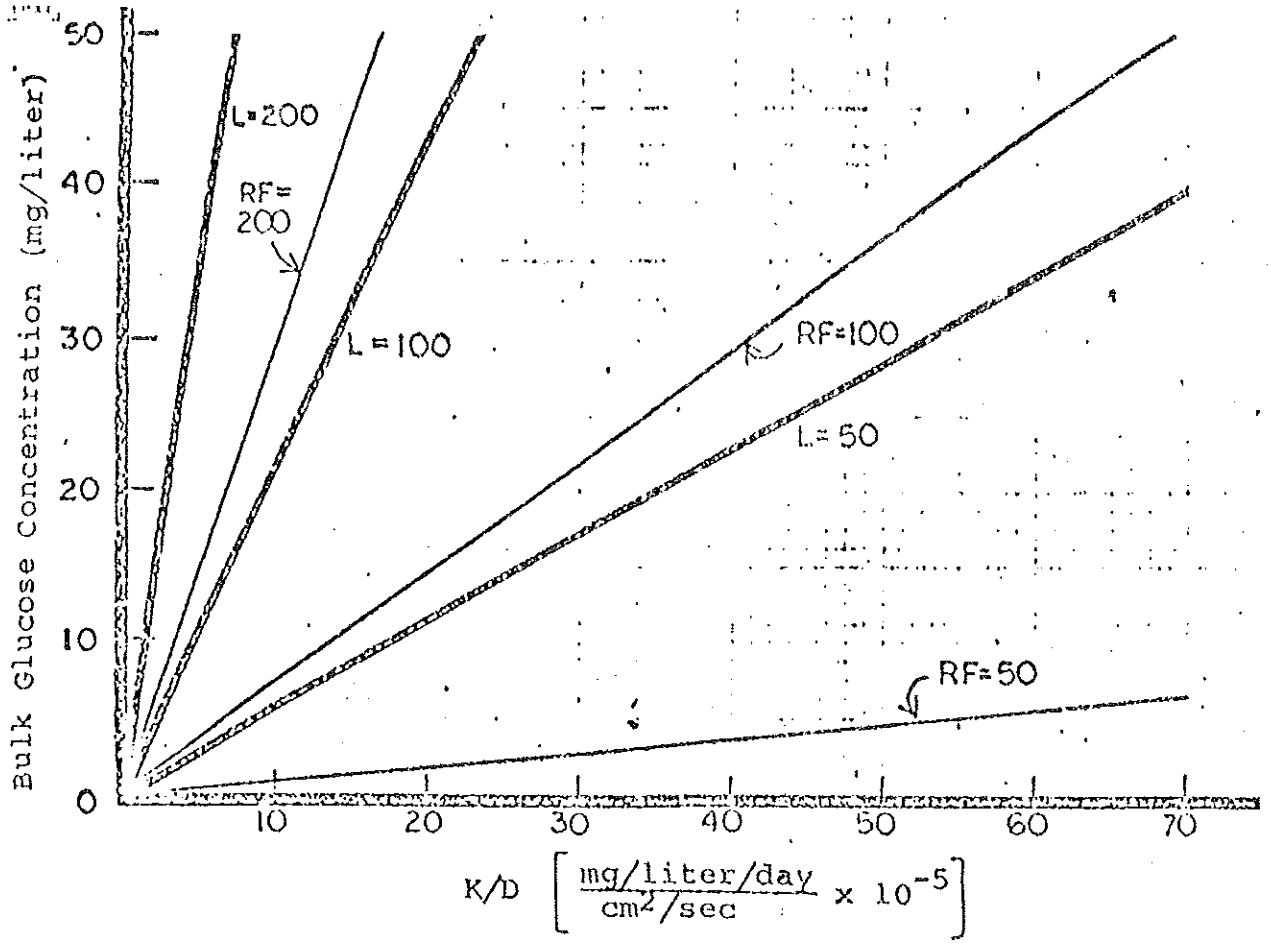

Figure 1. Relationship between Bulk Glucose Concentration, Microbial Aggregate Size (Thickness for a Fixed-Film or Radius for Fluidized Particle), and K/D (Reaction Rate Coefficient/Diffusion Coefficient) Necessary to Maintain Maximum Reaction Rate.

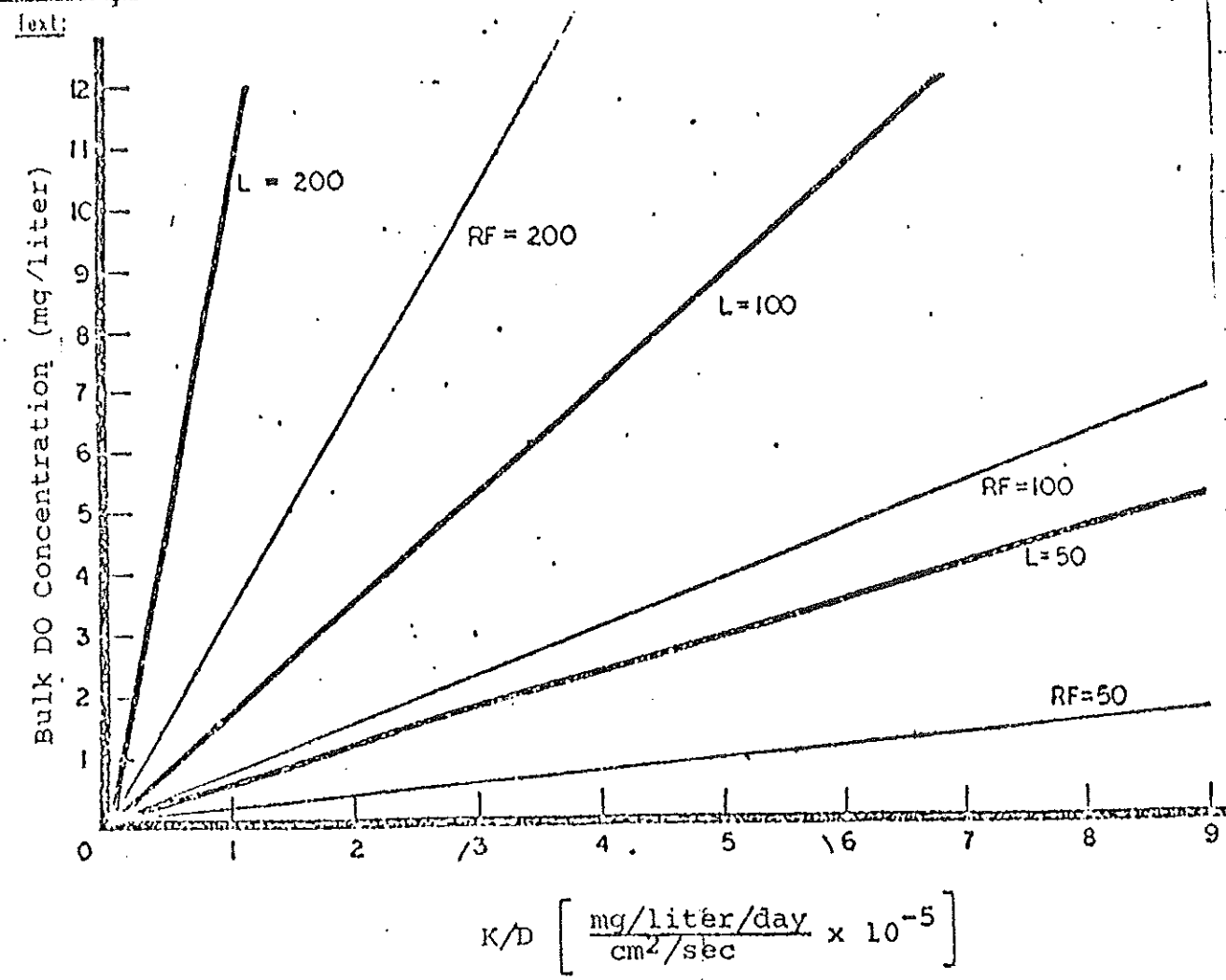

Figure 2. Relationship between Dissolved Oxygen Concentration, Microbial Aggregate Size, and K/D Necessary to Maintain Aerobic Conditions throughout the Microbial Aggregate.