Kinetics of assimilation of mixed substrates by heterogeneous bacteria

MAN M. VARMA, MALCOLM C. WEEKES, AND ALLEN F. CALVERT

THE GOAL OF THE ENVIRONMENTAL PROTECTION AGENCY, essentially set by PL 92-500, is that the discharge of pollutants into navigable waters be eliminated by 1985.[1] In reality, the goal of zero discharge of pollutants may not be attainable, quantitatively and qualitatively, unless the wastewaters are accorded a higher degree of treatment and unless a search for new and innovative treatment methods is made and the basic kinetics of treatment are understood.

The basic mathematical equations for the first- and second-order reactions are

$$\pm\frac{dy}{dt} = k_1\phi(y) \qquad (1)$$

$$\pm\frac{dy}{dt} = k_2\phi(y)^2 \qquad (2)$$

in which y = concentration of the substrate added or removed; t = time of exposure; and k_1 and k_2 = reaction rate (proportionality) coefficients. These equations describe the progressive use of organic matter by heterogeneous bacteria.

Both k_1 and k_2 are proportionality coefficients describing the relationship between 5-day biochemical oxygen demand (BOD) and the ultimate BOD. Not only are the units of k different, but, depending on the equation used, the ultimate BOD will also vary. Stoichiometrically, however, the ultimate BOD is fixed. The accurate evaluation of k and the order of reaction is essential for the design of treatment units, because the ultimate BOD is dependent on them. Reaction kinetics have traditionally been based on oxygen uptake. Studies on metabolism have been made by Varma and Reid [2] and Varma and Nepal.[3] The former study used an indirect method, and the latter used only glucose as a substrate. Wastewater is a conglomerate substrate composed of carbohydrates and proteinaceous matter. Sawyer [4] established that the carbon:nitrogen (C:N) value in the substrate affects the k value.

The objective of this study was to evaluate the kinetics of assimilation of mixed soluble (proteinaceous and carbonaceous) substrates by heterogeneous bacteria in the log growth phase at various C:N and food:microorganism (F:M) ratios. The substrates used were glucose and glutamic acid, glucose $^{14}C(U)$ and glutamic acid $^{14}C(U)$ being used for metabolic studies.

METHODOLOGY

The investigation consisted of growing the heterogeneous microorganisms under controlled conditions and harvesting them at the inflection point for kinetic studies. Respiration and metabolic studies were conducted under parallel conditions. Details of the assay were reported earlier.[3]

RESPIRATION AND METABOLIC STUDIES

A known weight of dry cells harvested at the inflection point was suspended in the nutrient water so that 1 ml of the cell suspension contained 5 mg of dry cell. For respirometric studies, 1 ml of known concentrations of plain glucose and glutamic acid was used.* This provided varying C:N values.

Parallel metabolic studies were conducted by using a metabolic shaker,† except that in this portion of the experiment,

* Gilson Respirometer, Gilson Medical Electronics, Inc., Middleton, Wis.

† Dubnoff Metabolic Shaker, Curtain Scientific, Rockville, N. Y.

tagged glucose $^{14}C(U)$ and glutamic acid $^{14}C(U)$ were used. The CO_2 produced was absorbed on 0.2-ml phenethylamine. The shaking speed of the respirometer and metabolic shaker was kept constant at 120 oscillations/min at all times.

Three metabolic flasks were withdrawn every hour, and 1 ml of 0.1 *M* H_2SO_4 was injected into the reaction mixture in one of these flasks, which was allowed to stay on the metabolic shaker for an additional 45 min in order to release any entrapped CO_3. The hanging well was then removed from this flask and transferred to a glass vial containing 15 ml of the cocktail solution.[3] The $^{14}CO_2$ was counted in a liquid scintillation counter.‡

The reaction mixture from the other two flasks was centrifuged. Each metabolic flask was rinsed with a known volume of water, and this was also transferred to the respective centrifuge tube. After being centrifuged for 15 min, the supernatant was decanted into a labeled beaker, and the cells at the bottom of the centrifuge tube were resuspended in 5 ml of water. Ten-ml portions of supernatant, as well as resuspended cells, were transferred to glass vials,

‡ Packard, Dowers Grove, Ill.

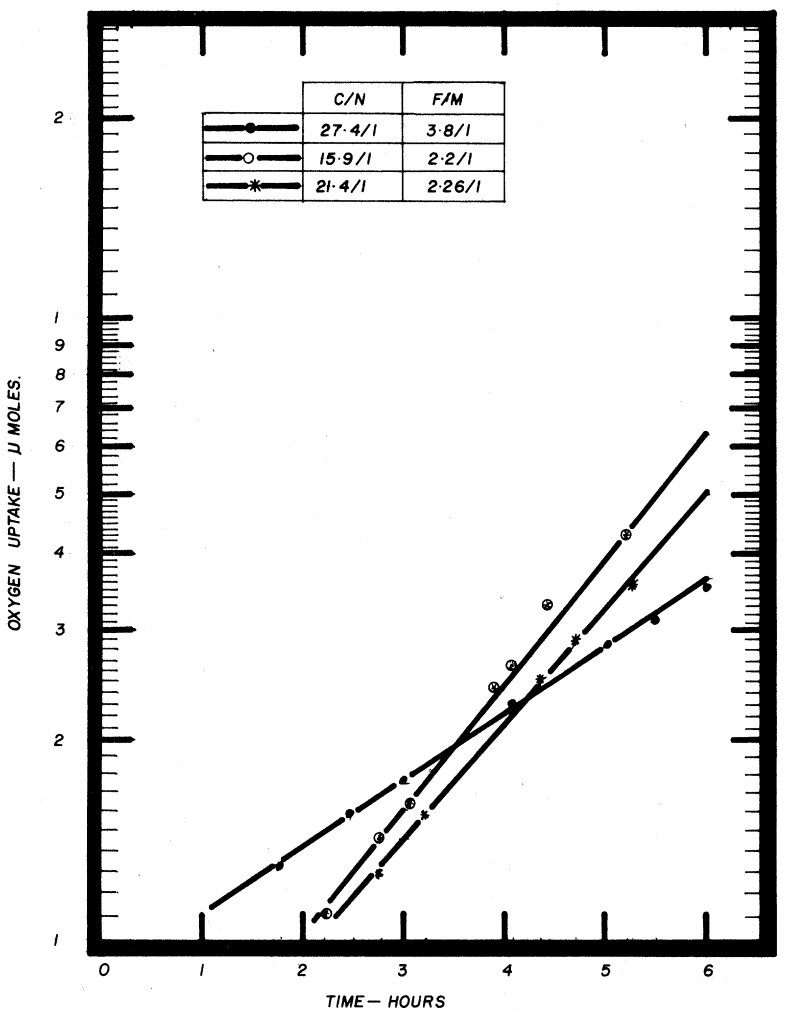

FIGURE 1.—Oxygen uptake at various C:N and F:M values.

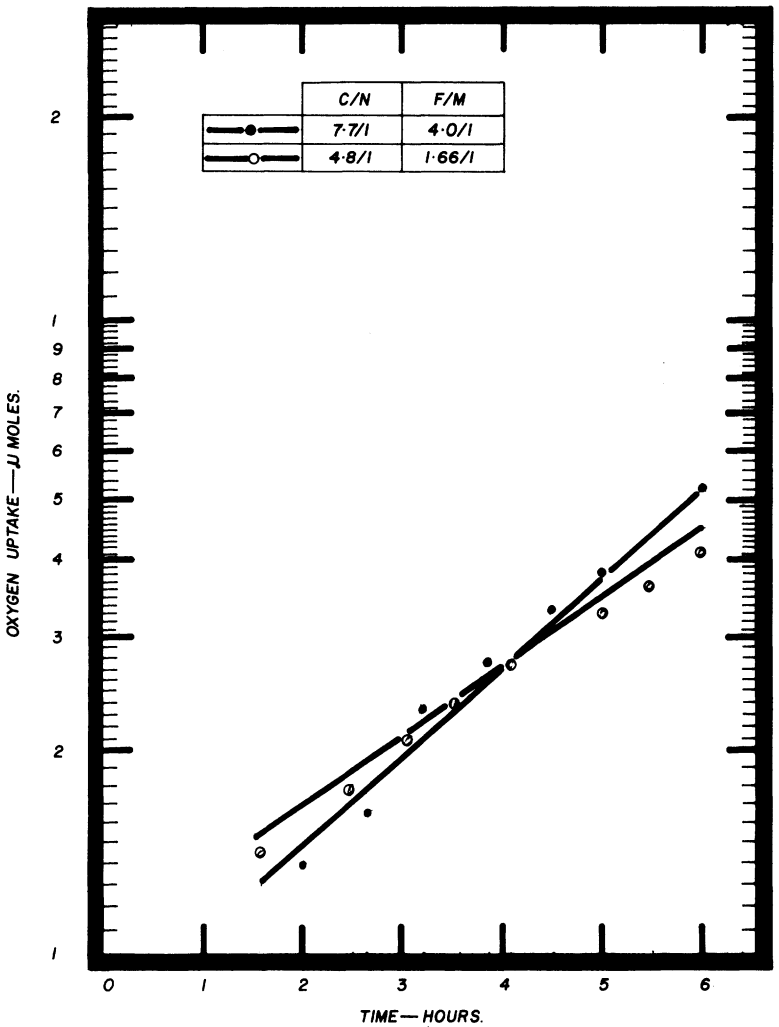

FIGURE 2.—Oxygen uptake at various C:N and F:M values.

each containing 15 ml of the cocktail solution previously described.[3] Cell counts and the concentration of the substrate in the supernatant were measured on the liquid scintillation counter by counting ^{14}C. All of the studies were conducted at a temperature of 25°C.

DISCUSSION

Respiration studies were performed at three different C:N and F:M ratios. At the beginning of the test, the pH in these experiments was 7.2, and at the end it was 6.4. (Figures 1 and 2). In order to eliminate the effect of pH fluctuations, these experiments were repeated with a predetermined amount of phosphate buffer added to maintain a constant pH of 7.2 (Figures 3 through 5). The k values are summarized in Table I.

Many factors control reaction kinetics, but the most important are pH, temperature, complexity and composition of the substrate, age of the microorganisms, and C:N ratio. To obtain the best treatability, these factors require control within an optimum range. In this study, all factors were kept constant except the C:N and F:M ratios.

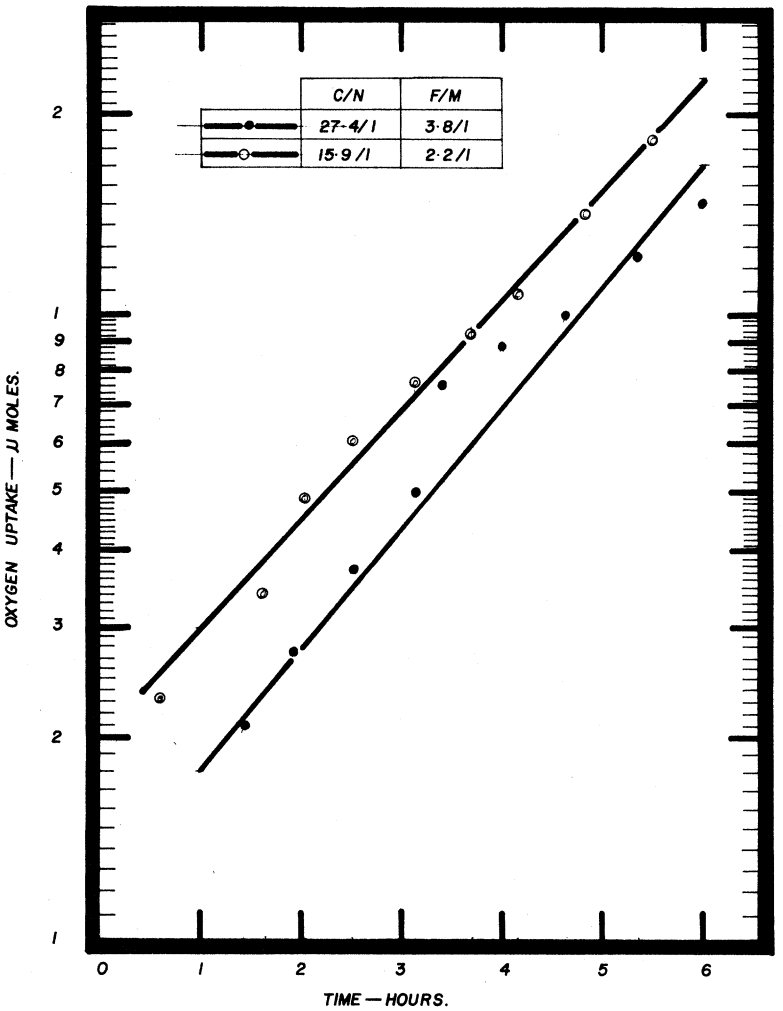

FIGURE 3.—Oxygen uptake by buffered substrate at various C:N and F:M values.

The results, in two sets of five experiments each, indicate that, at controlled pH, the k values were consistently higher than at fluctuating pH. The maximum k in either case was at a C:N of 21.4, the corresponding F:M was 2.26; however, the maximum difference in two k values occurred at a C:N of 21.4.

TABLE I.—Comparison of k-Values at Controlled and Fluctuating pH

C:N	F:M	k Values	
		Fluctuating pH	Constant pH
4.8	1.66	1.29	1.34
7.7	4.00	1.38	1.39
15.9	2.20	1.45	1.50
21.4	2.26	1.48	1.59
27.4	3.80	1.25	1.47

Figure 6 is a plot of various k values versus corresponding C:N and F:M ratios. The k value increased as C:N increased; thereafter, it reached a peak and then sharply declined. A similar pattern was observed with changes in F:M values.

At C:N of 21.4 and 15.9, F:M values were approximately the same, and the difference in k was also insignificant. Similarly, the difference in F:M at C:N of 7.7

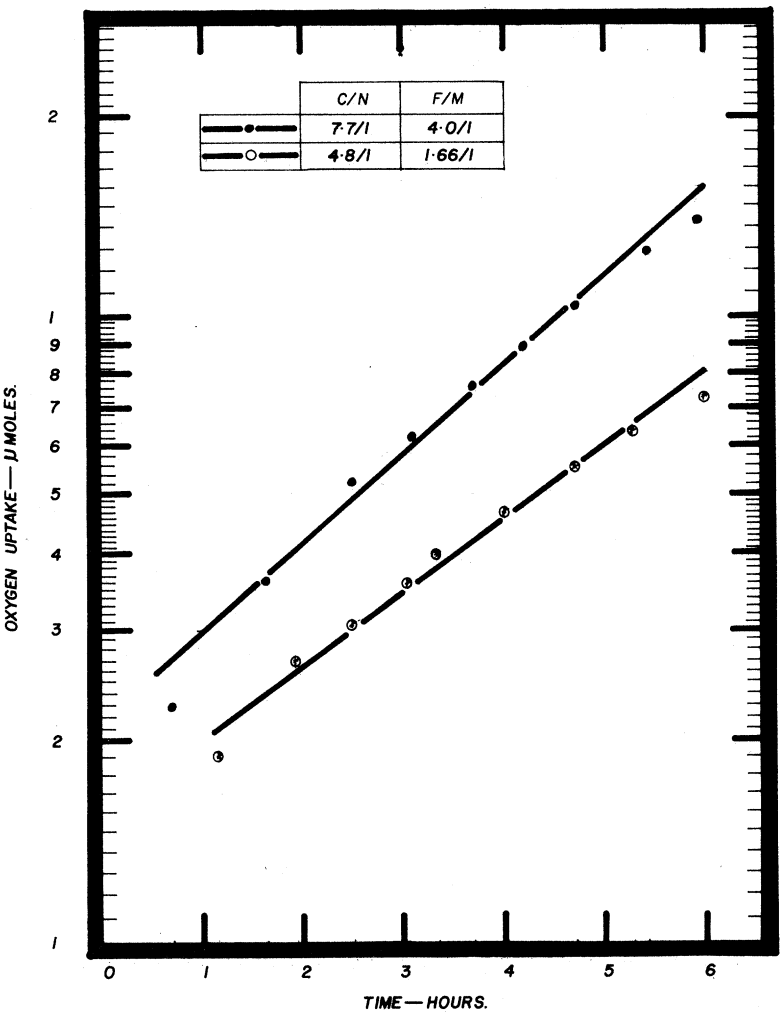

FIGURE 4.—Oxygen uptake by buffered substrate at various C:N and F:M values.

and 27.4 was very slight; however, the k value in the former was significantly higher, which indicates that best results are obtained at an optimum C:N range. At a C:N of 4.8, k was minimum, and at this point the carbonaceous substrate was also the least. The linearity of the plot may be attributed to the optimum balanced conditions prevailing in the reaction vessel.

RECOVERY OF $^{14}CO_2$

Microorganisms metabolize the substrate to produce photoplasm and liberate energy that is ultimately used to reduce the substrate to more stable end products, CO_2 and H_2O. The production of $^{14}CO_2$ corresponds to that portion of the substrate that was reduced to the end products; it does not represent the synthesized portion. The production of $^{14}CO_2$ was studied at three selected C:N values, as is shown in Figures 7 through 9. The comparative kO_2 and kCO_2 are summarized in Table II. The k was maximum at a C:N of 21.4 and minimum at an F:M of 7.7. The reaction rate was the fastest with the lowest concentration of nitrogen. The pattern of the $^{14}CO_2$ recovery is essentially similar to the oxygen uptake plot. The hourly respiratory quotient varied from 0.86 to 1.02.

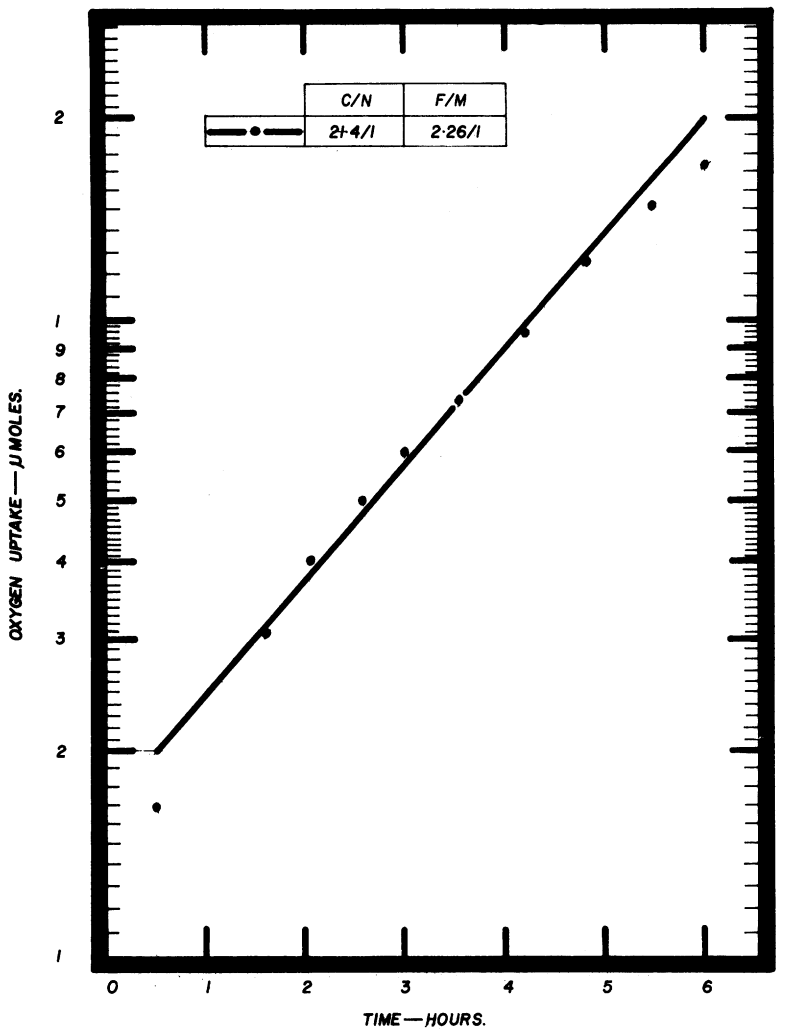

FIGURE 5.—Oxygen uptake by buffered substrate.

CELL PRODUCTION

In a reaction vessel, cells constitute a dynamic component of the system and continuously process the substrate for synthesis, thereby increasing in their population. At the same time, they destroy a portion of their structure to yield energy for normal functions. There is a definite relationship between the use of food and the increase in cellular protoplasm. The cell production, if $C_5H_7NO_2$ is assumed to be the empirical formula for cellular protoplasm, for various C:N ratios is summarized in Table III and superimposed in Figures 6 through 9. The best growth rate occurred between 2 and 5 hr, which corresponds to Rahn's findings.[5] The reaction coefficient for the cell growth varied from 1.25 to 1.47; the overall maximum rate was at a C:N value of 21.4. These results are in accordance with $^{14}CO_2$ recovery.

TABLE II.—Comparison of O_2 Uptake with $^{14}CO_2$ Production Rates

C:N	F:M	kO_2	kCO_2
7.7	4.00	1.40	1.38
15.9	2.20	1.50	1.43
21.4	2.26	1.58	1.49

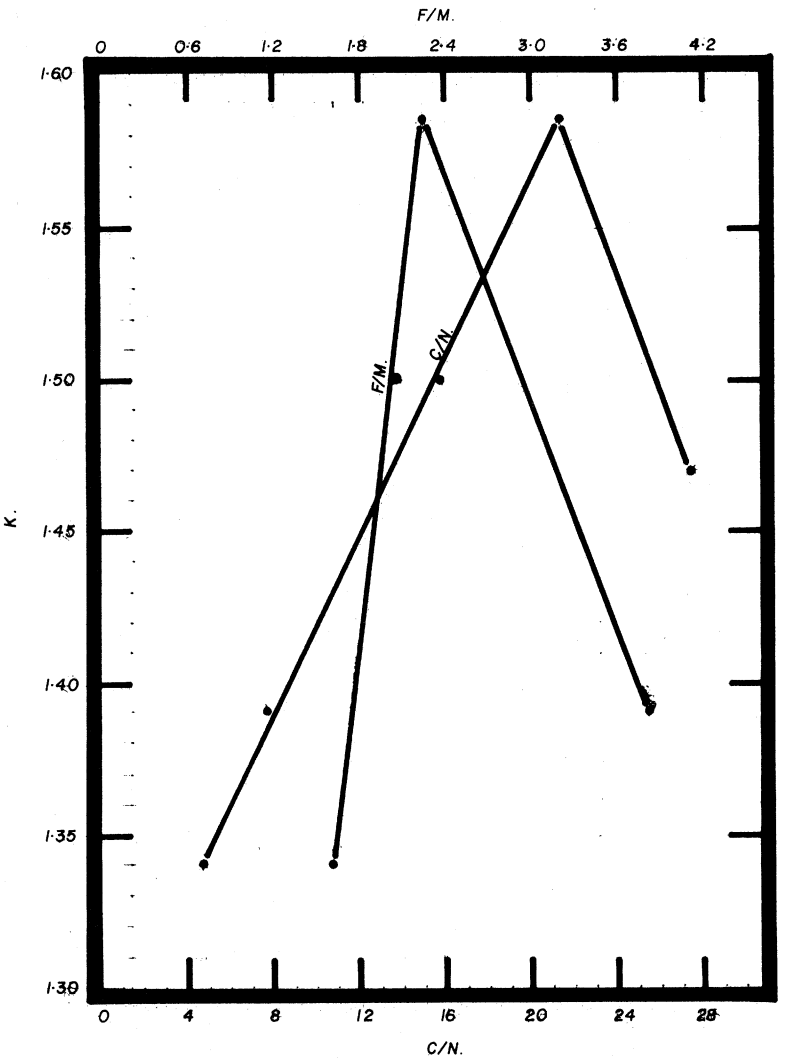

FIGURE 6.—Values of *k* at various F:M and C:N values.

The unstable oxygen-demanding portion of the substrate that is not synthesized by the microorganism is discharged into rivers and streams. This is of great concern to engineers because of the pollution danger. Measurements of unused substrate in reaction studies are essential for material balance as well as for determining the order of the reaction. In a true first order, t $0.5 = 0.693/k$.

The quantity of the used substrate was measured by separating the cells (empirical formula $C_5H_7NO_2$) and counting the radioactivity in the supernatant. Table IV shows the fraction of the substrate remaining at the end of each hour.

The percentage of metabolized substrate varied from 85 to 38.2, depending on the C:N and F:M ratios. It is interesting to note that, while the composition and complexity of the substrate remained unaltered, the maximum use occurred at an F:M of 2.26, when the C:N was 21.4. When the quantity of nitrogen was increased, the rate of metabolism decreased; further increase of nitrogen and F:M ratio did not improve

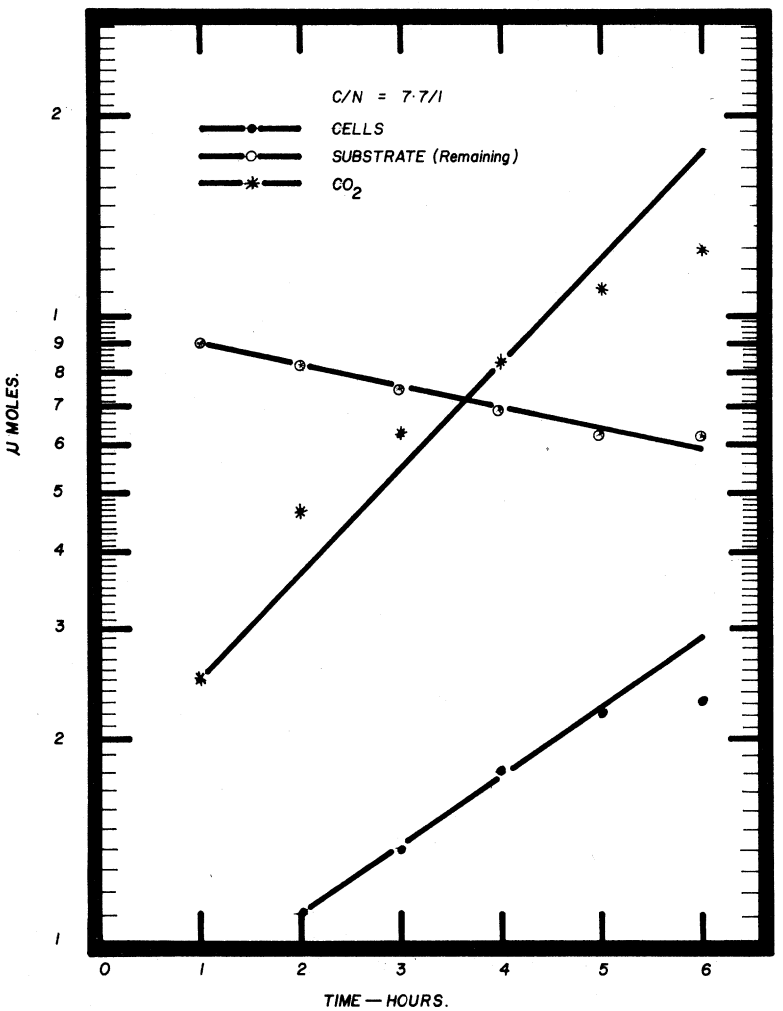

FIGURE 7.—CO_2, new cell production, and unconverted substrate.

the rate of metabolism. The F:M ratio of 2.26 and the C:N value of 21.4 seem to be the optimum conditions for better treatability results.

TABLE III.—Cell Production

Time (hr)	C:N 7.7 μ-M of Cells	C:N 15.9 μ-M of Cells	C:N 21.4 μ-M of Cells
1	6	5	5
2	11	10	10
3	14	15	15
4	18	21	22
5	22	22	24
6	23	25	28
	k = 1.25	k = 1.42	k = 1.47

REACTION KINETICS

Biochemical reactions proceed with a wide variety of speeds, and the usefulness

TABLE IV.—Percentage of Substrate Left Unconverted After Each Hour

Time (hr)	Percentage Unconverted Substrate in Vessel		
	C:N 7.7	C:N 15.9	C:N 21.4
1	90	84.4	83.7
2	82.5	69.0	66.0
3	75.5	54.0	54.7
4	69.0	35.5	33.5
5	63.0	33.5	23.0
6	61.8	31.5	15.0

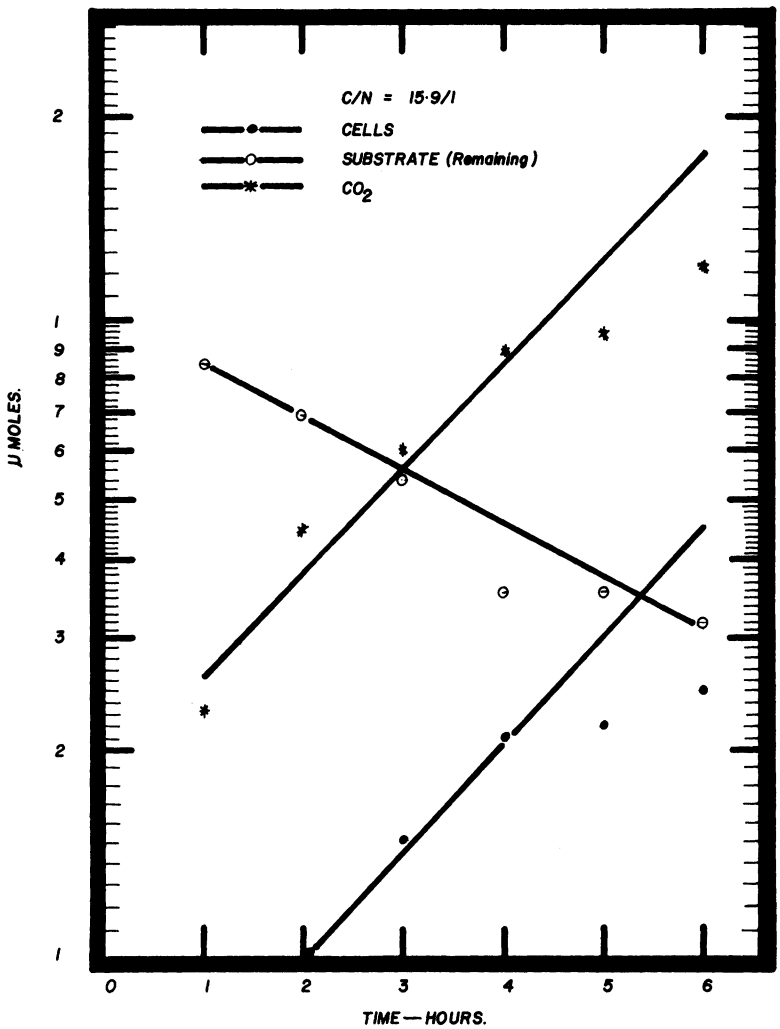

FIGURE 8.—CO_2, new cell production, and unconverted substrate.

of the specific reaction depends on how fast it takes place under the given condition. In the design of treatment plants, it is often necessary to calculate the ultimate oxygen demand based on the standard BOD test; it is therefore necessary to know the order of the reaction. Woodward,[6] in reviewing the work of Oxford and Ingram,[7] suggested that perhaps BOD data could be analyzed from the second-order equation. Young and Clark [8] fitted the BOD data that followed "a type" of second-order equation.

The data obtained in this research were analyzed for first-order as well as for second-order equations.

The test for a first-order reaction was made by plotting log $a/a - x$ versus time,[9] in which a = the initial amount of substrate and x = the amount of substrate reaction in time, t.

A straight line was obtained for all C:N values studied, which indicates that the data follow the first-order pattern. The second-order test was made by plotting $1/c$ versus time, in which $c =$ the concentration of the substrate at time, t. This plot showed that, in general, the substrate removal does not follow a second-order reaction pattern, although the C:N value of 7.7 seems to conform to the sec-

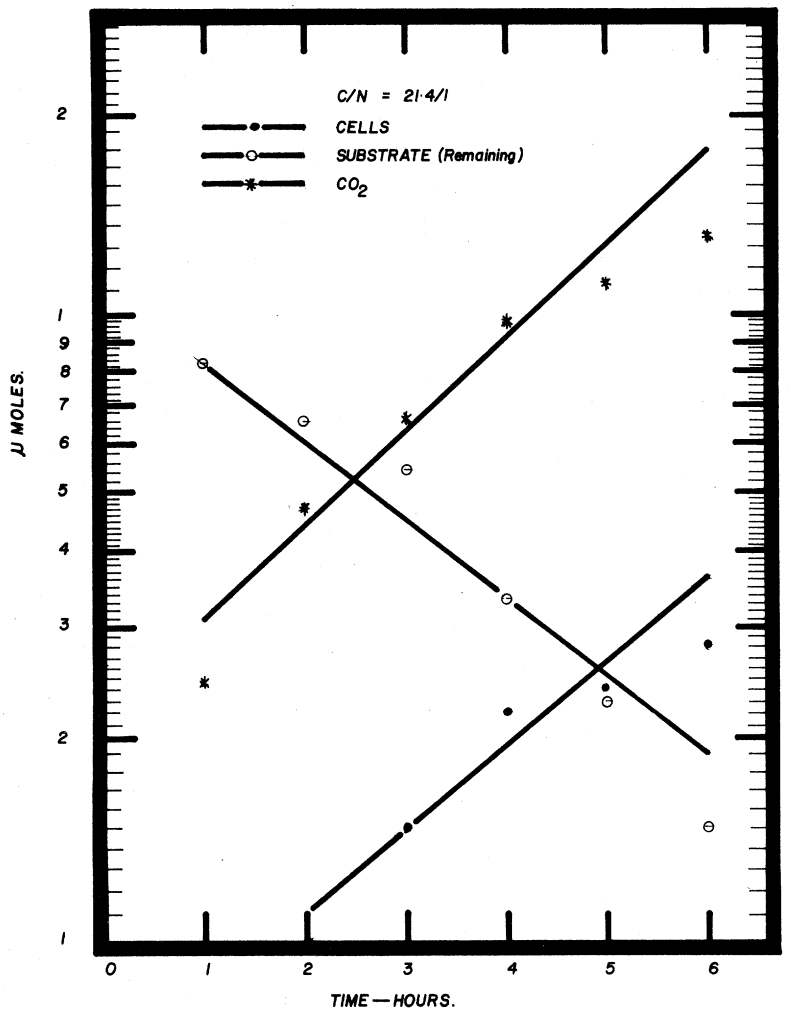

FIGURE 9.—CO_2, new cell production, and unconverted substrate.

ond-order reaction pattern as well as it does to the first-order pattern. The other two C:N values investigated showed substrate removal patterns that were not similar to the second-order reaction.

The staggered results indicate that, in BOD studies, the order of reaction is neither truly first- nor second-order, but perhaps lies somewhere between the two limits. Wastewater is a conglomerate substrate composed of varying amounts of organic carbon, nitrogen, fats, and proteins in large amounts of water. In order to confirm the validity of the reaction order, more studies must be performed under controlled conditions and using a mixed known substrate. Eventually, these studies should be repeated by using raw wastewater.

It must be pointed out at this stage that BOD determinations using the dilution method [10] presuppose first-order reactions and thus it is no wonder that the results fit in this equation.

CONCLUSIONS AND RECOMMENDATIONS

1. Maintaining a constant pH of 7.2 was more favorable to bacterial activity than having an initial pH of 7.2 and allowing this to fall as the reaction progressed.

2. In addition to the quantity of substrate (F:M), the nature of the substrate represented by the various C:N values is also important in determining the reaction rate in wastewater stabilization.

3. The respiratory quotient for all C:N values was 0.92, which indicates a similarity in the metabolic and respiratory patterns of the substrates using heterogeneous bacteria.

4. Labeled tritium could also be used to give information on cell quantity and unused substrate because the liquid scintillation counter also measures tritium. These results should be compared with ^{14}C studies.

5. Similar studies should be performed to trace the behavior pattern during the 6-hr period, staring 6 hr after the bacteria are brought in contact with the substrate, that is, ending the studies 12 hr after they begin.

6. Flora from countries with different diets, such as the Eskimo high fat diet, should be examined under the conditions used in this study. This would show the effect, if any, of fatty substrates on bacterial populations.

ACKNOWLEDGMENTS

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Authors. Man M. Varma, Malcolm C. Weekes, and Allen F. Calvert are, respectively, professor and director, Bio-Environmental Engineering and Sciences, Howard University, Washington, D. C.; environmental engineer, Catalytic, Inc., Philadelphia, Pa.; and associate professor, Medical Genetics Unit, Howard University. Weekes was formerly associated with Howard University.

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