Wat. Sci. Tech. Vol. 26, No. 3-4, pp. 743–751, 1992.
Printed in Great Britain.

0273–1223/92 $15.00

EXPLANATION OF BIOLOGICAL MEANING OF THE S_O/X_O RATIO IN BATCH CULTIVATION

P. Chudoba*, B. Capdeville* and J. Chudoba**

**Unité de Recherche Traitement Biologique des Eaux, Département de Génie des Procédés Industriels, Institut National des Sciences Appliquées, Complexe Scientifique de Rangueil, 31 077 Toulouse, France*
***Department of Water Technology and Environmental Engineering, Prague Institute of Chemical Technology, Technicka 5, 166 28 Prague 6, Czechoslovakia*

ABSTRACT

It is shown that the most important parameter in batch cultivation of mixed cultures is the ratio of the initial substrate concentration to the initial biomass concentration (S_O/X_O as COD/biomass). When the ratio is sufficiently low (below 2-4 depending on the mixed culture history) no cell multiplication takes place during the exogenous substrate removal. Under these conditions, a biomass increase is mostly due to the synthesis of storage polymers. It is also shown that the observed yield, Y_{obs}, decreases with increasing S_O/X_O ratio. Under the high S_O/X_O conditions, more energy is spent for cell multiplication, which results in greater part of substrate being oxidized. Batch cultivation at high S_O/X_O ratios results also in higher concentrations of microbial polymers produced as waste products of mixed culture microorganisms. It is concluded that for the biodegradation studies with the aim to obtain kinetic constants it is necessary to work at low S_O/X_O ratios to prevent mixed culture microorganisms from substantial multiplication. This is necessary because cell multiplication during batch cultivation of mixed culture changes the proportion among slow-growers and fast-growers. This is the only way to obtain the kinetic constants which are representative of the original mixed culture.

KEYWORDS

Batch, S_O/X_O ratio, energy level, substrate removal, biomass growth, cell multiplication, kinetic constants.

INTRODUCTION

The use of batch cultivation in biological wastewater treatment research has been described very often by numerous authors for different final objectives. This way of cultivation is frequently used for the determination of kinetic constants of activated sludge microorganisms (Peil and Gaudy, 1971; Cech et al., 1984; Gaudy et al., 1988; Grady et al., 1989; Chudoba et al., 1989). The need for data on the biodegradability of different organic compounds has led numerous other researchers to determine the biodegradation kinetics through different studies conducted under batch conditions (Gaudy et al., 1967; Gates and Marlar, 1968; Edwards and Wilke, 1968; Gaudy, 1975; Simkins and Alexander, 1984, 1985; Braha and Hafner, 1987; Tampleton and Grady, 1988; Chang, 1988).

The variations in relationship between biomass growth and substrate removal rate, and in cell yield during batch experiments have been studied by Mc Whorter and Heukelekian (1962), Fujimoto (1963), Rao and Gaudy (1966), Chudoba (1969), Gaudy and Ramanathan (1971), Varma and Nepal (1972), Ramanathan and Gaudy (1972), Gaudy and Srinivasaraghavan (1974), Seto and Alexander (1985), Chang (1988), Chudoba et al. (1991a) and Chudoba P. (1991).

It is thus obvious that a great number of various experimental data and results can be found in the literature that cannot be compared because of different operational conditions used. In order to make them suitable for a more correct interpretation, the data obtained from batch experiments have to be related to a universal parameter. According to Pitter and Chudoba (1990), such a parameter might be the ratio of the initial substrate concentration S_o to the initial biomass concentration X_o. This ratio is one of the most important parameters in batch cultivation, determining whether or not cell multiplication will take place during the exogenous substrate removal (Speece et al., 1973; Grau et al., 1975; Cech and Chudoba, 1983; Chudoba, 1985, 1989; Pitter and Chudoba, 1990; Chudoba et al., 1991a,b; Chudoba P., 1991).

It is then evident that S_o/X_o ratio can play the role of a relating parameter, useful in interpretation of different results obtained in batch experiments. Its importance has been underlined by many authors, notably by Fujimoto (1963), Downing and Knowles (1966), Rao and Gaudy (1966), Chudoba (1968, 1969, 1989, 1991), Gaudy and Ramanathan (1971), Speece et al. (1973), Gaudy and Srinivasaraghavan (1974), Cech and Chudoba (1983), Seto and Alexander (1985), Chang (1988), Gaudy et al. (1988), Pitter and Chudoba (1990) and Chudoba et al. (1991a,b).

However, there is no explanation in the literature for the particular role of the S_o/X_o ratio from the energetic point of view. In other words, what is the real biological significance of this ratio? What is the exact role played by it, with respect to the energy distribution and/or the initial energy level? Even if some authors accepted the importance of this ratio for the correct interpretation of batch experimental data (Pitter and Chudoba, 1990), some questions stay always unanswered.

Consequently, the main goal of this paper is to give some supplementary explanation of the real biological meaning of S_o/X_o ratio, as far as the initial energy level in batch experiments and its consequent distribution are concerned.

BASIC ASSUMPTIONS AND THEORETICAL DEVELOPMENT

During a batch experiment, an initial substrate concentration (S_o) is put into contact with an initial quantity of microbial culture (X_o), called also inoculum. The parameter S_o represents thus a carbon and energy source for biosynthesis, while X_o represents a source of carbon and energy consumption. After being put into contact with the substrate, microorganisms of the mixed culture start to remove the substrate, which is demonstrated by changes in substrate removal and biomass growth curves or lines.

From the physiological point of view, it is necessary to distinguish microbial growth and cell multiplication phases (Speece et al., 1973; Daigger and Grady, 1982; Pitter and Chudoba, 1990; Chudoba et al., 1991a,b) and storage and growth responses.

According to Pitter and Chudoba (1990), microbial growth reflects an increase in biomass with (multiplication) or without (storage or accumulation) the increase in cell number. In other words, microbial growth means the transport of extracellular substrates into the cell, oxidation of a portion to obtain energy, and the use of another portion to synthesize all components of the biomass in the proper portion (Daigger and Grady, 1982). On the other hand, storage response involves only the transport of substrates, the oxidation of a smaller portion, and the synthesis of the storage polymers like carbohydrate or lipid.

The main difference between these two responses is obviously the amount of energy produced by catabolic pathways and required by different metabolic processes. Consequently, the nature of the new biomass synthesized must be different for both storage and cell multiplication processes. At the same time, the quantity of substrate oxidized per unit of biomass synthesized, and thus the ratio between catabolism and anabolism, varies in relation with S_o/X_o ratio. It is obvious that the quantity of substrate oxidized per unit of biomass synthesized will be greater for a growth response (higher S_o/X_o) than for storage response (Rao and Gaudy, 1966; Daigger and Grady, 1982). Thus, the role of S_o/X_o ratio, as well as its importance for batch cultivation is underlined.

Storage or accumulation processes have been observed most usually during batch cultivation when microbial growth is limited by low amount of utilizable carbon and energy source, with respect to the inoculum

concentration. Thus, at low S_o/X_o ratio, a relatively high amount of biomass is supplied with a low quantity of substrate. The initial energy level is then low as well, and the increase in cell mass reflects only the increase in molecular polymer content in the biomass. Consequently, weight changes do not necessarily reflect similar changes in cell number. Due to the fact that the cell replication is a process energetically enough demanding, no, or only negligible cell multiplication takes place at low S_o/X_o ratios during exogenous substrate removal (Speece et al., 1973; Pitter and Chudoba, 1990; Chudoba et al., 1991a,b; Chudoba, 1991).

At higher S_o/X_o ratio, a relatively low amount of biomass is supplied with a higher quantity of substrate. The initial energy level is higher, and thus sufficient for different synthetic reactions which take place during cell replication cycle, like enzyme, protein and nucleic acids syntheses. Consequently, the number of microorganisms increases during the exogenous substrate removal, which is indicated by increasing rates of substrate removal and biomass growth. It is obvious that the portion of the substrate consumed for energy production by catabolic processes will be higher as well, because of higher energetic demand of cell multiplication and maintenance processes. This is summarized in Table 1 with data from Stouthamer (1973), indicating adenosine triphosphate (ATP) requirement for the formation of microbial cells from glucose, ammonia and salts.

TABLE 1. ATP Requirement for the Formation of Microbial Cells from Glucose.

Macromolecule	Content (%)	ATP required moles x 10(-4)/g cells
Polysaccharide	16.6	20.52
Protein	52.4	191.40
Lipid	9.4	1.40
RNA	15.7	23.00
DNA	3.2	5.76

The macromolecular composition of microbial cell material is taken from a study of Morowitz (1968). It can be seen that the largest amount of energy is required for the formation of proteins, and especially for the polymerization of the amino acids to build proteins as molecules - carriers of information.

The threshold between low and high values of S_o/X_o ratio is not defined strictly, and depends on various factors. Nevertheless, according to Speece et al. (1973), Pitter and Chudoba (1990) and Chudoba et al. (1991a,b), an interval between 2 and 4 may be considered as a correct estimation.

APPLICATION OF PRESENT THEORY

Basic kinetic response of a batch experiment is the substrate removal and biomass growth curves. From time to time, depending on the experimental system used, the oxygen consumption and/or carbon dioxide production curves can be obtained (Gaudy et al., 1988; Grady et al., 1989; Chudoba et al., 1991a,b). The interpretation of different shapes of these curves depends strictly on the S_o/X_o ratio, applied in the system. Also the character of substrate used (single-component or multicomponent) will influence the above curves.

Single-component substrate

Examples are given in Figs. 1 and 2 (Chudoba, 1969). The mixed culture used for batch experiments was cultivated semicontinuously (fill-and-draw) on a mixture of glucose (0.667 g $l^{-1}d^{-1}$) and peptone (1.333 g $l^{-1}d^{-1}$). Technological parameters of the cultivation were as follows : volume 6 litres, aeration period 22 h, sedimentation period 2 h, volume of supernatant removed daily 4 litres, mean biomass retention time 3 days.

In spite of the fact that a fully acclimated culture was used, apparent lag phases of about 1 day were detected at the highest initial S_o/X_o ratio. This is the time required for multiplication of an initially small population of the microorganisms involved to values causing a detectable decrease of a given substance. In these cases, the apparent lag phases were also affected to a certain extent by insufficient CO_2 concentrations since the cultivation proceeded in a mineral medium (Pitter and Chudoba, 1990).

It can be also seen from both figures that when the S_o/X_o ratio was below 3, both substrates were removed linearly, indicating no cell multiplication. At the S_o/X_o= 8 with glucose and S_o/X_o= 8.5 with alanine, the synchronized division took place as indicated by a break point on COD removal lines. The synchronized division in mixed cultures is discussed in detail elsewhere (Chudoba et al., 1991b).

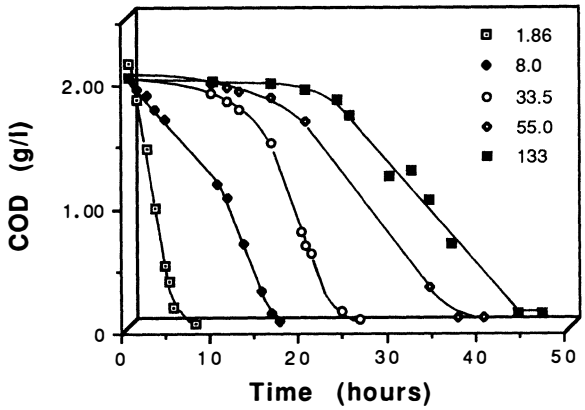

Fig. 1. Courses of glucose removal (as COD) by mixed culture at different initial S_o/X_o ratios. History of the mixed culture is given in the text (Chudoba, 1969).

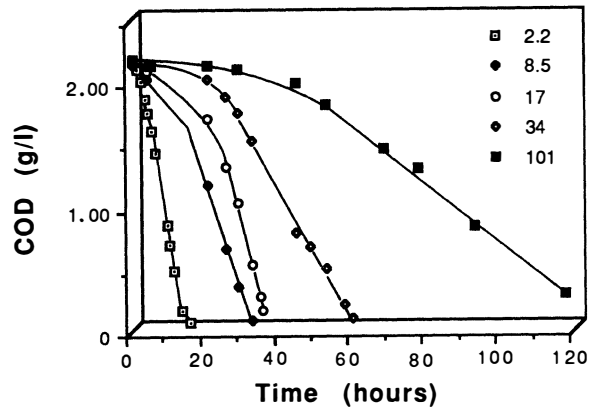

Fig. 2. Courses of alanine removal (as COD) by mixed culture at different initial S_o/X_o ratios. History of the mixed culture is given in the text (Chudoba, 1969).

Figure 3 shows the dependence of Y_{obs} on the S_o/X_o ratio. The lower values of Y_{obs} observed at higher S_o/X_o ratios are due to the fact that more energy is required for cell multiplication in proliferating systems. Similar conclusions were drawn by Chudoba et al. (1991a).

Multicomponent substrate

Figure 4 presents substrate removal and biomass growth curves, obtained in a batch system with mixed culture (activated sludge) fed on a multicomponent substrate (Chudoba, 1991), at low S_o/X_o ratio. The mixed culture used for batch experiments originated from a laboratory continuous-flow unit with technological parameters of the cultivation as follows : volume of the aeration tank 5 litres, hydraulic retention time 4 h, mean biomass retention time 5 days, recirculation ratio 0.8. The system was fed on a mixture of peptone, sodium acetate, methanol, saccharose and yeast extract.

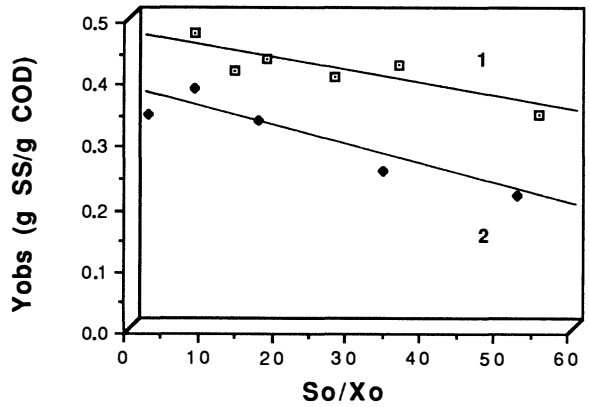

Fig. 3. Relationship between Y_{obs} and S_o/X_o ratio. 1- glucose, Y_{obs}= 0.47-0.00216 S_o/X_o; 2-alanine, Y_{obs}= 0.38-0.00325 S_o/X_o. History of the mixed culture is given in the text (Chudoba, 1969).

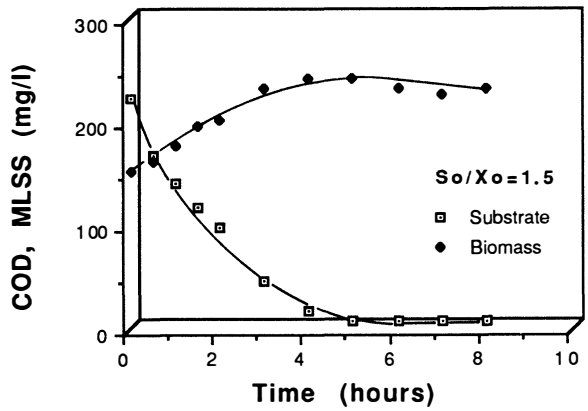

Fig. 4. Substrate removal and biomass growth in a system without cell multiplication at low S_o/X_o ratio. History of the mixed culture is given in the text (Chudoba, 1991).

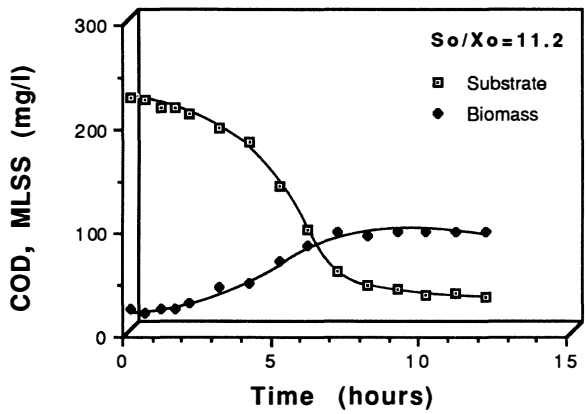

Fig. 5. Substrate removal and biomass growth in a system with cell multiplication at high S_o/X_o ratio. History of the mixed culture is given in the text (Chudoba, 1991).

Both curves show decreasing rates, typical for a system without cell multiplication during exogenous substrate removal. On the other hand, if the inoculum is small (S_o/X_o high), the growth and substrate consumption curves have sigmoidal shapes (Figure 5). The rates of biomass growth and substrate removal increase in the initial stage of the experiment and after reaching the maximum, decrease. The example shown in Figure 5 represents a multicomponent substrate removal by a mixed culture of microorganisms at high S_o/X_o ratio.

The increase in cell number (multiplication) is indicated by an acceleration of both biomass growth and substrate removal rates (Speece et al., 1973). The multiplication may be synchronized (Chudoba et al., 1991b), depending on history of the mixed culture used as inoculum in a batch experiment. In this case, the acceleration of both substrate removal and biomass growth rates is not consecutive, but occurs instantaneously, in a relatively short interval. This is indicated by a break point on both curves (Figs. 1 and 2).

The variation of substrate removal and biomass growth curves with S_o/X_o ratio has been observed already by numerous authors, namely by Fujimoto (1963), Downing and Knowles (1966), Chudoba (1969), Schaezler et al. (1971), Speece et al. (1973), Simkins and Alexander (1984), Seto and Alexander (1985), Pitter and Chudoba (1990), Chudoba et al. (1991a,b) and Chudoba (1991). However, the above mentioned theory concerning initial energy level in connection with S_o/X_o ratio gives the real theoretical bases for correct estimation of processes being able to prevail in a batch system during exogenous substrate removal.

Microbial polymers formation

Now it is a well known fact that the production of soluble microbial polymers (SMP) is a common phenomenon whose intensity depends on cultivation conditions (Pitter and Chudoba, 1990). The polymers are formed both during the growth and during the starvation and degradation of microorganisms. Their amounts are proportional to the substrate degraded and, in the batch culture, to the S_o/X_o ratio (Chudoba, 1969). This is demonstrated in Fig. 6, in which the residual soluble COD (S_R) as a percentage of the initial COD (S_0) is plotted against the S_o/X_o ratio. A more detailed discussion of this phenomenon is published elsewhere (Pitter and Chudoba, 1990).

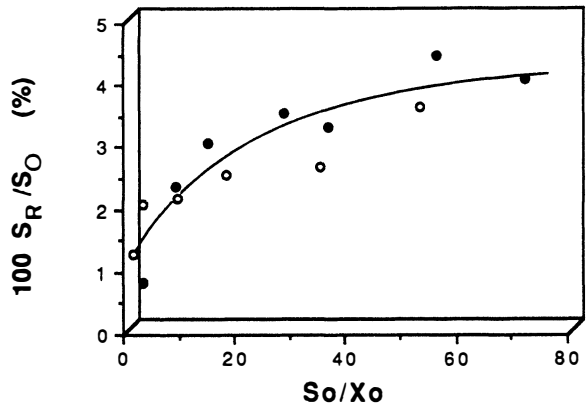

Fig. 6. Relationship between 100 S_R/S_0 and the S_o/X_o ratio obtained in batch systems with cell multiplication (see Figs. 1 and 2). Full circles - glucose, empty circles - alanine.

DISCUSSION

The basic question is whether batch experiments with mixed cultures should be carried out at low or high S_o/X_o ratios. Some authors (Gaudy et al., 1988; Tampleton and Grady, 1988; Grady et al., 1989) hold the view that they should be carried out in the proliferating systems with high S_o/X_o ratio, in which manifold multiplication of original cells takes place. The answer to this question will depend on for what reasons the batch experiments are carried out. If they are carried out exclusively for scientific reasons, the high

S_o/X_o ratios can be kept. If the results of batch experiments should be used in practice, the low S_o/X_o ratios should be kept. In domestic wastewater treatment practice the following actual S/X ratios are in different continuous systems :

Completely-mixed systems

S_o= 500 mg COD l^{-1} , X = 2000 mg l^{-1} , S_e= 20 mg COD l^{-1}.
As in these systems the actual concentration of available substrate is approximately the same as that in the effluent (S_e), the actual S/X ratio in the aeration tank is 20/2000 = 0.01.

Systems with plug flow pattern (e.g. compartmentalized)

S_o= 500 mg COD l^{-1} , X = 2000 mg l^{-1} , S_e= 20 mg COD l^{-1} , R = 1.
The available substrate concentration (S_m) in mixed liquor at the inlet part of the aeration tank is :

$$S_m = \frac{S_o + RS_2}{1 + R} = \frac{500 + 1x20}{1 + 1} = 260 \text{ mg COD } l^{-1}$$

The S_m/X ratio is then 260/2000 = 0.13.

This is the reality in wastewater treatment practice. Consequently, we ask at what S_o/X_o ratio should batch experiments with mixed cultures be carried out? In our opinion, they should be carried out at low S_o/X_o ratios without substantial cell multiplication. The kinetic constants obtained only under these conditions can be safely used for the activated sludge plant design and operation.

Table 2 shows the differences between kinetic constants obtained in batch systems cultivated under low and high S_o/X_o ratios.

TABLE 2. Growth Constants Obtained for Different Substrates and Mixed Cultures Cultivated Batchwise at High (Peil and Gaudy, 1971) and Low (Chudoba et al., 1985) So/Xo Ratios.

Substrate	High S_o/X_o		Low S_o/X_o	
	μ_{max}, h^{-1}	K_s, mg l^{-1}	μ_{max},(3) h^{-1}	K_s, mg l^{-1}
Glucose (1)	0.49	29	0.140	20.8
Glucose (2)	0.38	11	0.034	2.0
Acetic acid (1)	0.36	41	0.032	2.2
Acetic acid (2)	0.29	47	0.017	0.7
Glutamic acid (1)	0.78	47	0.035	3.0
Glutamic acid (2)	0.59	95	0.020	0.9
Alanine (1)	0.33	27	0.039	3.2
Alanine (2)	0.18	15	0.010	0.8

(1) Peil and Gaudy (1971): Dispersed growth of sewage microorganisms cultivated semicontinuously (enrichment technique) at Θ_x= 1.1 d. Chudoba et al. (1985): Flocculated mixed culture cultivated continuously in a selector system at Θ_x= 4.3 d.

(2) Peil and Gaudy (1971): Dispersed growth of sewage microorganisms cultivated semicontinuously (enrichment technique) at Θ_x= 1.1 d (Data from a later period). Chudoba et al. (1985): Filamentous mixed culture cultivated continuously in a completely mixed reactor at Θ_x = 4.3 d.

(3) For the low S_o/X_o experiments, the maximum specific growth rate, μ_{max}, was calculated according to the following relation: $\mu_{max} = Y_{obs} \cdot q_{max}$, where q_{max} is the maximum specific substrate removal rate.

Though the nonlinear curve-fitting technique enables us to calculate the kinetic constants from the data obtained under high S_o/X_o ratios, these constants cannot be reliably used for the design of the real activated sludge treatment plants.

Mixed culture of microorganisms (e.g. activated sludge) usually contains different bacterial species and predators (protozoa, rotifers, etc.) which differ in their growth constants. We can divide them approximately into two groups: slow-growers and fast-growers. Their proportion in any mixed culture is given by the technological parameters of cultivation, above all by the mean cell retention time. If we admit substantial cell multiplication during batch cultivation, we automatically change the proportion between slow-growers and fast-growers. Though some kinetic constants are obtained, they are not representative ones for the original mixed culture.

This is the main reason why the kinetic constants of mixed culture microorganisms should be obtained from the systems without substantial cell multiplication.

It should be also emphasized the primary reason for wastewater purification is to remove organic impurities and not to cultivate biomass. Consequently, the maximum specific substrate removal rate, q_{max}, should be determined rather than the maximum specific growth rate, μ_{max}. The latter loses in the mixed cultures, containing many different species and predators with different values of μ_{max}, its physical sense.

CONCLUSION

It has been shown that S_o/X_o ratio is one of the most important parameters in batch cultivation. It determines whether or not cell multiplication will take place during exogenous substrate removal. It plays then an important role in interpretation of results obtained during batch kinetic studies, notably as the initial energy level indicator. According to the value of this ratio, it is possible to estimate which processes will take place during batch experiment (accumulation of storage polymers versus cell multiplication). Thus, it has been concluded that for the biodegradation studies with the aim to obtain kinetic constants, it is necessary to work at low S_o/X_o ratios. This is the only way to obtain the biokinetic constants representative for the original mixed culture.

REFERENCES

Braha, A. and Hafner, F. (1987). Use of lab batch reactors to model biokinetics. Wat. Res., 21, (1), 73-81.

Cech, J. S. and Chudoba, J. (1983). Influence of accumulation capacity of activated sludge micro-organisms on kinetics of glucose removal. Wat. Res., 17, (6), 659-666.

Cech, J. S., Chudoba, J. and Grau, P. (1984). Determination of kinetic constants of activated sludge micro-organisms. Wat. Sci. & Tech., 17, (2/3), 259-272.

Chang, J. (1988). Etude cinétique et modelisation de la croissance d'une population mixte sur substrat complexe par couplage d'un réacteur discontinu à un spectromètre de masse. PhD.thesis, INSA Toulouse, France.

Chudoba, J. (1968). Formal discussion of : Linear substrate removal in the activated sludge process. Proc. of 4th IAWPRC Biennial Conference, Paper n° II-4, Prague, Czechoslovakia.

Chudoba, J. (1969). Residual organic matter in activated sludge process effluents V. - Effect of the initial food-to-microorganisms ratio. Sci. Pap. Inst. Chem. Technol., Prague, F-15, 23-34.

Chudoba, J. (1985). Quantitative estimation in COD units of refractory organic compounds produced by activated sludge microorganisms. Wat. Res., 19, (1), 37-43.

Chudoba, J., Cech, J. S., Farkac, J. and Grau, P. (1985). Control of activated sludge filamentous bulking V. Experimental verification of a kinetic selection theory. Wat. Res. , 19, 191-196.

Chudoba, J. (1989). Discussion of : Effect of culture history on the determination of biodegradation kinetics by batch and fed-batch techniques. J. Wat. Pollut. Control Fed., 61, (3), 367-369.

Chudoba, J., Albokova, J. and Cech, J.S. (1989). Determination of kinetic constants of activated sludge microorganisms responsible for degradation of xenobiotics. Wat. Res., 23, 1431-1438.

Chudoba, P. (1991). Etude et intérêt du découplage énergétique dans les processus d'épuration des eaux par voie biologique. Procédé OSA. PhD. thesis, INSA Toulouse, France.

Chudoba, P., Chevalier, J.J., Chang, J. and Capdeville, B. (1991a). Effect of anaerobic stabilization of activated sludge on its production under batch conditions at various S_o/X_o ratios. Wat. Sci. & Tech.,

23, (4/6), 917-926.
Chudoba, P., Chang, J. and Capdeville, B. (1991b). Synchronized division of activated sludge micro-organisms. Wat. Res., 25, (in press).
Daigger, G.T. and Grady, C.P.L. jr. (1982). The dynamics of microbial growth on soluble substrates. A unifying theory. Wat. Res., 16, 365-382.
Downing, A.L. and Knowles, G. (1966). Population dynamics in biological treatment plants. Proc. of 3rd IAWPRC Biennial Conference, Paper n° 7, Munich, Germany.
Edwards, V.H. and Wilke, C.R. (1968). Mathematical representation of batch culture data. Biotechnol. & Bioeng., 10, 205-232.
Fujimoto, Y. (1963). Kinetics of microbial growth and substrate consumption. J. Theoret. Biol., 5 171-191.
Gates, W.E. and Marlar, J.T. (1968). Graphical analysis of batch culture data using the Monod expressions. J. Wat. Pollut. Control Fed., 40, (11), R 469-R 476.
Gaudy, A.F. jr. (1975). Basic and applied kinetic concepts for design of activated sludge processes. Prog. in Wat. Tech., 7, (1), 113-125.
Gaudy, A.F. jr. and Ramanathan, M. (1971). Variability in cell yield for heterogeneous microbial populations of sewage origin grown on glucose. Biotechnol. & Bioeng., 13, 113-123.
Gaudy, A.F. jr. and Srinivasaraghavan (1974). Effect of specific growth rate on biomass yield of heterogeneous populations growing in both continuous and batch systems. Biotechnol. & Bioeng., 16, 423-427.
Gaudy, A.F. jr., Ramanathan, M. and Rao, B.S. (1967). Kinetic behavior of heterogeneous populations in completely mixed reactors. Biotechnol. & Bioeng., 9, 387-411.
Gaudy, A.F. jr., Yang, P.Y., Bustamante, R. and Gaudy, E.T. (1973). Exponential growth in systems limited by substrate concentration. Biotechnol. & Bioeng., 15, 589-596.
Gaudy, A.F. jr., Rozich, A.F., Garniewski, S., Moran, N.R. and Ekambaram, A. (1988). Methodology for utilizing respirometric data to assess biodegradation kinetics. Proc. of 42nd Ind. Waste Conf., Purdue Univ., Lafayette, Indiana, 573-584.
Grady, C.P.L. jr., Dang, J.S., Harvey, D.M., Jobbagy, A. and Wang, X.-L. (1989). Determination of biodegradation kinetics through use of electrolytic respirometry. Wat. Sci. & Tech., 21, (8/9), 957-968.
Grau, P., Dohanyos, M. and Chudoba, J. (1975). Kinetics of multicomponent substrate removal by activated sludge. Wat. Res., 9, 637-642.
McWhorter, T.R. and Heukelekian, H. (1962). Growth and endogenous phases in the oxidation of glucose. Proc. of 1st IAWPRC Biennial Conference, London, England.
Morowitz, H.J. (1968). Energy flow in biology : biological organization as a problem in thermal physics. Academic Press, New York.
Peil, K.M. and Gaudy, A.F. (1971). Kinetic constants for aerobic growth of microbial populations selected with various single compounds and with municipal wastes as substrates. Appl. Microbiol., 21, 253-256.
Pitter, P. and Chudoba, J. (1990). Biodegradability of organic substances in the aquatic environment. CRC Press, Boca Raton, USA.
Ramanathan, M. and Gaudy, A.F. jr. (1972). Sludge yields in aerobic systems. J. Wat. Pollut.Control Fed., 44, (3), 441-450.
Rao, B.S. and Gaudy, A.F. jr. (1966). Effect of sludge concentration on various aspects of biological activity in activated sludge. J. Wat. Pollut. Control Fed., 38, (5), 794-812.
Schaezler, D.J., McHarg, W.H. and Busch, A.W. (1971). Effect of the growth rate on the transient responses of batch and continuous microbial cultures. Biotechnol. & Bioeng. Symp., J. Wiley & Sons, Inc. (Ed)., N°2, 107-129.
Seto, M. and Alexander, M. (1985). Effect of bacterial density and substrate concentration on yield coefficients. Appl. & Environmental Microbiol., 50, (5), 1132-1136.
Simkins, S. and Alexander, M. (1984). Models for mineralization kinetics with the variables of substrate concentration and population density. Appl. & Environmental Microbiol., 47, (6), 1299-1306.
Simkins, S. and Alexander, M. (1985). Nonlinear estimation of the parameters of Monod kinetics that best describe mineralization of several substrate concentrations by dissimilar bacterial densities. Appl. & Environmental Microbiol., 50, (4), 816-824.
Speece, R.E., Engelbrecht, R.S. and Aukamp, D.R. (1973). Cell replication and biomass in the activated sludge process. Wat. Res., 7, 361-374.
Stouthamer, A.H. (1973). A theoretical study on the amount of ATP required for synthesis of microbial cell material. Antonie van Leeuwenhoek, 39, 545-565.
Tampleton, L.L. and Grady, C.P.L. jr. (1988). Effect of culture history on the determination of bio-degradation kinetics by batch and fed-batch techniques. J. Wat. Pollut. Control Fed., 60, (5), 651-658.
Varma, M.M. and Nepal, J.K. (1972). Kinetics of soluble substrate assimilation. J. Wat. Pollut. Control Fed., 44, (12), 2316-2324.