ured model of bacterial growth
sts with activated sludge
ne-stage and two-stage chemostat

A. Harder

Structured model of bacterial growth and tests with activated sludge in a one-stage and two-stage chemostat

Promotor : Dr. P.G. Fohr, hoogleraar in de waterzuivering aan de Landbouwhogeschool te Wageningen

Co-promotor: Ir. J.A. Roels, buitengewoon lector in de biomathematica aan de Technische Hogeschool te Delft

A. Harder

Structured model of bacterial growth and tests with activated sludge in a one-stage and two-stage chemostat

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. H.C. van der Plas,
hoogleraar in de organische scheikunde,
in het openbaar te verdedigen
op vrijdag 18 mei 1979
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen

pudoc

Centre for Agricultural Publishing and Documentation

Wageningen - 1979

Abstract

Harder, A. (1979) Structured model of bacterial growth and tests with activated sludge in a one-stage and two-stage chemostat. Agric. Res. Rep. (Versl. landbouwk. Onderz.) 886, ISBN 90 220 0702 2, (vii) + 114 p., 38 figs, 16 tables, 164 refs, Eng. and Dutch summaries. Also: Doctoral thesis, Wageningen.

A kinetic model for a growing culture of micro-organisms was developed that correlated the biochemical structure of cells with quantitative physiological behaviour. The three-compartment model was adequate for simulation of continuous, batch and transient experiments with activated sludge fed on vanillin. The model assumed three basic compartments in a cell: (1) a building-block for synthesis of energy-rich compounds, small metabolites, DNA and storage materials; (2) the protein-synthesizing machinery; (3) the pool of enzymes and other proteins. The concept of viability was incorporated by postulating irreversible conversions of the three compartments to inert fractions.

The two-stage activated-sludge process was improved and a mathematical model was developed for kinetic optimalization of the process. The two-stage process was better than the one-stage process in rate of vanillin breakdown. The kinetic coefficients of the second stage were affected by concentration and composition of intermediate substrate. Increase of maximum yield coefficient and simultaneous decrease of maintenance coefficient of the second stage with decreasing concentration of intermediate substrate were predicted by fitting the biochemical data of the second stage to the model. The production of biomass in the two-stage system under optimum kinetics was always greater than that of the one-stage system. The presence of acetate allowed higher rates at low concentrations of substrate, reducing the kinetic advantage of the two-stage process.

The predominant populations of bacteria in the chemostat altered if the specific growth rate was increased by a factor 4 or when acetate was added to the vanillin-basal medium. Much of the sludge flocs were built up by fibrillar cellulose-like exopolymers and may play an essential role in the mechanism of flocculation.

Free descriptors: acetate, activated-sludge process, floc structure, identification of micro-organisms, intermediate substrate, optimalization of the two-stage activated-sludge process, structured model of microbial growth, the three-compartment model, vanillin.

Cover: Impressions of an activated-sludge floc surrounding the three-compartment model by H. Harder sr.

This thesis will also be published as Agricultural Research Reports 886.

Aan mijn ouders
Aan Ingrid
Aan Nathan

Dankwoord

Allen, die aan de wording van dit proefschrift hebben bijgedragen, wil ik graag met dit woord vooraf bedanken.

In de eerste plaats ben ik veel dank verschuldigd aan mijn ouders voor hun goede zorgen tijdens mijn vlegeljaren en de mogelijkheden, die zij mij gaven een studie te beginnen. Het verheugt mij, dat mijn vader iets van zijn tekentalent kan tonen op de omslag van deze dissertatie.

Ingrid, meer dan enig ander heb jij de ups en downs van mijn promotie-onderzoek meegemaakt. Ik ben je erg dankbaar voor je interesse, je acceptatie om gedurende lange tijd het leven van een onbestorven weduwe te leiden en je optimisme in de goede afloop van het onderzoek.

Mijn promotor, Prof.Dr. P.G. Fohr, dank ik zeer voor de gelegenheid die hij mij bood, het onderzoek aan het actief-slib op een fundamentelere manier aan te pakken dan gebruikelijk. Uw waardevolle suggesties en de bepaling van mijn aandacht ook bij de praktische problemen in de technologie van de waterzuivering, heb ik zeer op prijs gesteld.

Bijzondere dank ben ik verschuldigd aan mijn co-promotor Ir. J.A. Roels. Beste Joop, allereerst ben ik je zeer erkentelijk, dat je in een laat stadium nog wilde participeren in het onderzoek. Van de vele discussies rond het thema van de modelvorming en van je mathematische benadering van de kinetiek heb ik veel geleerd.

Voorts gaat mijn dank uit naar mevrouw Dr. M.H. Deinema voor haar bereidheid het microbiologisch onderzoek aan het actief-slib te begeleiden en te laten uitvoeren op de vakgroep Microbiologie. Ook dank ik haar voor het kritisch lezen van een deel van het manuscript.

Een woord van dank gaat ook uit naar Ir. H. Akse van de vakgroep Proceskunde. Beste Henk, ik dank je zeer voor het schrijven van de subroutine DVOGER en voor de vele avonden, die we verder nog samen achter een terminal van de Landbouwhogeschool computer hebben doorgebracht.

Zonder de hulp van de medewerkers van de vakgroep Waterzuivering zou dit onderzoek niet mogelijk zijn geweest. Graag noem ik Jo Jacobs, die een jaar lang technische assistentie verleende in het laboratorium en Dr.Ir. Bram Klapwijk, die een deel van het manuscript las en opbouwende kritiek leverde.

Zeer erkentelijk ben ik de studenten Ir. G. Butijn, Ir. F. Dijkman, mevrouw Ir. C. de Graaff, J. Steketee, Ir. H. Slijkhuis en mevrouw H. Wolferink, die in het kader van hun doctoraal studie aan dit onderzoekproject meewerkten. Beste Gert, Frens, Carolien, Jaap, Herman en Hanneke het was leerzaam, vruchtbaar en fijn met jullie dit onderzoek te ontwikkelen.

Voorts dank ik ook Prof.Dr. C. Veeger van de vakgroep Biochemie voor de faciliteiten

die hij mij gaf om in 'blessuretijd' de laatste hand te leggen aan mijn proefschrift.

Ook de heren van de technische dienst ben ik veel dank verschuldigd; de heren A. van Amersfoort (vakgroep Waterzuivering) en W.J. Ackerman (werkplaats Biotechnion) bouwden de laboratorium installaties, de heer W.Ch. Melger (vakgroep Organische Chemie) gaf goede raad bij de gaschromatografische analyses aldaar, de heren C. Rijpma en M. Schimmel (tekenkamer Biotechnion) tekenden de figuren, de heer H.G. Elerie (Technische en Fysische Dienst van de Landbouwhogeschool) drukte de electronenmicroscopische foto's af en stelde ze beschikbaar en de heer A. Wessels (vakgroep Microbiologie) verzorgde de foto's van de slibvlokken.

De vele formules in deze dissertatie maakten het typen niet alleen tot een kunde maar meer nog tot een kunst. Ester Boersma-van Olderen, Ellen Stooker, Janny Toppenberg-Fang (vakgroep Biochemie) en de dames van de typekamer van de Landbouwhogeschool met name mevrouw A.W. Wigman-van Brakel ben ik zeer dankbaar voor hun noeste vlijt en accuratesse.

De redactionele begeleiding van de medewerkers van Pudoc heb ik zeer gewaardeerd. In het bijzonder dank ik de heer J.C. Rigg voor de correctie van de Engelse tekst en voor zijn zorg om de gebruikte symbolen en de definities in overeenstemming te brengen met de geldende conventies.

Curriculum vitae

De auteur, geboren op 2 december 1949 te Naarden, bezocht eerst de christelijke ULO te Naarden van 1962 tot 1966 en behaalde vervolgens het HBS-B diploma in 1969 aan het Christelijk Commenius College te Hilversum. Daarna volgde hij een studie aan de Landbouwhogeschool te Wageningen (richting Moleculaire Wetenschappen, chemische specialisatie) van 1969 tot januari 1975. Het vakkenpakket in de doctoraalfase bestond uit biochemie (verzwaard hoofdvak), microbiologie (hoofdvak), organische chemie (bijvak) en waterzuivering (toegepast bijvak). Van 1975 tot 1978 werd een promotie-onderzoek uitgevoerd op de vakgroep Waterzuivering van de Landbouwhogeschool onder leiding van de hoogleraar Prof.Dr. P.G. Fohr en Ir. J.A. Roels, lector in de biomathematica aan de Technische Hogeschool te Delft. Vanaf september 1978 is de auteur verbonden aan de vakgroep Biochemie van de Landbouwhogeschool als post-doctoraal medewerker.

Contents

1 General introduction

1.1 TO A STRUCTURED MODEL FOR THE ACTIVATED-SLUDGE PROCESS

1.1.1 Presuppositions; a personal statement

When scientists develop models of that aspect of reality which they normally call nature they assume the existence of physical, chemical and biochemical principles, which can be formulated in a verbal or mathematical way and which are discovered by means of measurements. The same truth has been expressed poetically in the following words:

> 'What has been, that will be;
> what has been done that will be done.
> Nothing is new under the sun'.
>
> (Ecclesiastes 1:9)

The search for and discovery of these principles lead to the joy of man for:

> 'Great are the works of the Lord,
> exquisite in all their delight'.
>
> (Psalms 111:2)

What is more, in this search for, ultimate discovery and use of those principles embedded in nature, man fulfils his dual cultural vocation 'to cultivate and care for' the earth (Genesis 2:16; 3:15-19). However the 'fear of the Lord is the beginning of wisdom: a good understanding have all they that do his commandments' (Psalms 111:10).

The spiritual motivation behind the research in this thesis, lies in the second of the dual cultural vocation.

1.1.2 Criticism of activated-sludge models

A model is a simplified image of reality that describes only restricted aspects of a system or process. It is based on a set of hypotheses which seem important according to our present knowledge. By manipulating and testing the model, one hopes to gain an insight and a grasp of the real system.

In the microbial world, verbal and mathematical models of growth are generated by a combination of three principles: (Fredrickson et al., 1970): 'First, well-established physical, chemical and biological principles may provide certain parts of the model. Second, some hypotheses of the model may be plausible inferences from existing data.

Finally, the remaining part of the model are guesses but one hopes educated ones'.

On these principles, Monod (1942) and Herbert et al. (1956) formulated models for microbial growth on which most published models of growth of activated sludge have been based. Classically two relationships were defined by Monod and extended by Herbert for growth of micro-organisms in relation to a limiting nutrient. First

$$\mu = \mu_{max} \frac{C_S}{K_S + C_S} - k_d \qquad (1)$$

where μ, the specific growth rate, is the production of biomass per unit time and biomass, μ_{max} is the maximum specific growth rate obtained when the nutrient factor, $C_S/(K_S + C_S)$ approaches 1, K_S is the half-velocity constant and k_d is a decay term. Second, by definition μ relates through the yield factor, Y, to the specific rate of substrate consumption per unit biomass, q_S, as

$$\mu = Y.q_S \qquad (2)$$

With Eqn 1 and Eqn 2, it is possible to deduce all the models for the kinetics of activated sludge found in the literature. Several investigators use Eqn 1 (for example, Ramanathan & Gaudy, 1971; Chiu et al., 1972; Srinivasaraghavan & Gaudy, 1975; Bonotan-Dura & Young, 1976; Marais & Ekama, 1976; Ekama & Marais, 1977; Daigger & Grady, 1977).

The model of Lawrence & McCarty (1970) is obtained by substituting Eqn 1 for μ in Eqn 2.

Independently Eckenfelder (1961) and McKinney (1962) postulated that the kinetic process of substrate removal in activated sludge may be approximated by pseudo-first order kinetics. Recently Goodman & Englande (1974) reviewed the approaches of Eckenfelder and McKinney and concluded that they were the same, and that only the nomenclature was different. By assuming that in practice $K_S >> C_S$, the Eckenfelder/McKinney model may be derived from the Lawrence/McCarty model by ignoring C_S in the denominator.

However, there are many objections to the use of these simple models and the relevance of the coefficients in the equations, i.e. μ_{max} and K_S. Some objections are as follows:

Equation 1, minus the k_d term was originally used to describe the relationship between the substrate concentration, S, and the velocity of a single enzymatic reaction. This equation has been successfully applied to growth of micro-organisms, because the specific growth rate, μ, may be proportional to the rate of a single enzymatic reaction which forms a bottle-neck in metabolism (Ierusalimskii, 1967; Dabes et al., 1973). The values of the coefficients, μ_{max} and K_S reflect this rate limiting step. In activated sludge, however, we are concerned with an undefined mixed culture of flocculently growing micro-organisms and the specific growth rate would be proportional to the combined result of a number of different bottle-necks. Figure 1 is a graphical representation of possible bottle-necks (Q_1 - Q_4) in activated sludge.

Q_1 a bottle-neck in the diffusion of nutrients into the floc, which means there are less nutrients in the innerpart of the flocs than would be expected from the concentrations outside (Baillod & Boyle, 1970; Sladká & Zahrádka, 1971; Krul, 1977).

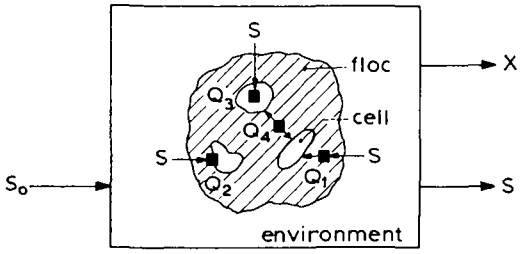


Fig. 1. Graphical representation of possible bottle-necks in an activated-sludge floc. S_0, incoming substrate; S, substrate in the system, X, biomass; Q_1, diffusion limitation; Q_2, limiting steps of transport of nutrient into a cell; Q_3, limiting step in metabolism and Q_4, limitation caused by bacterial interaction.

Q_2, Q_3 limiting steps in the transport of the nutrients into the cell (by diffusion or active transport) and in the cellular metabolic activity, respectively (van Uden, 1967; Dawes et al., 1976).

Q_4 limitation caused by interaction of different kinds of organisms in the activated sludge for instance inhibition, competition for nutrients, predation, parasitism and synergism (Gall, 1970; Painter & Bungay, 1971; Veldkamp & Jannasch, 1972; Veldkamp, 1976).

Relevant environmental conditions include temperature, *pH*, shear force (Morand, 1964), residence time of sludge, the method used to mix substrate and sludge (Rensink, 1974; 1977), ratio of food to micro-organism (Downing, 1966), composition and concentration of limiting nutrients. They determine for instance the microbial species in the culture, floc formation and the geometry of the floc. In other words, the bottle-necks in the biosystem are also determined by the environmental conditions. Thus whether the kinetic coefficients in the activated-sludge models are constant is uncertain. They cannot represent a limiting step because there are several, they occur at different levels and they may be expected to shift as a result of physico-chemical,biochemical and population changes (Jones, 1973; Chudoba et al., 1973).

The cellular composition of any micro-organism is influenced markedly by the growth-supporting environmental conditions and it may be expected that the micro-organisms will behave differently under the different conditions (Herbert, 1961; Neidthart, 1963; Maaløe & Kjeldgaard, 1966; Tempest & Dicks, 1967).

Models that do not account for cellular composition expressed in the 'physiological state' of the culture will only be adequate if the physiological state is balanced, i.e. in steady state, or does not effect the phenomenon studied.

But in unbalanced growth as occurs, for example, after a nutritional shock, the cellular composition of the various micro-organisms changes in time, and the dynamic behaviour is poorly explained by the models of Eqns 1 and 2 (George & Gaudy, 1973; Chi & Howell, 1976; Krishnan & Gaudy, 1976). Therefore, during the last ten years, models that take account for the structure of bacterial cells were developed (Powell, 1969; Young et al., 1970; van Dedem & Moo-Young, 1973; Chase, 1977; Roels, 1978).

A structured model (Chapter 2) was developed to take account of such criticism. It models the dynamics of microbial composition and formulates an n-th order equation (for

substrate consumption) as is extensively used in chemical reactor kinetics (Levenspiel, 1971). Meaningful quantitative studies on microbial cultures generally can only be made with cultures growing under rigidly controlled conditions (Tempest, 1970). An adequate chemostat for research on cultivation of activated sludge was therefore developed (Chapter 3). Equipment and analytical methods are described. Chapter 4 summarizes a first approach of experimental verification of the proposed structured model.

1.2 DUAL-STAGE ACTIVATED-SLUDGE PROCESS

Special attention has been paid in this thesis to the two-stage activated-sludge process. In the last two decades, the conventional activated-sludge system has been modified in several ways as a consequence of the need for the treatment of specific wastes and of the high quality of effluents required. Several arguments for the use of the dual-stage system have been mentioned in the literature.

1.2.1 When and where dual-stage systems are used

At least two biological stages are required for the optimum treatment of highly concentrated sewage and industrial waste waters (C_{SO} > 0.5 g BOD_5/l, Imhoff, 1977), wastes difficult to degrade biologically and liquors containing substances which are degradable but toxic to activated sludge (Lindner, 1957; Imhoff, 1955; Dietrich, 1968; Paul, 1969; Brouzes, 1973, Klapproth, 1976; Imhoff, 1977; Ilićs, 1977).

Two-stage activated-sludge systems do an excellent job on wastes from yeast industry (Wu & Kao, 1976), sulphite-pulping industry with lignin derivatives (Dubach, 1966, 1968), kraft bleachery industry (Liao & Dawson, 1975), food industries such as cider making, slaughterhouse (Imhoff, 1977), and dairy factories (Paul, 1969), chemical industries with organic compounds such as methanol, formaldehyde, methylene chloride, formate, trioxane, citric acid, and pigments (Heinicke, 1967), coke ovens and gas works with phenols, thiocyanates, thiosulphates and cyanides (Abson & Todhunter, 1961). The metabolism of compounds difficult to degrade occurs especially at the second stage (Heinicke, 1967). Moreover variations in inlet concentrations of substrate are smoothed out at this stage and a high quality of effluent can be achieved (Klee, 1970; Lohmann, 1975).

1.2.2 Appropriate cultures in both stages

In a two-stage activated sludge process it is possible to maintain appropriate cultures in both stages. The population in the first stage is high-loaded and metabolizes energy-rich organic compounds that are easily metabolized. The population in the second stage is low loaded and oxidizes energy-poor organic compounds that are more difficult to degrade (Jannasch & Mateles, 1974). In general, therefore the first-stage and second-stage populations are characterized by fast growers and slow growers, respectively. An example of this is given by Heinicke (1967). Trioxane was metabolized for 25% in a one-stage process, but for 70% in the second stage of a two-stage system, because of the low loading of this stage.

A second example of population differentiation, not directly related to our problem, is to build up a two-sludge system with preliminary denitrification in the first stage (Klapwijk, 1978) to maintain a predominately protozoal sludge in the second phase for possible improvement of the quality of effluent (Dubach, 1966, 1968). Toxic interactions between some of the bacteria and organic material present in the waste can also be eliminated by isolation of appropriate cultures at the different stages. Phenols are toxic for thiobacilli. To purify a mixture of phenols, thiocyanates, thiosulphates and cyanides, impurities of coke-ovens and gas-works waste, Abson & Todhunter (1961) used a three-stage system. In the first stage phenols were metabolized by *Vibrio*, *Pseudomonas* and *Actinomyces* species and in the second stage *Thiobacillus* species were present to eliminate the thiocyanates, thiosulphates and cyanides. The third stage was used for nitrification of ammonia. Inherent to the two-stage activated-sludge process, the amount of sludge produced will be less than that produced by conventional one-stage systems, because of low cell efficiency of biomass production and high overall specific maintenance/decay rates (Downing, 1966; Wu & Kao, 1976).

1.2.3 Dimensioning of two-stage activated-sludge plants

The dimensioning of five two-stage activated sludge plants are summarized in Table 1. The first stage is high loaded with q_S, i.e. net consumption rate of substrate (of BOD_5) divided by the concentration of biomass (of dry matter) of 0.011 - 0.040 g/g.h. In contrast, the second stage is low loaded and has net consumption rates of 0.002 - 0.006 g/g.h. To obtain high qualities of effluent of BOD_5, (C_S 0.015 - 0.040 g/l), Imhoff (1977) prefers a net consumption rate for the second stage of less than 0.006 g/g.h. In spite of high loading at the first stage, in the Gütersloh plant for example (Table 1), a fractional reduction of 0.84 is achieved with a net consumption rate of 0.028 g/g.h.

In general, a conventional single-stage activated-sludge plant is low loaded and a fractional reduction of 0.95 may be expected with a mean net consumption rate, q_S of 0.008 g/g.h. When comparing the net consumption rates of the two systems based on the same mass of activated sludge, for example in the Gütersloh plant (Table 1), the rate in the first stage of the dual system might be three times that in the conventional system (0.028/ 0.008 = 3.5). In other words depending on the size of the second stage, a two-stage activated-sludge system may be designed smaller or it can be used to purify more waste water (Imhoff, 1955; Heinicke, 1967; Dietrich, 1968; Paul, 1969; Klee, 1970; Lohmann, 1975).
However, the use of a high rate of substrate removal in waste treatment is inherently unstable and requires close and accurate process control (Bargman et al., 1957; Jung, 1960, van Luven, 1972).

1.2.4 Research on the two-stage system

Thus little fundamental work has been done to improve the two-stage process. For example, the total net consumption rates of the plants summarized in Table 1 have not been optimalized (Wuhrmann, 1968).

Table 1. Design of two-stage activated-sludge systems; C_{SO}, incoming substrate of BOD_5; C_S, substrate concentration of BOD_5; q_S, rate of consumption of substrate; t, hydraulic retention time; C_X, concentration of biomass; η, fractional reduction of substrate; V_1 and V_2, working volumes of first and second stages, respectively; (1) Imhoff (1977); (2) Paul (1969); (3) Klapproth (1976).

		Gütersloh (1)	*Osnabrück (1)*	*Leverkusen (1)*	*Haldem (2)*	*Ingelheim (3)*
Stage 1						
C_X	(g/l)	7.3	6.0	6.8	4.1	7.4
C_{SO}	(g/l)	0.600	0.380	0.750	1.28	2.440
t	(h)	2.5	1.3	6.0	23.4	13.2
q_S	(g/g.h)	0.028	0.040	0.012	0.011	0.024
C_S	(g/l)	0.096	0.065	0.250	0.256	0.122
η_1		0.84	0.83	0.67	0.80	0.95
Stage 2						
C_X	(g/l)	2.6	6.2	8.6	5.5	5.5
C_{SO}	(g/l)	0.096	0.065	0.250	0.256	0.122
t	(h)	4.5	2.3	12	22	9
q_S	(g/g.h)	0.006	0.003	0.002	0.002	0.002
C_S	(g/l)	0.030	0.016	0.050	0.009	0.045
η_2		0.69	0.75	0.80	0.95	0.58
$\Sigma\eta$		0.95	0.96	0.94	0.99	0.98
V_1/V_2		0.5	0.5	0.5	1.0	1.5

To tackle this problem a mathematical model optimalizing the kinetic design of a two-stage process was formulated and tested (Chapter 5). In our experiments a single organic compound was used as limiting substrate. Chapter 5 describes also the influence of acetate on the biodegradation of the model compound used in the two-stage system; acetate is assumed to be representative for easily biodegradable compounds.

Finally, the microbiology of the high-loaded first stage of the two-stage system. They clarify the building up of the floc structure, and the interrelationships between the different microbial species in the activated sludge during their composition for the substrate (Chapter 6).

2 Materials and methods

2.1 ACTIVATED SLUDGE

The original mass culture was obtained from the municipal waste treatment plant at Zeist near Utrecht. The organic loading rate of COD in mixed liquid suspended solids (MLSS) of this plant was 0.5 g/g.d. For 4 weeks, the activated sludge was continuously adapted to the basal medium with vanillin as carbon and energy source (organic loading rate of carbon 0.01 g/g.h). A slightly yellow well-settling sludge was obtained.

For the continuous flow experiments described in Chapter 6, sodium acetate and ammonium sulphate were added to the vanillin-basal medium and the activated sludge was again adapted to this new medium for 4 weeks. A grey well-settling sludge was achieved.

2.2 VANILLIN-BASAL MEDIUM

Experiments (Chap. 4 and 5) were conducted in a basal medium used by Gaudy et al. (1967) in slightly modified form with added vanillin. Vanillin ($C_8H_8O_3$) was purchased from Merck (Germany). The composition per litre demineralized water was as follows vanillin 1.0 g; $(NH_4)_2SO_4$ 0.5 g; $MgSO_4.7H_2O$ 0.1 g; $MnSO_4.H_2O$ 0.01 g; $FeCl_3.6H_2O$ 0.015 g; $CaCl_2$ 0.025 g; K_2HPO_4 1.070 g; KH_2PO_4 0.527 g; and tap water 100 ml.

The ingredients of the basal medium were prepared in the four following stock solutions to prevent precipitation in storage:
buffer solution: K_2HPO_4; KH_2PO_4; $(NH_4)_2SO_4$
salt solution 1: $FeCl_3.6H_2O$
salt solution 3: $CaCl_2$

The mass ratio of carbon to nitrogen in this medium was calculated as 6.0. The nutrient solution was not sterilized but every day freshly prepared in 30-litre batches to prevent initial bacterial degradation of the limiting nutrients.

2.3 SODIUM ACETATE-VANILLIN-BASAL MEDIUM

In the experiments described in Chapter 6, sodium acetate trihydrate was added to the vanillin-basal medium (Section 2.2). The ammonium sulphate concentration was increased too in order to keep constant the carbon to nitrogen ratio at 6.0.

2.4 MEASUREMENTS IN THE SUSPENDED MATTER

Generally 30-ml or 50-ml samples of the activated sludge, quickly cooled down to 0 ^{o}C, were centrifuged in a Sorval Highspeed centrifuge (15 000 x g_n, 15 min, 0 ^{o}C).

The precipitate was washed by resuspending in phosphate buffer of 50 mmol/l 0 °C, adjusted to pH = 7,2 and recentrifuging. After three washings, the biomass suspension 30-ml or 50-ml was used for the different assays. Dry mass and protein determinations were carried out immediately after sampling and washing of the biomass.
The rest of the biomass suspension was stored at -20 °C for estimation of DNA, RNA and total carbohydrate.

2.4.1 Total dry weight

Washed biomass suspension, 10 ml, was transferred to small weighing bottles and dried at 105 °C, 15 h. The dry mass was corrected for the phosphate buffer salts. Contamination of salts out of the medium could be neglected after washing three times.

2.4.2 Concentration of DNA (deoxyribonucleic acid)

The extraction of DNA out of activated sludge was used according to Speece et al. (1973). Washed cell suspension, 15 ml, was centrifuged (25 000 x g_n, 15 min, 0 °C) and resuspended in distilled water, 0 °C. This suspension was centrifuged (25 000 x g_n, 15 min, 0 °C) and resuspended in 15 ml of NaCl (cold) 1.5 mol/l. Subsequently the suspension was sonified (20 kHz, 40 W, 5 min, 0 °C) using a Branson Sonifier.

Duplo samples of 4 ml were mixed with 0.2 ml of $HClO_4$ 0.5 mol/l and heated at 70 °C for 15 min, shaking the tubes intermittently. After cooling in cold tap-water, the samples were centrifuged (50 000 x g_n, 60 min, 0 °C). The supernatant liquor of every sample was refrigerated at 0 °C and the precipitate resuspended in 4 ml of $HClO_4$ 0.5 mol/l. Subsequently the same procedure of heating and centrifuging was repeated and both supernatant liquors of the first and second extraction were collected and well mixed.

The concentration of DNA in the extracted samples was determined by the method of Burton (1956).
The method is based on a colour reaction of the deoxypentose moieties of DNA with diphenylamine in a mixture of acetic and sulphuric acid at 100 °C (Dische reaction). The sensitivity of the method is increased by addition of acetaldehyde to the diphenylamine reagent.

Acetaldehyde-diphenylamine reagent:
Diphenylamine (Merck) 1.5 g is dissolved in 100 ml of concentrated acetic acid and 1.5 ml of H_2SO_4 (concentrated) added. This reagent must be stored in the dark. Just before starting the colour reaction 0.5 ml acetaldehyde (0.2 acetaldehyde, concentrated, diluted in 10 ml distilled water) is added to the reagent.

To a 2-ml aliquot (in duplicate) of the combined supernatant liquors, 4 ml of freshly prepared acetaldehyde-diphenylamine reagent was added, thoroughly mixed and the colour was developed at 30 °C for 16 h.

Standard solutions of DNA (calf thymus, Merck, Batch 6407936) of five concentrations (0 - 200 mg/l) had been included in duplicate in every experiment and prepared as the combined supernatant liquors of the activated-sludge samples. The absorbance

was read at 600 nm wavelength (Cecil CE 272 Spectrophotometer).

A straight line through the origin for concentration of biomass DNA versus different dilutions of an activated-sludge sample was obtained. Results were expressed as mass fraction of DNA in dry mass.

2.4.3 Concentration of RNA (ribonucleic acid)

The concentration of RNA in samples of activated sludge had been measured by the method of Herbert et al. (1971).

To 5 ml of washed cell suspension, 2.5 ml of NaOH 3,0 mol/l was added. In a stoppered tube, the mixture was heated for 10 min in a boiling waterbath and cooled in cold tap-water. Sonification (20 kHz, 40 W, 10 min, 0 ^{o}C) of the washed cell suspension instead of hydrolysation with NaOH resulted in equal amounts of RNA in the samples.

Estimation of RNA in extracted samples of activated sludge was based on a colour reaction of the pentose moieties of RNA. In the reaction pentose is converted to furfural on treatment with HCl at 100 ^{o}C which produces a green complex with orcinol catalysed by $FeCl_3$.

Orcinol reagent:

$FeCl_3.6H_2O$ 0.90 g is dissolved in 1 litre of HCl (concentrated).

Orcinol (BDH) 1.0 g is dissolved in 100 ml distilled water and kept at 0 ^{o}C. The orcinol reagent immediately prepared before use is obtained when 1 volume orcinol solution is added to 4 volumes of $FeCl_3$/HCl solution.

Duplicate aliquot volumes of 0.5 ml of hydrolysed samples were taken and made up to 1 ml with distilled water. Then 3 ml freshly prepared orcinol reagent was added and well mixed. The mixtures were heated for 10 min in a boiling waterbath and cooled in tap water. The absorbance was measured at 670 nm wavelength.

A reagent blank and standard RNA (sodium ribonucleate, pure extract of yeast, Koch-Light Laboratories LTD, Batch 4946t) were put up simaltaneously. Linear dependency was observed between RNA concentration in sludge and dilution of one sample of activated sludge. Interference of DNA and hexoses with RNA could be neglected.

2.4.4 Concentration of protein

Activated-sludge protein was estimated by the Folin-Ciocalteu method as described by Lowry et al. (1951). In applying it to total protein in whole micro-organisms, the modification of Herbert et al. (1971) was used. At high concentrations of protein in the samples (> 0.5 g/l), the simpler biuret method of Robinson & Hogden was used as described by Herbert et al. (1971).

The first step in both assays must be the quantitative solubilization of protein from the cells. Therefore 10 ml of washed cells suspension was mixed with 10 ml of NaOH 1.0 mol/l (Folin-Ciocalteu assay) or 5 ml of NaOH 3.0 mol/l biuret assay). The mixture was heated to 100 ^{o}C for 5 min, cooled in cold water and the protein could be assayed. Egg albumin (Merck) was used as reference.

2.4.5 Total concentration of carbohydrate

Total carbohydrate was estimated by the Anthrone method of Scott & Melvin as described by Hodge & Hofreiter (1962).

To 5.0 ml of a washed cell suspension, 2.5 ml of NaOH 3.0 mol/l was added and heated in a boiling waterbath for 10 min. After cooling in tap water, a sample of 0.5 ml was used for estimation of total carbohydrate (anthrone, Merck).
A reagent blank and a set of reference carbohydrate solutions (D(+)-glucose, Merck) were treated in the same way.

2.4.6 Rate of consumption of oxygen

Oxygen consumption of the activated sludge fed on vanillin, vanillic acid or protocatechuic acid was measured in a Voith Sapromat by the method of Liebmann & Offhaus (1966).

2.5 CHEMICAL ASSAYS IN THE SUPERNATANT

2.5.1 Total organic carbon. TOC

Supernatant liquor of a centrifuged sample (25 000 x g_n, 15 min, 0 ^{o}C) was acidified to pH 2-3 with 0.5 ml of HCl (concentrated) and 3 min flushed with N_2 to blow out all the inorganic carbon compounds. Aliquots of 50 µl were converted in an oven to CO_2 at 870 ^{o}C with Co on pumice-stone as a catalyst. The produced CO_2 could be detected in an infrared analyser (Beckman Infrared Analyser, Model 865).

A set of reference solutions of potassium hydrogen phthalate, (Merck) were treated in the same way as the samples.

2.5.2 Total concentrations of aromatics

The total aromatic concentration of supernatant liquor acedified to pH 2.0 with HCl (concentrated) could be photometrically measured at 280 nm wavelength. Vanillin was used as the reference compound at 280 nm wavelength, pH 2.0.

2.5.3 Vanillin, vanillic acid and protocatechuic acid

Generally organic compounds solved in aqueous solution are poorly detected by gas-liquid chromatographic (GLC) due to the large interference of water in the column. However, as the use of GLC is limited to volatile samples, it is not applicabe in direct analysis of most substituted, non-volatile and thermally unstable phenols.

Therefore in our experiments, samples were freeze-dried and subsequently N,N-bis (trimethylsilyl) trifluoroacetamide (BSTFA) derivatives were prepared by the method of van de Casteele et al. (1976) in order to improve GLC of phenolic compounds in the effluent. The procedure was as follows:

Samples (100 - 1000 ml) of activated-sludge culture were centrifuged (25 000 x g_n, 15 min, 0 °C) and acidified to pH 2 with HCl 5 mol/l.

After freeze-drying (New Brunswick), the residue was silylated with BSTFA (Pierce). First, 500 µl of gallic acid in methanol 0.6 g/l was injected into a reaction vial of 1 ml and subsequently the methanol was evaporated. Gallic acid was used as a tracer in the trimethylsilylation of the residues.
Then duplicate portions of the homogenized freeze-dried residue with 0.4 - 0.8 mg of C were weighed and 300 µl BSTFA added. Then the mixture was maintained for 10 min at 125 °C in a sealed vial. After cooling, 10-µl samples of the reaction mixture were injected directly into the gas-liquid chromatograph (Hewlett Packard 5700 A).

The GLC was equipped with a metal column and flame ionization detector. The column (length 2.0 m; inner diam. 3.175 mm) was packed with 2.3 g Chromosorb W HP 100/120 coated with 8 % Dow Chemical - 430.
Volume flow rates of the carrier gas nitrogen of hydrogen and of air were 30, 30 and 300 ml/min, respectively.
The injection port temperature and the detector temperature were 250 °C. The column oven temperature was programmed as a function of time (isotherm inital period 160 °C, 4 min; 160 - 240 °C, programme power 4 °C/min; isotherm final period 240 °C, 2 min). The analysis time amounted to 26 min.

A standard micture of vanillin, 0.1 mg; vanillic acid (Merck), 0.1 mg; protocatechuic acid (Merck), 0.1 mg and gallic acid (Merck), 0.1 mg and 350 µl BSTFA was always simultaneously silylated and analysed.

The peak areas were calculated with a Spectra Physics Autolab Computing Integrator.

A V.G. MM 70 - 70 gas chromatograph- mass spectrometer was used to determine the mass of the parent ion of the BSTFA derivatives in the samples (by the Laborartory of Organic Chemistry of the Agricultural University).

2.5.4 Acetic acid

Acetic acid could be assayed directly from the aqueous phase of centrifuged (25 000 x g_n, 15 min, 0 °C) samples of activated-sludge culture.

The analyses were performed on a Becker Gas Chromatograph Model 417 equipped with a glass column and flame-ionization detector. The glass column (length 1.0 m, inner diam. 4.0 mm) was packed with Chromosorb W - AW (80 - 100 mesh) coated with 20 % Tween 80. Volume flow rates of the carrier gas nitrogen saturated with formic acid of hydrogen and of air were 60, 45 and 350 ml/min, respectively.
The injection port temperature and the detector temperature were 170 °C. The column temperature was 115 °C.

2.5.5 Ammonia, nitrite and nitrate

Ammonia in the supernatant liquor of samples centrifuged (25 000 x g_n, 15 min 0 °C) samples was estimated by the method of Nessler (NEN 3235, Part 6.1.1). Nitrite was assayed by the method of Griess-Romijn-van Eck, (NEN 3235, Part 6.3). Nitrate was measured by the sodium salicylate method, (NEN 3235, Part 6.4).

2.5.6 Dissolved oxygen

Concentration of dissolved oxygen in the mixed culture was monitored with a galvanic cell oxygen analyser (PS Scientific Co.).

2.5.7 pH

A Knick pH-meter was used for pH measurements. For automatic control of pH during experiments (pH 7.2), a Knick pH-meter could be connected through a relay (Shinko) with a pump dosing NaOH or HCl both 3.0 mol/l into the culture.

2.6 EXPERIMENTAL EQUIPMENT

2.6.1 Batch equipment

The batch experiments used the experimental equipment shown in Figure 2. This unit provided a working volume of 15 l.

The temperature was 20 ^{o}C, deviating 0,5 ^{o}C and the pH was adjusted automatically to 7.2, deviating 0.2 by adding the NaOH or HCl.

The rotational speed (Heidolph stirrer) was maintained at 250 min^{-1} and the air flow rate to the reactor was so maintained to keep volume fraction of dissolved oxygen in the culture fluid at 3 mg/l or larger.

2.6.2 Activated-sludge chemostat with recycling of biomass

The chemostat of activated sludge employed in this research consisted of a completely-mixed reactor and a separator, as shown in Figure 3.

The reactor (A) and the separator (B) were made from poly(methylmethacrylate) (PMMA) tubing, of inner diam. 190 mm and 75 mm respectively. It could be used because sterilization of the equipment was not necessary. The reactor tube is embedded in a box

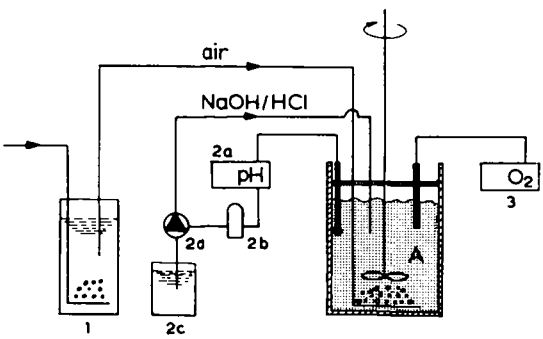


Fig. 2. Pictoral diagram of the batch system. 1, waterbath; 2a, pH-meter; 2b, relay; 2c, base or acid reservoir; 2d, base or acid pump and 3, O_2-meter.

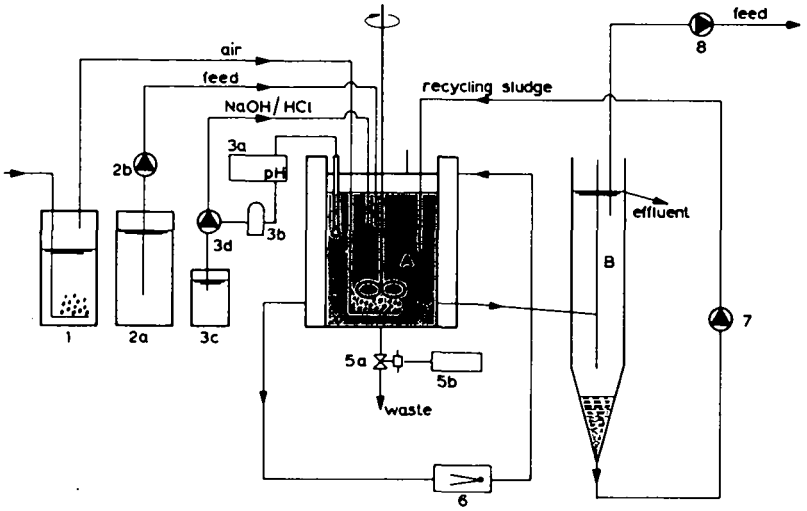


Fig. 3. Diagram of the activated-sludge chemostat with recycling of biomass. A, reactor; B, separator; 1, waterbath; 2a, feed reservoir; 2b, feed pump; 3a, pH-meter, 3b, relay; 3c, base or acid reservoir; 3d, base or acid pump; 5a, magnetic valve; 5b, pulse/pause switch; 6, cryostat/thermostat; 7, recycling pump and 8, feed pump.

of PMMA sheet (10 mm). In this way, distilled water could be circulated through the double wall by using a cryostat/thermostat (Haake) and the reactor temperature maintained at 20 ^{o}C, deviating 0.5 ^{o}C.

It was observed that by stirring and vigorous aerating, activated sludge in the reactor precipitated on the wall just above the surface of the culture liquor. It was partially dried up and could be hardly resuspended in the culture. The problem has been solved by covering the reactor in order to increase the relative humidity.

Figure 4 shows that the cover (E) lies on a PMMA ring (D) which can be fixed at different levels in the reactor tube. So the working capacity of the reactor could be varied from 1.0 - 6.0 litres.

Continuous discharge of activated sludge was hampered by flocculent growth of the micro-organisms. The measured concentration of the wasted biomass was always less than

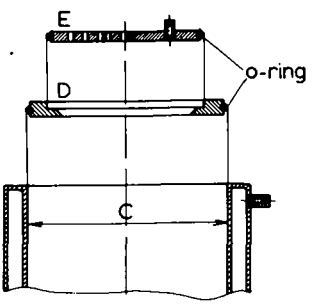


Fig. 4. Construction of the cover of the reactor tube (C). E, cover; D, PMMA ring which can be fixed at different levels in the reactor tube (S).

in the reactor. The only satisfactory solution was to discharge the biomass by gravity through a magnetic valve of 16 mm orifice (ASVA) connected with a pulse-pause switch (Schleicher). A continuous way of wasting off biomass could be well approached by short pulse-pause time intervals.

pH 7.2 could be automatically controlled (Chap. 2.5.7).

The rotational speed (Heidolph stirrer) was maintained at 250 min^{-1} and the daily controlled concentration of dissolved oxygen was always 3 mg/l or larger. The complete mixing of fluid in the reactor was checked by periodically checking that substrate concentration in the reactor and the separator were equal.

The feed of the culture was pumped (Heidolph/WAB pump) continuously into the chemostat. The reactor effluent flowed near the bottom of the reactor tube in order to prevent short circuiting, by gravity to the separator (volume 1.7 l). The precipitated activated sludge was pumped back continuously into the reactor.

Every day, reactors and separators were cleaned to prevent growth on walls, because this could effect the rate of reaction, especially in the continuous-flow experiments at dilution rates near wash-out. The silicon tubes for feeding medium were cleaned daily and the other tubes weekly. In our two-stage experiments the feed of the second stage was obtained by pumping effluent continuously from the separator of the first-stage.
The second-stage chemostat was a duplicate of the unit described above.

2.7 PARAMETER ESTIMATION

A Hewlett Packard 65 pocket calculator with prerecorded programs (HP 65 Stat Pac 1) was used for fitting straight line-, power- and exponential relationships by the least squares method. Balance equations of the three-compartment model and the partial differential equation of the two-stage model were solved on the DEC SYSTEM-1090 TIMESHARING of the Agricultural University, Wageningen, The Netherlands.

3 Three-compartment model

3.1 INTRODUCTION

In this chapter a structured model for a growing culture of activated sludge which takes account for the dynamics of biochemical composition of the micro-organism will be developed. As described in Section 3.2 this model can be envisaged as an extension of a two-compartment model developed by Williams (1967, 1975) and recently discussed and fundamentally improved by Roels & Kossen (1978) and Roels (1978). Problematically in the formulation of two-compartment models is, that they are lacking in biochemical background of the subdivision of both compartments. Defining a three-compartment model such a problem may be overcome.

A set of mass-conservation equations was developed (Section 3.3.1). The constitutive equations, i.e. expressions for the rates of the conversion processes, were defined (Section 3.3.2) and the model was applied to a chemostat with recycling of biomass (Section 3.3.3). Conclusions, i.e. the complete set of differential and steady-state equations, are summarized in Section 3.4.

3.2 VERBAL DESCRIPTION OF THE THREE-COMPARTMENT MODEL

The proposed three-compartment model can be expressed schematically (Fig. 5). The fundamental assumption of this model is that a cell comprises three basic compartments: (1) a building-block for synthesis, for instance of energy-rich compounds (ATP, GTP), small metabolites (amino acids), DNA and storage materials. This section of the cell is called, the R Compartment

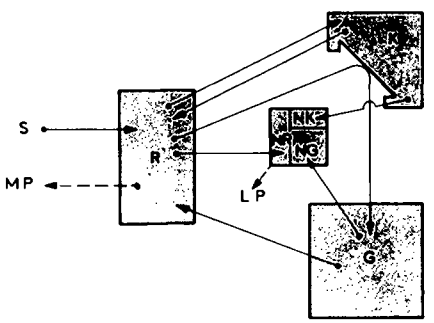


Fig. 5. Diagram of the three-compartment model. S, growth-limiting nutrient; MP, excreted metabolic products; LP, excreted lysis products; NR, loss of R Compartment; NK, loss of K Compartment and NG, loss of G Compartment.

(2) the protein-synthesizing machinery of the cell denoted as the K Compartment
(3) the pool of enzymes and other proteins; the G Compartment.

The main item of this structured model is the K Compartment, which consists mainly of ribosomal-ribonucleic acid (r-RNA), 80-83% and converts R Component (i.e. amino acids) to G Component (i.e. enzymes and other proteins).

Koch (1971) analysed the energy requirements for protein synthesis in bacteria and concluded that the ribosome is the most expensive item in the protein-synthesizing machinery. Therefore Koch argued that ribosomes (80-83% of K Compartment) should be used at highest efficiency. So it has to be expected generally that the net content of prokaryotic RNA is proportional to the growth rate of the micro-organisms and increases with increasing growth rate (Maaløe & Kjeldgaard, 1966; Pace, 1973; Nierlich, 1978). A graphical representation of this physiological phenomenon is shown in Fig. 6, using data from Table 2.

This RNA synthesis is all the more remarkable for its non-specificity. It matters little whether the medium is rich in RNA precursors, carbohydrates or amino acids: the initial response to a change in the nutritional composition of the medium is frequently a changed rate of RNA accumulation (Neidthard, 1963; Koch, 1970; Tempest, 1970).

Interpreting these observations in our model (Fig. 5), with a shift in growth rate, a faster response of the K Component would precede the changing conversion rate from R Component to G Component (i.e. shift in pool of enzymes).

Fig. 6 shows that independent on the species of micro-organisms the K content of cells will be a linear function of the specific growth rate (μ) which depends fully upon the general growth-supporting ability of the environment. From this it may be expected

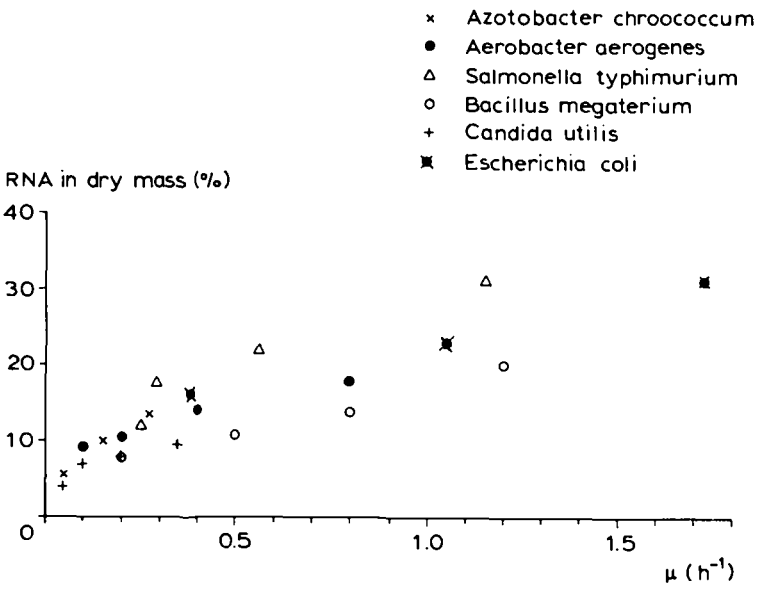


Fig. 6. Growth of different micro-organisms in continuous culture. RNA in dry mass as function of specific growth rate.

Table 2. Biochemical composition (DNA, RNA and protein) of species of micro-organisms and the mass fractions w_R, w_K and w_G in dry mass at different specific growth rates

Organisms	Limiting substrate	μ (h^{-1})	°C	DNA[1]	RNA(w_K)[1]	Protein (w_G)[1]	w_R[1]	Reference
Azotobacter vinelandii	mannitol or ammonia	0.05	30	-	5.4	57	37.6	Dalton & Postgate (1969)
		0.15		2.0	10.2	57	32.8	
		0.27		1.8	13.8	63	23.2	
Aerobacter aerogenes	glycerol	0.10	35	4.2	9.4	73	17.6	Tempest et al. (1965)
		0.20		4.4	10.5	72	17.5	
		0.40		4.3	14.1	73	12.9	
		0.80		3.1	18.3	68	13.7	
Escherichia coli	acetate/ glucose/ broth	0.38	37	3.2	16.3	80	3.7	Forchhammer & Lindahl (1967)
		1.05		2.3	22.9	75	2.1	
		1.73		2.6	30.7	67	2.3	
Salmonella typhimurium	glucose/ broth	0.25	37	4.0	12.0	83	5.0	Maaløe & Kjeldgaard (1966)
		0.29		3.7	18.0	78	4.0	
		0.56		3.7	22.0	74	4.0	
		1.15		3.5	31.0	67	2.0	
Bacillus megaterium	casein/ mannitol	0.20	35	3.1	8.0			Herbert (1961)
		0.50		2.8	10.8			
		0.80		2.5	14.0			
		1.20		2.3	20.0			
Candida utilis	glucose	0.05	30	0.35	4.2	35.2	60.6	Brown & Rose (1969)
		0.10		0.30	6.8	34.4	58.8	
		0.20		0.30	7.6	30.0	62.4	
		0.35		0.52	9.5	30.6	59.9	

1. as % of dry mass.

that also a mixture of micro-organisms, for example activated sludge will respond in the same way.

The R Compartment is produced by uptake of externally available nutrients (S) which can be a mixture of fresh medium, excreted metabolic intermediates (MP) and products of cells lysis (LP).

Part of it may also function as a store for materials. For example when nitrogen limits growth and carbon is in excess, the conversion from R Component to G Component is inhibited. Then in the R Compartment, the carbon surplus will be transformed to polysaccharides or lipids, or both (Dawes & Senior, 1973).

Of the total energy consumed by prokaryotes, the maintenance energy can be an important fraction especially at low specific growth rates (Pirt, 1975; Stouthamer, 1976, 1977). It has to be supplied to maintain the ordered state of micro-organisms, even when growth stops. In the proposed model, one can to distinguish the two rates i.e. the energy requirements in turnover of proteins (G Components) and RNA (K Components). Both components are assumed to degrade to R Component by a quantitative process of for example depolymerisation.

The viability (ability to grow) of a culture of micro-organisms and thus its metabolic activity depends upon the range of specific growth rate (μ) (Tempest et al., 1967; b; Weddle & Jenkins, 1971; Grady & Roper, 1974; Rawlings & Woods, 1977). For activated sludge in laboratory culture, Weddle & Jenkins found the increasing viability to be $\mu < 0.04\ h^{-1}$, 20 - 70%; $0.04 < \mu < 0.08\ h^{-1}$, 70-100% and $\mu > 0.08\ h^{-1}$, 100%.

The conversion of viable cells to non-viable (i.e. inability to grow) or dead cells can be incorporated in our model by postulating conversions considered irreversible from components R, K and G to the components NR, NK and NG, respectively.
In activated sludge, loss of active biomass may also occur by consumption by predators.

3.3 MATHEMATICAL APPROACH

3.3.1 The balance equations

Modelling a bio-engineering system, our mathematical approach is based on matrix representation which provide a well-ordered and systematic analysis of the biosystem (Fredrickson et al., 1970; Roels & Kossen, 1978, Roels, 1978).

The system of a growing microbial culture is thought to be a collection of whole microbial cells, the biotic phase, growing in a growth-supporting environment, the abiotic phase. The growth-limiting nutrient in this system is assumed to be the carbon and energy source, (S).
Let us assume that the system is built up by seven components (Fig. 5). This biosystem can be represented by a general state vector $\bar{C}$ of seventh order.

Recently Fredrickson (1976) and Roels & Kossen (1978) pointed out that the dilution of intracellular components brought about by expansion (growth) of the biomass has been overlooked in some structured growth models. For example, Roels & Kossen calculated from the model of Williams (1967, 1975) that the rate of synthesis of the structural/genetic compartment would be proportional to the second power of the number of cells. A fourfold increase in the rate of synthesis of that compartment is then predicted if the number of micro-organisms has been doubled. Roels and Kossen (1978) concluded this to be inconsistent with existing experimental evidence, and showed this to result from an incorrect model structure.

Recently Chase (1977) developed a dynamic kinetic model of the activated sludge

process in which the rate of substrate utilization is controlled by the concentration of enzymes in the biomass. However, according to the formalism of Fredrickson (1976) the model of Chase is not intrinsic: the term representing the dilution of the pool of enzymes brought about by growth of the biomass is absent in the dynamic equations for enzyme concentration. The equilibrium rate of synthesis of enzyme in batch is in the model of Chase given as (his notation):

$$\left(\frac{dz}{dt}\right)_{synthesis} = k_2 . z_{equilibrium} \tag{3a}$$

in which z is the specific concentration of enzymes, (mass fraction of enzymic protein) in the cells and k_2 is a rate constant. If intrinsic variables be used, Equation 3a should be written as (notation of Chase): $(d(z.X)/dt)_{syn} = k_2\, z_{equil.}.X$ where X is the concentration of biomass. After partial differentiation of the left side of this intrinsic equation, writing $(dz/dt)_{syn}$ explicitly, we have

$$\left(\frac{dz}{dt}\right)_{syn} = k_2 . z_{equil.} - \mu . z_{equil.} \tag{3b}$$

in which $\mu . z_{equil.}$ represents the dilution of the mass fraction of enzymes, for example in dry matter, caused by growth.

In order to overcome the problems discussed above Fredrickson and Roels & Kossen proposed to distinguish two types of reactions in a biological system:
(1) Reactions of the biotic components, i.e. components within the microbial cells, which are governed by the mass fractions of the components in dry mass, thus expressed as intrinsic variables
(2) Reactions of the abiotic components, i.e. components outside the microbial cells, which are controlled by the mass concentrations of it in culture fluid. In dense cultures, however, the concentration of abiotic components in culture fluid must in fact be taken as the concentration of abiotic components per unit volume of abiotic medium.

To define the two types of reactions in a microbial culture, the general state vector $\overline{C}$ of the biosystem must be divided into a state vector of abiotic components $\overline{Y}$ and a state vector of biotic components $\overline{X}$. In our specific model only in the abiotic phase, one component S has been defined and thus $\overline{Y}$ is of the first order. In the biotic phase six components R, K, G, NR, NK and NG have been defined resulting in a biotic state vector $\overline{X}$ of sixth order.
In vector notation we obtain:

$$\overline{C} = \left[\overline{Y}\ \overline{X}\right] \tag{4}$$

In unstructured models it is assumed that conversions take place between biotic components in terms, for instance of dry mass or protein and the abiotic components in the medium. However, in structured models, mutual conversions between biotic components can be distinguished too.

In our model, eight types of conversion processes will be distinguished and are graphically shown in Fig. 5:

1. Conversion of S to R	$S \rightarrow Y_{SR}R$	rate r_{SR}	(5)
2. Conversion of R to K: RNA synthesis	$R \rightarrow Y_{RK}K$	rate r_{RK}	(6)
3. Conversion of R to G governed by the K Compartment: protein synthesis	$R \rightarrow Y_{RG}G$	rate r_{RG}	(7)
4. Turnover of K to R	$K \rightarrow R$	rate r_{KR}	(8)
5. Turnover of G to R	$G \rightarrow R$	rate r_{GR}	(9)
6. Loss of R by conversion to NR: shift to non-viability or death	$R \rightarrow NR$	rate r_{NR}	(10)
7. Loss of K by conversion to NK: shift to non-viability or death	$K \rightarrow NK$	rate r_{NK}	(11)
8. Loss of G by conversion to NG: shift to non-viability or death	$G \rightarrow NG$	rate r_{NG}	(12)

In the first three conversion processes (Eqns 5-7), allowances are made for substrate consumed in energy supplying reactions or substrate mass given off as CO_2, H_2O or other metabolites. The yield coefficients Y_{SR}, Y_{RK} and Y_{RG} are defined as amount of a biotic component divided by substrate or component converted and are expressed as mass ratios.

The turnover processes (Eqns 8 and 9) are assumed to be pure depolymerization reactions in one-to-one relationships. Quantitative conversions for the loss or decay process (Eqns 10-12) are assumed too. The stoichiometric coefficients of the components on the left of Eqns 5-12 are considered negative.

The rates of the 8 conversion reactions in the microbial system may be presented by a vector $\overline{r}$ of eight order:

$$\overline{r} = [r_{SR}\ r_{RK}\ r_{RG}\ r_{GR}\ r_{KR}\ r_{NR}\ r_{NK}\ r_{NG}] \tag{13}$$

The stoichiometric matrix of the abiotic components of the defined conversion reactions (Eqns 5-12) α' will be of order 8 x 1 and can be written in the form

$$\alpha' = \begin{bmatrix} -1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix} \tag{14}$$

Subsequently the stoichiometric matrix of the biotic components α of the order 8 x 6 (i.e. 8 conversion reactions and 6 components) is given by:

$$\alpha = \begin{bmatrix} Y_{SR} & 0 & 0 & 0 & 0 & 0 \\ -1 & Y_{RK} & 0 & 0 & 0 & 0 \\ -1 & 0 & Y_{RG} & 0 & 0 & 0 \\ +1 & 0 & -1 & 0 & 0 & 0 \\ +1 & -1 & 0 & 0 & 0 & 0 \\ -1 & 0 & 0 & +1 & 0 & 0 \\ 0 & -1 & 0 & 0 & +1 & 0 \\ 0 & 0 & -1 & 0 & 0 & +1 \end{bmatrix} \quad (15)$$

Finally we have to define the vectors of flow rates of the different components into the system after which the different balance equations of mass of the six components can be formulated.

First, the vector of flow rate of the abiotic component S can be expressed as

$$\bar{\phi}_Y = [\phi_S] \quad (16)$$

The expression for ϕ_S (g/l.h) depends on the technical system defined. For example, in a batch system $\phi_S = 0$. In Section 3.3.3 ϕ_S will be defined for a chemostat with recycling of biomass.

The increase in the abiotic component S in the system is given by the abiotic balance.

Accumulation of S per unit time t per unit volume	=	Conversion of S per unit time t per unit volume	+	Net flow of S per unit time t per unit volume	(17)

The kinetics of the abiotic processes are completely defined by the vector of reaction rates (Eqn 13) and the stoichiometric matrix of the abiotic components α' (Eqn 14). Using matrix notation, Equation 17 can now be reformulated and the balance equation of S becomes:

$$\frac{d\bar{Y}}{dt} = \bar{r}.\alpha' + \bar{\phi}_Y \quad (18)$$

Substituting for $\bar{r}$, Eqn 13; for α', Eqn 14 and for $\bar{\phi}_Y$, Eqn 16 in Eqn 18 and the balance equation of S in the culture is given by

$$\frac{dC_S}{dt} = -r_S + \phi_S \quad (19)$$

where C_S is the concentration of component S in culture.

Secondly the vector of flow rates of the biotic components in the system ϕ_R, ϕ_K, ϕ_G, ϕ_{NR}, ϕ_{NK} and ϕ_{NG} has to be formulated. However, we must bear in mind that the biotic phase of the system is thought to be a collection of whole microbial cells. So biotic substances entities can enter or leave the system only as components of the cells. In a

distributed model, the biotic components may be expressed in organic dry mass of the culture (g/g).

To express the biotic components in terms of fractions of dry mass, the state vector of these components $\overline{X}$ in Equation 4 may be reformulated as follows

$$\overline{X} = \overline{W}.C_X \tag{20}$$

where the new state vector of biotic components $\overline{W}$ is defined as

$$\overline{W} = \left[\frac{C_R}{C_X} \quad \frac{C_K}{C_X} \quad \frac{C_G}{C_X} \quad \frac{C_{NR}}{C_X} \quad \frac{C_{NK}}{C_X} \quad \frac{C_{NG}}{C_X}\right] \tag{21}$$

or

$$\overline{W} = \left[w_R \quad w_K \quad w_G \quad w_{NR} \quad w_{NK} \quad w_{NG}\right] \tag{22}$$

where w_i is the mass fraction in dry mass of a biotic component i.
The elements of $\overline{W}$ are not fully independent because

$$w_R + w_K + w_G + w_{NR} + w_{NK} + w_{NG} = 1 \tag{23}$$

The vector of flow rates of the biotic components as mass fractions of dry mass into the system can be written now as

$$\overline{\phi}_X = \phi_X.\overline{W} \tag{24}$$

where ϕ_X is the flow rate of whole cells into the culture.

Using again the principle of balance equation for each of the biotic-phase components, the balance for the biotic component is

Accumulation of a biotic component per unit time t per unit volume	=	Conversion of a biotic component per unit time t per unit volume	+ Net flow of a biotic component per unit time t per unit volume

(25)

The kinetics of any process in which the biotic components of the system participate are completely defined by the vector of reactions rates (Eqn 13) and the matrix of stoichiometric coefficients of the biotic components α (Eqn 15).
Using matrix notation, Equation 25 can now be reformulated as follows

$$\frac{d\overline{X}}{dt} = \frac{d(\overline{W}.C_X)}{dt} = \overline{r}.\alpha + \overline{\phi}_X \tag{26}$$

Partial differentiation of the left of Eqn 26 and substituting Eqn 24 for $\overline{\phi}_X$, the balance equation of the biotic components becomes

$$C_X \frac{\partial \overline{W}}{\partial t} + \overline{W} \frac{\partial C_X}{\partial t} = \overline{r}.\alpha + \phi_X.\overline{W} \tag{27}$$

If the balance equations of each of the elements of biotic state vector $\overline{W}$ are added, we may then write the balance equation of biotic components in the system as

$$C_X\left(\frac{\partial \omega_R}{\partial t} + \frac{\partial \omega_K}{\partial t} + \frac{\partial \omega_G}{\partial t} + \frac{\partial \omega_{NR}}{\partial t} + \frac{\partial \omega_{NK}}{\partial t} + \frac{\partial \omega_{NG}}{\partial t}\right) + \left(\omega_R + \omega_K + \omega_G + \omega_{NR} + \omega_{NK} + \omega_{NG}\right)\frac{\partial C_X}{\partial t} =$$

$$= (\overline{r}.\alpha).\overline{1} + \phi_X(\omega_R + \omega_K + \omega_G + \omega_{NR} + \omega_{NK} + \omega_{NG}) \qquad (28)$$

where $\overline{1}$ is the unit column vector of order 6.
There the sum of the elements of $\overline{W}$ is equal to unity (Eqn 23), the overall change in time will be zero. Then Equation 28 can be simplified and the balance equation of dry mass in the system in vector notation becomes

$$\frac{\partial C_X}{\partial t} = (\overline{r}.\alpha).\overline{1} + \phi_X \qquad (29)$$

This vector notation can be transformed back into a balance equation for dry mass by multiplying out the matrix and substituting for $\overline{r}$ and α, with Equations 13 and 15, respectively.
The result of this operation is

$$\frac{\partial C_X}{\partial t} = Y_{SR}.r_{SR} + r_{RK}(Y_{RK} - 1) + r_{RG}(Y_{RG} - 1) + \phi_X \qquad (30)$$

The series of biotic components expressed as mass fractions of dry mass in the culture is found by substituting Equation 29 for $\partial C_X/\partial t$ into Eqn 27. In matrix notation the balance equations of biotic components as mass fractions of dry mass are given by

$$\frac{\partial \overline{W}}{\partial t} = \frac{1}{C_X}\{\overline{r}.\alpha - ((\overline{r}.\alpha).\overline{1})\overline{W}\} \qquad (31)$$

This compact notation can be transformed back into the different balance equations of each of the biotic components by multiplying out the matrix and writing separate expressions for the elements of the state vecter $\overline{W}$. These operations result in the following series of equations

$$\frac{\partial \omega_R}{\partial t} = \frac{1}{C_X}\{Y_{SR}.r_{SR} - r_{RK} - r_{RG} - r_{GR} - r_{KR} - r_{NR} - (\frac{\partial C_X}{\partial t} - \phi_X)\omega_R\} \qquad (32)$$

$$\frac{\partial \omega_K}{\partial t} = \frac{1}{C_X}\{Y_{RK}.r_{RK} - r_{KR} - r_{NK} - (\frac{\partial C_X}{\partial t} - \phi_X)\omega_K\} \qquad (33)$$

$$\frac{\partial \omega_G}{\partial t} = \frac{1}{C_X}\{Y_{RG}.r_{RG} - r_{GR} - r_{NG} - (\frac{\partial C_X}{\partial t} - \phi_X)\omega_G\} \qquad (34)$$

$$\frac{\partial \omega_{NR}}{\partial t} = \frac{1}{C_X}\{r_{NR} - (\frac{\partial C_X}{\partial t} - \phi_X)\omega_{NR}\} \qquad (35)$$

$$\frac{\partial \omega_{NK}}{\partial t} = \frac{1}{C_X}\{r_{NK} - (\frac{\partial C_X}{\partial t} - \phi_X)\omega_{NK}\} \qquad (36)$$

$$\frac{\partial \omega_{NG}}{\partial t} = \frac{1}{C_X}\{r_{NG} - (\frac{\partial C_X}{\partial t} - \phi_X)\omega_{NG}\} \qquad (37)$$

Now that we have derived a set of balance equations of a growing culture of micro-organisms based on the postulates of the three compartment model (Section 3.2) (Eqns 19, 29 and 32-37), we must postulate constitutive equations for the rates of the biochemical processes (Eqn 13) and define flow rates of substrate ϕ_S and biomass ϕ_X.

3.3.2 The constitutive equations

3.3.2.1 Rate of conversion of substrate S to component R: r_{SR}

Constitutive equations are expressions for the rates of conversion processes in the biosystem in terms of the elements of the biotic and abiotic state vector and other intensive variables like pH and temperature. For example, in order to describe substrate kinetics in activated sludge, Monod-like relationships are commonly used in sanitary engineering. However, these relationships are only of limited use (Section 1.1.2) and would be inadequate, as will be shown in Chapter 4 if for example, population shifts occur (Jones, 1973) or if enzymatic shifts in metabolism of the micro-organisms occur at fast growth rates (Shehata & Marr, 1971).

Complicated sequences of biochemical processes may be approached classically by transforming to a set of linear constitutive equations.
However, this linearization procedure for modelling a conversion process at different substrate concentrations will be only a good approximation in a small region of the substrate concentration. Savageau (1976) therefore, proposed to approximate the constitutive equation of a rate of a conversion by taking the natural logarithm of this rate and to develop this function with the Taylor series method. Postulating r_{SR} to be a function of C_S and C_X, $\ln r_{SR}(C_S, C_X)$ may be expanded in a Taylor series about a concentration C_S, taken as $\tilde{C}_S$ and C_X, taken as $\tilde{C}_X$. The tilde denotes a steady-state quantity. The Taylor series of $\ln r_{SR}(C_S, C_X)$ is now given by

$$\ln r_{SR}(C_S,C_X) = \ln r_{SR}(\tilde{C}_S,\tilde{C}_X) + \left(\frac{\partial \ln r_{SR}}{\partial \ln C_S}\right)_{\tilde{C}_S,\tilde{C}_X} \cdot (\ln C_S - \ln \tilde{C}_S)$$

$$+ \left(\frac{\partial \ln r_{SR}}{\partial \ln C_X}\right)_{\tilde{C}_S,\tilde{C}_X} \cdot (\ln C_X - \ln \tilde{C}_X) \qquad (38)$$

If $\ln k_{SR}$ is defined as

$$\ln k_{SR} = \ln r_{SR}(\tilde{C}_S,\tilde{C}_X) - \left(\frac{\partial \ln r_{SR}}{\partial \ln C_S}\right)_{\tilde{C}_S,\tilde{C}_X} \cdot \ln \tilde{C}_S - \left(\frac{\partial \ln r_{SR}}{\partial \ln C_X}\right)_{\tilde{C}_S,\tilde{C}_X} \cdot \ln \tilde{C}_X \qquad (39)$$

where k_{SR} is denoted as the rate constant of the substrate utilization process about $\tilde{C}_S$ and $\tilde{C}_X$. The ordinary derivatives of $\ln r_{SR}$ with respect to C_S and C_X respectively, about C_S, taken as $\tilde{C}_S$ and C_X, taken as $\tilde{C}_X$ may be defined as

$$a_S = \left(\frac{\partial \ln r_{SR}}{\partial \ln C_S}\right)_{\tilde{C}_S, \tilde{C}_X} \tag{40}$$

and

$$a_X = \left(\frac{\partial \ln r_{SR}}{\partial \ln C_X}\right)_{\tilde{C}_S, \tilde{C}_X} \tag{41}$$

whereas a_S and a_X are denoted as the formal orders of the substrate utilization reaction. Substituting Equations 39-41 into the Taylor series of $\ln r_{SR}$ (Eqn 38), we have

$$\ln r_{SR} = \ln k_{SR} + a_S \ln C_S + a_X \ln C_X \tag{42}$$

or

$$r_{SR} = k_{SR} \cdot C_S^{a_S} \cdot C_X^{a_X} \tag{43}$$

If r_{SR} is assumed to be independent of limitation of substrate diffusion to the micro-organisms, for example by flocculent growth or high concentration of the growing culture, r_{SR} is proportional to C_X, i.e. $a_X = 1$ and we may define the constitutive equation of the conversion of component S to component R in the culture as

$$r_{SR} = k_{SR} \cdot C_S^{a_S} \cdot C_X \tag{44}$$

The boundary conditions of the Equation 44 can be defined if C_S is increased until the derivative of $\ln r_{SR}$ with respect to C_S (Eqn 40) at C_X constant, becomes equal to zero i.e. $a_S = 0$, above a fixed concentration of C_S. This means that r_{SR} approaches its maximum $r_{SR} = r_{SR,max}$, and becomes only dependent on the concentration of dry mass C_X. With respect to these boundary conditions, i.e. $C_S = C_{S,max}$ and $a_S = 0$, Equation 39 can be written as:

$$\ln k_{SR} = \ln r_{SR,max} - \ln C_X \tag{45}$$

or

$$k_{SR} = r_{SR,max} / C_X \tag{46}$$

In fact, by the method of Savageau a 'n^{th} order rate equation' (Eqn 44) has been obtained which is extensively used in chemical reactor design (Levenspiel, 1971). This type of constitutive equation for substrate utilization by micro-organisms was already adopted by Grau et al. (1975) and by Benefield & Randall (1977) to fit their kinetic experiments. The reasoning presented here can be assumed a rational for the application of such an equation in the present case.

3.3.2.2 Rate of conversion of R Component to K Component and G Component, respectively: r_{RK} and r_{RG}

In the two-compartment model of Roels & Kossen (1978) and Roels (1978), the kinetic expression for the conversion rate of the synthetic section (K Compartment) to the structural genetic section (G Compartment) is thought to be a bimolecular reaction:

$$r_{KG} = k_{KG} \cdot w_K \cdot w_G \cdot C_X \tag{47}$$

in which w_K, w_G are the mass fractions of K and G Components. With this constitutive equation, their model predicts a linear relationship between the steady state of the K Component and specific growth rate (μ). It shows a strong resemblance to Figure 6. However a quantitative verification with data from Table 2 shows that the increase in K Component as a function of μ, as predicted by the model of Roels & Kossen (1978) and Roels (1978) is too pronounced. In actual practice a slower increase is found. Further the 'maintenance factor' of G Compartment, m_G was calculated to be a factor 10 or more as high as that experimentally found (Pirt, 1975).

So it is uncertain whether r_{RK} and r_{RG} in our model are simply proportional to w_R, w_K and w_G in such complicated metabolically regulated conversion processes between the compartments as assumed by Roels & Kossen (1978) and Roels (1978). Therefore in the model proposed:

1) r_{RK} is postulated to be a function of the mass fraction of R Component in cells, representing the content of building block for synthesis, for instance of macromolecular compounds (RNA, DNA, enzymes), and of the mass fraction of K Component, i.e. the protein-synthesizing machinery. Presumably RNA synthesis will be governed by the contents of R Component and K Component in cells (Section 3.2). Mathematically

$$r_{RK} = f(w_R, w_K)\ C_X \tag{48}$$

2. r_{RG}, i.e. rate of synthesis of enzymes and other proteins, is thought to be governed by the protein-synthesizing machinery and the pool of enzymes itself (Section 3.2). This hypothesis may be formulated as

$$r_{RG} = f(w_K, w_G)\ C_X \tag{49}$$

3. The idea in this model is to approach both functions $f(w_R, w_K)$ and $f(w_K, w_G)$ with the Taylor series method for functions of two variables. The functions have to be defined with derivatives of 2nd order at least. If the net increase of biotic components is proportional to the growth rate μ (Table 2; Fig. 6), then the rate of synthesis of biotic components must be proportional to the second power of μ or second power of the net increase in a biotic component (Koch, 1971).

The Taylor series for $f(w_R, w_K)$ and for $f(w_K, w_G)$ are expanded about w_{RO}, w_{KO} and w_{GO}, i.e. the content of a biotic component in cells when growth stops ($\mu = 0$), and substituted in the Equations 48 and 49, respectively, r_{RK} and r_{RG} can be formulated as

$$r_{RK} = f(\omega_{RO}, \omega_{KO}) + f_{\omega_R}(\omega_{RO}, \omega_{KO})(\omega_R-\omega_{RO}) + f_{\omega_K}(\omega_{RO}, \omega_{KO})(\omega_K-\omega_{KO}) + \frac{1}{2!}\left[f_{\omega_R\omega_R}(\omega_{RO}, \omega_{KO})(\omega_R-\omega_{RO})^2 + 2f_{\omega_R\omega_K}(\omega_{RO}, \omega_{KO})(\omega_R-\omega_{RO})(\omega_K-\omega_{KO}) + f_{\omega_K\omega_K}(\omega_{RO}, \omega_{KO})(\omega_K-\omega_{KO})^2\right] \quad (50)$$

where f_{ω_R} and f_{ω_K} are the partial derivates of first order evaluated at $(\omega_{RO}, \omega_{KO})$. Similarly the partial derivates of second order are denoted by $f_{\omega_R\omega_R}$, $f_{\omega_R\omega_K}$ and $f_{\omega_K\omega_K}$, also evaluated at $(\omega_{RO}, \omega_{KO})$.

$$r_{RG} = f(\omega_{KO}, \omega_{GO}) + f_{\omega_K}(\omega_{KO}, \omega_{GO})(\omega_K-\omega_{KO}) + f_{\omega_G}(\omega_{KO}, \omega_{GO})(\omega_G-\omega_{GO}) + \frac{1}{2!}\left[f_{\omega_K\omega_K}(\omega_{KO}, \omega_{GO})(\omega_K-\omega_{KO})^2 + 2 f_{\omega_K\omega_G}(\omega_{KO}, \omega_{GO})(\omega_K-\omega_{KO})(\omega_G-\omega_{GO}) + f_{\omega_G\omega_G}(\omega_{KO}, \omega_{GO})(\omega_G- \omega_{GO})^2\right] \quad (51)$$

where f_{ω_K} and f_{ω_G} are the partial derivatives of first order evaluated at $(\omega_{KO}, \omega_{GO})$ and the partial derivatives of second order are denoted by $f_{\omega_K\omega_K}$, $f_{\omega_K\omega_G}$ and $f_{\omega_G\omega_G}$, evaluated at $(\omega_{KO}, \omega_{GO})$ too.

3.3.2.3 Rates of turnover of K Component and G Component respectively: r_{KR} and r_{GR}

According to Roels & Kossen (1978) and Roels (1978) a turnover of the compartments is assumed in order to give rise to maintenance processes in the microbial cells. Allowance for this feature can be made by assuming a first-order degradation of K and G Components to R Component. The two constitutive equations become

$$r_{KR} = m_K \cdot \omega_K \cdot C_X \quad (52)$$

and

$$r_{GR} = m_G \cdot \omega_G \cdot C_X \quad (53)$$

in which the specific maintenance rate; m_K and m_G, may be regarded as turnover rates of K Component and G Component, respectively.

3.3.2.4 Rates of loss of R, K, and G Components in NR, NK, and NG Components respectively: r_{NR}, r_{NK} and r_{NG}

Loss of viability of organisms in activated sludge (Weddle & Jenkins, 1971; Grady & Roper, 1974) causes a reduction in the active cell fraction of the biomass, especially with decreasing growth rate (Section 3.2).

In accordance with the concept of Sinclair & Topiwala (1970), the loss of viability is assumed to follow first-order kinetics with rate constants: k_{NR}, k_{NK} and k_{NG}.

The proposed constitutive equations for loss of cell components may be written as

$$r_{NR} = k_{NR} \cdot \omega_R \cdot C_X \tag{54}$$

$$r_{NK} = k_{NK} \cdot \omega_K \cdot C_X \tag{55}$$

$$r_{NG} = k_{NG} \cdot \omega_G \cdot C_X \tag{56}$$

where the rates are again proportional to mass fractions of whole cells in dry mass.

After defining the series of constitutive equations (Eqns 44, 50-56), the last step is to formulate expressions for the flow rates ϕ_S and ϕ_X.

3.3.3 Formulation of the flow rates of substrate ϕ_S and dry mass ϕ_X in a chemostat with recycling of biomass

Highest efficiency of conventional purification of waste water can be achieved by increasing the rate of purification per unit reactorvolume. This is possible if the biomass concentration is increased above the value possible in an once-through chemostat, that is $Y_{SR}(C_{SO} - C_S)$. The optimum concentration within environment limits is achieved by concentrating the biomass in a separator outside the reactor. Conventionally, the concentrated stream is fed back to the reactor and a part of it is discharged as surplus sludge. In this way, retention time of biomass and of medium can be regulated independently. For better control of biomass concentration in the reactor, surplus sludge was removed from the reactor directly rather than out of the recycling stream of biomass. The chemostat with activated sludge was stirred and aerated. Figure 7 shows the chemostat with recycling of biomass and its parameters.

The net flow rate of substrate into the system, ϕ_S, will be

	rate of incoming substrate per unit volume		rate of feedback substate per unit volume		rate of outflow substrate per unit volume		rate of waste substrate per unit volume	
ϕ_S =		+		-		-		(57)

$$\phi_S = (\phi_0 \cdot C_{SO} + \omega_3 \cdot \phi_1 \cdot C_S - \phi_1 \cdot C_S - \omega_1 \cdot \phi_0 \cdot C_S)/V \tag{58}$$

where ϕ_0 is the inflow rate of incoming substrate C_{SO}; ϕ_1 is the outflow rate of substrate in the reactor C_S; ω_3 is the mass fraction of ϕ_1 fed back and ω_1 is the mass fraction of ϕ_0 discharged from the reactor as waste biomass; V is the working volume of the reactor.

The outflow rate ϕ_1 can be written as

$$\phi_1 = \phi_0 + \omega_3 \cdot \phi_1 - \omega_1 \phi_0 \tag{59}$$

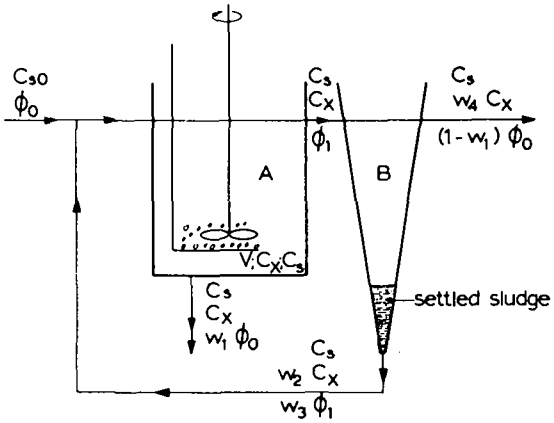


Fig. 7. Chemostat with recycling of biomass. A, reactor; B, separator; V, working volume of reactor; C_X, concentration of biomass; C_{SO}, concentration of incoming substrate; C_S, concentration of substrate in reactor; ϕ_0, flow rate of incoming substrate; ϕ_1, outflow rate of substrate in reactor; w_1, w_2, w_3 and w_4, mass fractions.

and writing ϕ_1 explicitly

$$\phi_1 = \frac{(1 - w_1)}{(1 - w_3)} \cdot \phi_0 \tag{60}$$

Substituting Eqn 60 for ϕ_1 in Equation 58, where $\phi_0 / V = D$ and D is the dilution rate of the reactor, we obtain

$$\phi_S = D.C_{SO} + \frac{w_3(1 - w_1)}{(1 - w_3)} . D.C_{SO} - \frac{(1 - w_1)}{(1 - w_3)} .D.C_{SO} - w_1.D.C_{SO} \tag{61}$$

and after rearrangement we can define ϕ_S as:

$$\phi_S = D(C_{SO} - C_S) \tag{62}$$

In the same way the net flow rate of biomass ϕ_X can be formulated. It will be for our system (Fig. 7)

$$\begin{matrix} \text{rate of incoming} \\ \phi_X = \text{biomass per} \\ \text{unit volume} \end{matrix} - \begin{matrix} \text{rate of waste} \\ \text{of biomass per} \\ \text{unit volume} \end{matrix} - \begin{matrix} \text{rate of outflow} \\ \text{of biomass per} \\ \text{unit volume} \end{matrix} \tag{63}$$

$$\phi_X = (w_3.\phi_1.w_2.C_X - w_1.\phi_0.C_X - \phi_1.C_X)/V \tag{64}$$

where $w_2.C_X$ is the fraction of biomass fed back into the reactor. Substituting Equation 60 for ϕ_1 into Equation 64 we have

$$\phi_X = \frac{w_2 \cdot w_3 (1 - w_1)}{(1 - w_3)} . D . C_X - w_1 . D . C_X - \frac{(1 - w_1)}{(1 - w_3)} . D . C_X \tag{65}$$

The biomass balance for the separator is given by

$$\phi_1 . C_X = (1 - w_1) \phi_0 . w_4 . C_X + w_3 . \phi_1 . w_2 . C_X \tag{66}$$

defining $w_4 . C_X$ as the fraction of biomass in the liquid stream leaving the separator. Substituting again for ϕ_1 (Eqn 60) and after rearrangement, w_2 may be written explicitly as

$$w_2 = \frac{1 - w_4 + w_3 . w_4}{w_3} \tag{67}$$

Substituting Equation 67 in Equation 65 and rewriting the right side of it, we obtain

$$\phi_X = - \{w_1 + (1 - w_1) w_4\} D . C_X \tag{68}$$

or

$$\phi_X = - w_D . D . C_X \tag{69}$$

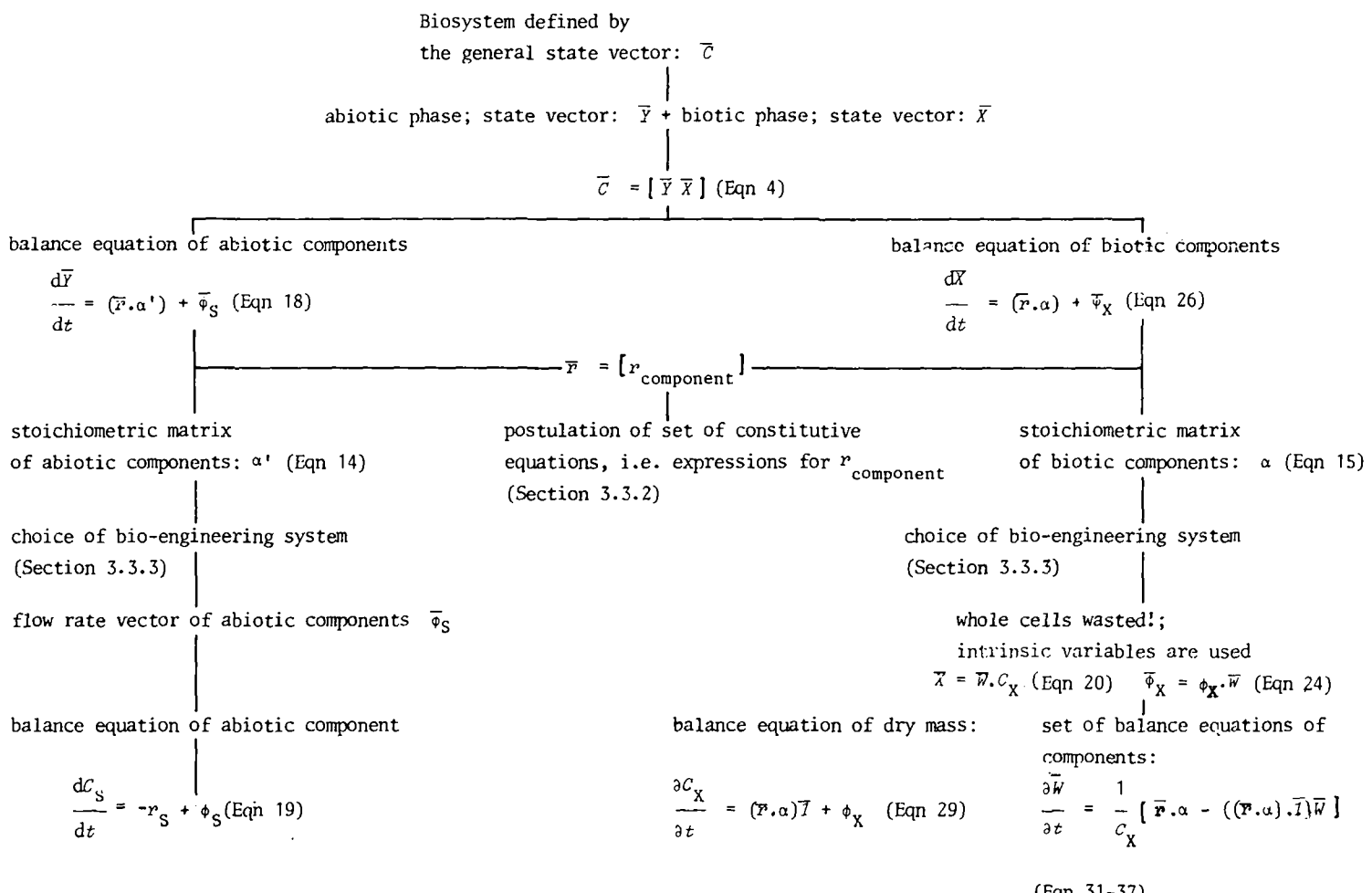
where w_D is called the hold-up factor, defined as $w_D = w_1 + (1 - w_1) w_4$.
It is the addition of the fraction rate of total discharge of biomass leaving the system with the effluent stream, $w_4(1 - w_1)$ and the fraction rate of discharge of biomass taken from the reactor directly as discharged activated sludge, w_1.

The factor w_4 defines the settling of activated sludge and can be $0 < w_4 < 1$. If $w_4 = 0$, there is ideal settling and if $w_4 = 1$ there is no settling at all in the separator. In practice, intermediate values are obtained.
Finally w_D defines the technical system used. If $w_D = 1$, a once-through system is designed and if $w_D = 0$, a batch system.

3.4 SUMMARY OF THE COMPLETE SET OF DIFFERENTIAL- AND STEADY-STATE EQUATIONS OF THE THREE-COMPARTMENT MODEL

Scheme 1 represents the mathematical development of the three-compartment model. The final result of this approach is the definition of the set of state and steady-state equations. In a steady state of the culture, the mass concentration of biomass, C_X, of growth-limiting substrate C_S and biotic mass fractions remain constant.

Scheme 1. Mathematical development of the structured model.



3.4.1 State equation of the limiting substrate concentration: C_S

If Equation 44 and 62 are respectively substituted for r_{SR} and ϕ_S, in Equation 19, the state equation of C_S can be defined as

$$\frac{dC_S}{dt} = - k_{SR}.C_S^{a_S}.C_X + D\ (C_{SO} - C_S) \tag{70}$$

3.4.2 Rate of uptake of substrate by R Compartment: q_{SR}

In steady state, $dC_S/dt = 0$ and the state equation of C_S (Eqn 70) can be reformulated as follows

$$\tilde{q}_{SR} = \frac{D(C_{SO} - \tilde{C}_S)}{\tilde{C}_X} = k_{SR}.\tilde{C}_S^{a_S} \tag{71}$$

in which $\tilde{q}_{SR}$ is defined as the total consumption rate of substrate divided by the concentration of biomass. The tilde denotes a steady-state quantity.

For quantitative kinetic analysis, the effect of altering $\tilde{C}_S$ on $\tilde{q}_{SR}$ is determined at different steady states of the culture. Then ln $\tilde{q}_{SR}$ may be plotted against in $\tilde{C}_S$ and k_{SR} and a_S determined. These calculated values of k_{SR} and a_S will be mean values of a series taken on the curve of $\tilde{q}_{SR}$ against $\tilde{C}_S$ (Eqn 39).

3.4.3 Steady-state equation of the concentration of biomass: $\tilde{C}_X$

If steady state in a culture is assumed, $dC_S/dt = 0$ and $\tilde{C}_X$ in Equation 70, written explicitly, becomes

$$\tilde{C}_X = \frac{D(C_{SO} - \tilde{C}_S)}{k_{SR}.\tilde{C}_S^{a_S}} \tag{72}$$

3.4.4 State and steady-state equations of the mass fraction of K Component in dry mass: w_K

Substituting into the balance equation for w_K in the reactor (Eqn 33), Equation 48 for r_{RK} and Equation 69 for ϕ_X, this balance equation can be reformulated as

$$\frac{\partial w_K}{\partial t} = \left[Y_{RK}.f(w_R,w_K).C_X - m_K.w_K.C_X - k_{NK}.w_K.C_X - r_X.w_K\right]\frac{1}{C_X} \tag{73}$$

In steady state when $\partial w_K/\partial t = 0$, the rate of conversion of R Component to K Component, $f(w_R,w_K)$ can be formulated from Equation 73 as

$$\tilde{f}(\omega_R,\omega_K) = \frac{1}{Y_{RK}}\left[m_K.\tilde{\omega}_K + k_{NK}.\tilde{\omega}_K + (r_X.\tilde{\omega}_K)/C_X \right] \quad (74)$$

where the tilde denotes assumption of steady state.

The rate of conversion of R Component to K Component, $r_{RK} = f(\omega_R,\omega_K)C_X$ is approximated with a second-order Taylor series for functions of two variables (Section 3.3.2.2; Eqn 50). This general definition of the constitutive equation of r_{RK} was used because of the uncertainty in regulation by ω_R and ω_K in such a complicated metabolically regulated conversion process. However experimentally it is difficult to determine the constants of the Taylor series. Therefore in the three-compartment model, the following assumption was made.

For growth in non-steady state r_{RK} is assumed to be a small oscillation about r_{RK} in steady state: $r_{RK} = f(\omega_R, \omega_K)C_X$ (Eqn 74). Then $r_{RK} \doteq f(\omega_R, \omega_K)C_X$ in non-steady state may be well approximated by the second-order Taylor series of the steady-state equivalent, i.e. $r_{RK} = \tilde{f}(\omega_R,\omega_K)\ C_X$. The balance equation of ω_K in the reactor (Eqn 73) can then be reformulated as

$$\frac{\partial \omega_K}{\partial t} = \left[Y_{RK}.\tilde{f}(\omega_R, \omega_K)C_X - m_K.\omega_K.C_X - k_{NK}.\omega_K.C_X - r_X.\omega_K \right] \frac{1}{C_X} \quad (75)$$

In Equation 75, the Taylor series of $\tilde{f}(\omega_R,\omega_K)$ as developed in Equation 50, can be solved by using the steady-state assumptions.

From experimental evidence (Table 2; Section 4.2.2.3) linear relationships between mass fractions of cells, ω_R, ω_K, ω_G and specific growth rate may be assumed in steady state. The increase in mass fraction with increasing specific growth rate for $\tilde{\omega}_R$, $\tilde{\omega}_K$ and $\tilde{\omega}_G$, respectively, becomes

$$\tilde{\omega}_R - \omega_{RO} = \alpha_R.\mu \quad (76)$$

$$\tilde{\omega}_K - \omega_{KO} = \alpha_K.\mu \quad (77)$$

$$\tilde{\omega}_G - \omega_{GO} = \alpha_G.\mu \quad (78)$$

where α_R, α_K and α_G are rate constants. Substituting Equations 76-78 into the formal Taylor series of r_{RK} (Eqn 50) where now $f(\omega_R,\omega_K).C_X = \tilde{f}(\omega_R,\omega_K).C_X$, we should have

$$r_{RK} = \left[\tilde{f}(\omega_{RO},\omega_{RK}) + (\tilde{f}_R.\alpha_R + \tilde{f}_K.\alpha_K)\mu + (\tfrac{1}{2}.\tilde{f}_{RR}.\alpha_R^2 + \tilde{f}_{KR}.\alpha_R.\alpha_K + \tfrac{1}{2}.\tilde{f}_{KK}.\alpha_K^2)\ \mu^2 \right] C_X \quad (79)$$

The 0 th order derivative for $\tilde{f}(\omega_R,\omega_K)$ with respect to $\tilde{\omega}_R$ and $\tilde{\omega}_K$ about $\tilde{\omega}_R = \omega_{RO}$ can be derived from Equation 74 if $r_X.\omega_K/C_X = \omega_D.D.\tilde{\omega}_K = \mu.\tilde{\omega}_K$, is set to zero. Then we may formulate

$$f(\omega_{RO},\omega_{KO}) = \frac{(m_K + k_{NK})\ \omega_{KO}}{Y_{RK}} \quad (80)$$

Substituting Equation 80 for $f(\omega_{RO},\omega_{KO})$ into Equation 79, the rate of conversion of R Component to K Component becomes

$$r_{RK} = \left[\frac{(m_K + k_{NK})\ \omega_{KO}}{Y_{RK}} + (\tilde{f}_R.\alpha_R + \tilde{f}_K.\alpha_K)\mu + \right.$$

$$\left. + (\tfrac{1}{2}.f_{RR}.\alpha_R^2 + f_{KR}.\alpha_R.\alpha_K + \tfrac{1}{2}\ f_{KK}.\alpha_K^2)\mu^2 \right] C_X \qquad (81)$$

The state equation of ω_K can be developed by substituting Equations 81 for $r_{RK} = f(\omega_R,\omega_K).C_X = \tilde{f}(\omega_R,\omega_K).C_X$ into Equation 73 and after rewriting the right we obtain

$$\frac{\partial \omega_K}{\partial t} = \frac{1}{C_X}\left[Y_{RK}\{\frac{(m_K + k_{NK})\ \omega_{KO}}{Y_{RK}} + (\tilde{f}_R.\alpha_R + \tilde{f}_K.\alpha_K)\mu + \right.$$

$$+ (\tfrac{1}{2}.\tilde{f}_{RR}.\alpha_R^2 + \tilde{f}_{KR}.\alpha_R.\alpha_K + \tfrac{1}{2}.\tilde{f}_{KK}.\alpha^2{}_K)\mu^2\}\ C_X$$

$$\left. - m_K.\omega_K.C_X - k_{NK}.\omega_K.C_X - r_X.\omega_K \right] \qquad (82)$$

Equation 82 can be solved by carrying out continuous-culture experiments.
The coefficients Y_{RK}, m_K, k_{NK}, ω_{KO}, α_R and α_K can be determined. The two Taylor-series constants in Equation 82 associated with μ and μ^2 respectively, can be easily calculated as shown in Appendix C.

3.4.5 State and steady-state equations for mass fraction of G Component in dry mass: ω_G

The same mathematical procedure can be followed as used in Section 3.4.4 if we start with Equations 34, 49 and 69. Here only the final results will be given.

$$r_{RG} = \left[\frac{(m_G + k_{NG})\ \omega_{GO}}{Y_{RG}} + (\tilde{f}^*_K.\alpha_K + \tilde{f}^*_G.\alpha_G)\mu + \right.$$

$$\left. + (\tfrac{1}{2}.\tilde{f}^*_{KK}.\alpha_K^2 + \tilde{f}_{KG}.\alpha_K.\alpha_G + \tfrac{1}{2}.\tilde{f}^*_{GG}.\ \alpha_G^2)\mu^2 \right] C_X \qquad (83)$$

and

$$\frac{\partial w_G}{\partial t} = \frac{1}{C_X}\Big[Y_{RG}\{\frac{(w_G + k_{NG})\, w_{GO}}{Y_{RG}} + (\tilde{f}^*_K.\alpha_K + \tilde{f}^*_G.\alpha_G)\,\mu +$$

$$+ (\tfrac{1}{2}.\tilde{f}^*_{KK}.\alpha_K^2 + \tilde{f}^*_{KG}.\alpha_K.\alpha_G + \tfrac{1}{2}.\tilde{f}^*_{GG}.\alpha_G^2)\,\mu^2\}\, C_X$$

$$- m_G.w_G.C_X - k_{NG}\, w_G.C_X - r_X.w_G\Big] \qquad (84)$$

3.4.6 State and steady-state equations of the non-viable or dead mass fractions in dry mass: w_{NR}, w_{NK} and w_{NG}

The state equations of the net productions of NR, NK and NG in time are found if Equations 54-57 are substituted for r_{NR}, r_{NK} and r_{NG}, respectively, and $\phi_X = -\, w_D.D.C_X$ (Eqn 69), into Equations 35-37, respectively, we may write

$$\frac{\partial w_{NR}}{\partial t} = \{k_{NR}.w_R.C_X - r_X.w_{NR}\}\,\frac{1}{C_X} \qquad (85)$$

$$\frac{\partial w_{NK}}{\partial t} = \{k_{NK}.w_K.C_X - r_X.w_{NK}\}\,\frac{1}{C_X} \qquad (86)$$

$$\frac{\partial w_{NG}}{\partial t} = \{k_{NG}.w_G.C_X - r_X.w_{NG}\}\,\frac{1}{C_X} \qquad (87)$$

In steady state if $\partial w_{NR}/\partial t = 0$; $\partial w_{NG}/\partial t = 0$ and $\partial C_X/\partial t = 0$, Equations 85-87 may be written, writing the non-viable or dead mass fractions explicitly

$$\tilde{w}_{NR} = k_{NR}.\tilde{w}_R.\,\frac{1}{\mu} \qquad (88)$$

$$\tilde{w}_{NK} = k_{NK}.\tilde{w}_K.\,\frac{1}{\mu} \qquad (89)$$

$$\tilde{w}_{NG} = k_{NG}.\tilde{w}_G.\,\frac{1}{\mu} \qquad (90)$$

3.4.7 State equation for mass concentration of biomass: C_X

If Equations 81 and 83 are substituted respectively for r_{RK} and r_{RG} into Equation 30 and $\phi_X = -\, w_D.D.C_X$ (Eqn 60) the state equation of C_X can be expressed as

$$\frac{\partial C_X}{\partial t} = Y_{SR} \cdot k_{SR} \cdot C_S^{a_S} \cdot C_X + \Big[\frac{(m_K + k_{NK})\ w_{KO}}{Y_{RK}} + (\tilde{f}_R \cdot \alpha_R + \tilde{f}_K \cdot \alpha_K)\ \mu +$$

$$(\tfrac{1}{2} \cdot \tilde{f}_{RR} \cdot \alpha_R^2 + \tilde{f}_{KR} \cdot \alpha_R \cdot \alpha_K + \tfrac{1}{2} \cdot \tilde{f}_{KK} \cdot \alpha_K^2)\ \mu^2 \Big] (Y_{RK} - 1)\ C_X +$$

$$\Big[\frac{(m_G + k_{NG})\ w_{GO}}{Y_{RG}} + (\tilde{f}^*_K \cdot \alpha_K + \tilde{f}^*_G \cdot \alpha_G)\ \mu +$$

$$(\tfrac{1}{2} \cdot \tilde{f}^*_{KK} \cdot \alpha_K^2 + \tilde{f}^*_{KG} \cdot \alpha_K \cdot \alpha_G + \tfrac{1}{2} \cdot \tilde{f}^*_{GG} \cdot \alpha_G^2)\ \mu^2 \Big] (Y_{RG} - 1)\ C_X - w_D \cdot D \cdot C_X \qquad (91)$$

3.4.8 Steady-state equation for mass concentration of limiting substrate: $\tilde{C}_S$

In steady state, $\partial C_X / \partial t = 0$ and dividing the terms of Equation 91 by C_X, we obtain for the concentration of $\tilde{C}_S$ in the culture

$$\tilde{C}_S = \sqrt[a_S]{\Big[\{ \frac{(m_K + k_{NK})\ w_{KO}}{Y_{RK}} + (\tilde{f}_R \cdot \alpha_R + \tilde{f}_K \cdot \alpha_K)\ \mu +$$

$$+ (\tfrac{1}{2} \cdot \tilde{f}_{RR} \cdot \alpha_R^2 + \tilde{f}_{KR} \cdot \alpha_R \cdot \alpha_K + \tfrac{1}{2} \cdot \tilde{f}_{KK} \cdot \alpha_K^2)\ \mu^2 \} (1 - Y_{RK})$$

$$+ \{ \frac{(m_G + k_{NG})\ w_{GO}}{Y_{RG}} + (\tilde{f}^*_K \cdot \alpha_K + \tilde{f}^*_G \cdot \alpha_G)\ \mu +$$

$$+ (\tfrac{1}{2} \cdot \tilde{f}^*_{KK} \cdot \alpha_K^2 + \tilde{f}^*_{KG} \cdot \alpha_K \cdot \alpha_G + \tfrac{1}{2} \cdot \tilde{f}^*_{GG} \cdot \alpha_G^2)\ \mu^2 \} (1 - Y_{RG})$$

$$+ w_D \cdot D \Big] / Y_{SR} \cdot k_{SR} \Big] \qquad (92)$$

3.4.9 State and steady-state equations for mass fraction of R Component in dry mass: w_R

Finally the change of w_R in time, $\partial w_R / \partial t$, will be zero minus the sum of changes in mass fractions of all the other components in time. For steady state, it is unity minus the sum of the mass fractions of all the other components, respectively.

4 Experiments with activated sludge for testing of the three-compartment model

4.1 VANILLIN AS CARBON AND ENERGY SOURCE

In our experiments, vanillin (3-methoxy-4-hydroxy-benzaldehyde) (molecular mass 152.14, white crystalline needles, solubility in cold water 10 g/l) was chosen as carbon and energy source. Vanillin is a metabolic intermediate in the breakdown of lignin (Dagley, 1971) which for example may be found in waste waters of paper pulp factories (Dubach, 1966; 1968). Vanillin may be regarded as a representative organic compound for many aromatics. By feeding activated sludge on the vanillin-basal medium (Section 2.2), a well-settling sludge was obtained that consisted of a variety of micro-organisms and some protozoa (Chap. 6).

Toms & Wood (1970) established both chemically and enzymically vanillin, vanillic acid (3-methoxy-4-hydroxy-benzoic acid) and protocatechuic acid (3,4-dihydroxy-benzoic acid) as definite intermediates in the degradation of trans-ferulic acid found in lignin. Our results confirmed these observations of Toms & Wood (1970).

Washed cell suspensions of vanillin-grown activated sludge (μ = 0.08 h^{-1}) in Sapromat flasks (Section 2.4.6) oxidized vanillin, vanillic acid and protocatechuic acid at respective rates of 2.5 mmol/g.h, 2.2 mmol/g.h and 1.9 mmol/g.h. The substance mass ratio of vanillin to dry mass was 2.5 mmol/g. Catechol was oxidized after a lag-phase of 2.0 h (Fig. 8).

From these results (Fig. 8), it can be calculated that each mole of vanillin consumed 1.5 mol O_2 more than the consumption of 1 mol of protocatechuic acid; 0.5 mol for carboxylation and 1 mol for demethoxylation (Toms & Wood, 1970). Vanillic acid consumed 1 mol of O_2 more than 1 mol of protocatechuic acid for demethoxylation. Protocatechuic acid was rapidly oxidized with consumption of 3.6 mmol of O_2 per mmol of substrate to give 3-carboxy-cis-cis-muconate as the first aliphatic intermediate (λ_{max} = 270 nm) (Fujisawa & Hayaishi, 1968).

The activity content of EC 1.13.11.3-protocatechuate 3,4-dioxygenase in crude extracts was determined according to Fujisawa (1970) and found to be 35-36 µmol protocatechuic acid converted per mg protein per minute and of EC 1.13.11.8- protocatechuate 4,5-dioxygenase (Wheelis et al., 1967) 0.0 µmoles/mg.min. Toms & Wood (1970) observed cleavage between carbon atoms 4 and 5 (meta cleavage) which is characteristic of *Pseudomonas acidovorans* and *Pseudomonas testosteroni* (Wheelis et al., 1967). Cleavage between carbon atoms 3 and 4 (ortho cleavage), also found in the activated sludge, is observed of all other species that oxidize protocatechuic acid (Stanier et al., 1977).

It may be suggested that the metabolic pathway in the activated sludge for conversion of vanillin be according to the view of Toms & Wood (1970). The further metabolism of protocatechuic acid by a 3,4-oxygenase would be expected to give rise to 1.0 mol

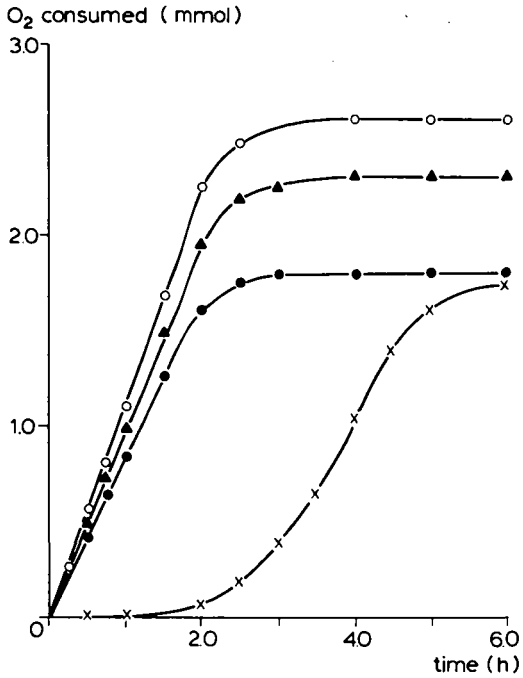
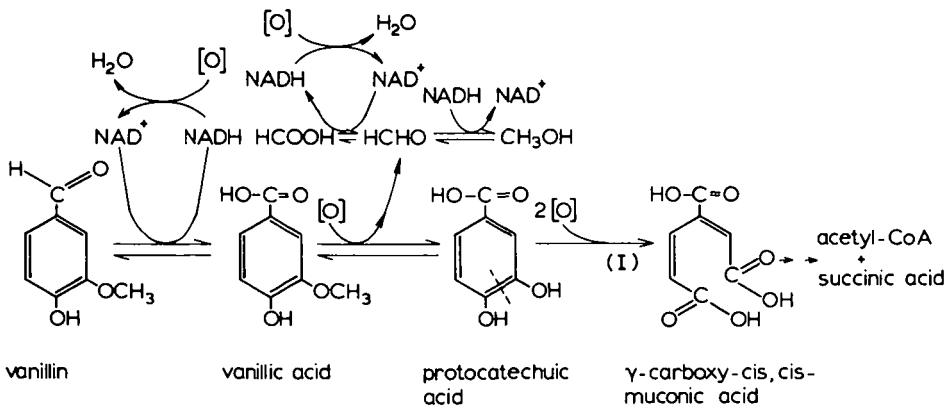


Fig. 8. Oxidation of aromatic compounds by washed cell suspensions of vanillin-grown activated sludge in Sapromat flasks. Each flask contained a final volume of 166 ml made up of basal medium minum $(NH_4)_2SO_4$, cell suspension 0.44 g and substrate 0.5 mmol. Results were corrected for endogenous respiration in the absence of substrate. o , vanillin; ▲ , vanillic acid; ● , protocatechuic acid; x , catechol.

of acetyl-CoA and 1.0 mol of succinic acid, both of which can immediately enter the tricarboxylic acid (TCA) cycle. Scheme 2 summarized the metabolic pathway of the conversion of vanillin into acetyl-CoA and succinic acid.



Scheme 2. Conversion of vanillin into protocatechuic acid as suggested by Toms & Wood (1970) with vanillin-grown activated sludge. Protocatechuic acid was found to be cleaved by EC 1.13.11.8 protocatechuate 4,5-dioxygenase (I).

Phenomenon of substrate inhibition or toxicity of vanillin at higher concentrations would complicate the kinetic studies of vanillin-grown activated sludge. Therefore the influence was examined of the initial concentration of vanillin on the rate of consumption. A series of Sapromat experiments (Section 2.4.6) was carried out, in which the rate of oxygen consumption was measured at different substance-mass ratios of aromatics to dry mass (Fig. 9).

Above aromatic substance-mass ratio of 3.0 mmol/g, the rate of oxygen consumption approached maximum, but no inhibition of consumption of vanillin was measured. In the experiments, such high aromatic loadings of the activated sludge occurred only near washout.

4.2 STEADY-STATE BEHAVIOUR OF ACTIVATED SLUDGE FED WITH VANILLIN IN THE CHEMOSTAT WITH RECYCLING OF BIOMASS

4.2.1 Procedures

The activated-sludge chemostat employed in this research (Section 2.6.2) was inoculated with activated sludge (Section 2.1) and adapted to the vanillin-basal medium (Section 2.2). Concentration of incoming substrate in carbon was: C_{S0} = 0.631 g/l. The reactor volume was 4 l. The oxygen concentration was maintained at 0.003 g/l or more (Section 2.5.6) and pH 7.2, by adding NaOH 3.0 mol/l automatically (Section 2.5.7). Maintenance of temperature 20°C, cleaning of the equipment and approached continuous discharge of biomass are described in Section 2.6.2.

The flow rate of recycling of biomass $\omega_3\phi_1$ was always equal to the flow rate of

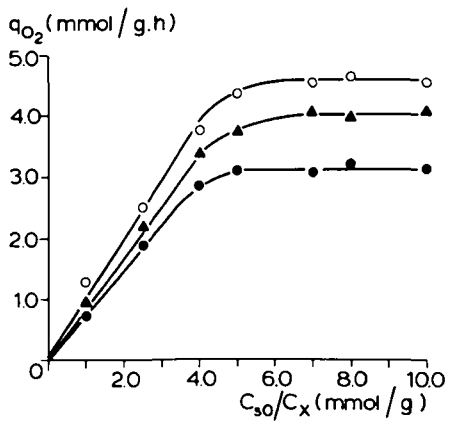


Fig. 9. Rate of oxygen consumption of aromatic compounds (q_{O_2}) by washed cell suspensions of vanillin-grown activated sludge. The substance content of substrate (C_{S0}) in biomass (C_X) increased from 1.0 to 8.0 mmol/g. Sapromat flasks contained a final volume of 166 ml made up of basal medium minus $(NH_4)_2SO_4$ (pH 7.2), cell suspension 0.44 g and substrate as indicated. Results were corrected for endogenous respiration. o , vanillin; ▲ , vanillic acid; ● , protocatechuic acid.

incoming substrate ϕ_0 (Fig. 7). During the experiments, no accumulation of activated sludge was observed in the separator B (Fig. 7) and all biomass in the system could be assumed to be in the reactor.

The specific growth rate μ ranged from low 0.02 h^{-1} where endogenous metabolism was appreciable to high 0.19 h^{-1} near washout, by increasing the dilution rate D from 0.064 to 0.608 h^{-1} and simultaneously increasing the flow of discharge of biomass $\omega_1\phi_0$ (Fig. 7). The hold-up factor ω_D could be maintained at 0.31, deviating 0.10. After an interval greater than three times the reciprocal of μ, reliable estimates of a steady state could be made.

Samples 30-ml or 50-ml of the contents of the reactor and separator units (Fig. 3) were cooled immediately in ice to stop the metabolic processes.
The samples were pre-conditioned (Section 2.4) and the suspended matter analysed for total dry mass (Section 2.4.1), DNA (Section 2.4.2), RNA (Section 2.4.3), protein (Section 2.4.4) and total carbohydrates (Section 2.4.5). The culture liquor was analysed for total organic carbon (Section 2.5.1).

4.2.2 Results and discussion

4.2.2.1 Influence of population or metabolic shifts on the Monod coefficients

Jones (1973) showed theoretically, using a hypothetical culture of ten micro-organisms each with its own particular substrate and kinetics (Table 3), that it may be possible to obtain straight-line relationships when using the Lineweaver-Burk method, $1/\mu$ is plotted against $1/C_S$ in order to calculate the Monod coefficients μ_{max} and K_S (Eqn 1). In discussing Monod kinetics, Jones concluded that the obtained values of the coefficients are determined by μ_{max} and K_S of the individual species. However, the kinetic coefficients calculated were only based on data in the flat region of the growth curve (μ = 0.001 - 0.0083 h^{-1}), and an overall μ_{max} of 0.31 h^{-1} was found, while eight species could grow with much higher specific growth rates (Table 3).

In their study of statistical analysis of optimum design of continuous-flow experiments, Johnson & Berthouex (1975) pointed out the necessity of obtaining data on the upswing of the growth curve if precise estimates of the kinetic coefficients and verification of usefulness of the model are required. In particular, data on the flat region of the growth curve are weighted much too heavy statistically if the Lineweaver-Burk method is used for determination of μ_{max} and K_S.

Using the values of μ_{max} and K_S of 'Jones mixed culture' (Table 3), data in the upswing of the growth curve could be calculated if Equation 1 (k_d = 0) was used. The calculated results are represented in a Lineweaver-Burk plot and shown in Figure 10. The arrow in Figure 10 indicates the total substrate concentration where washing out of some species starts. This Lineweaver-Burk plot is only linear over the lower range of substrate concentrations of carbon (C_S = 0.0004 - 0.093 g/l). At higher concentrations, it obviously departs from linearity, because the slowly growing micro-organisms are washed out.

Actually these tailing-off phenomena in mixed cultures were reported, for instance

Table 3 Kinetic coeffiencts used for calculation of substrate removal curves for a ten-component mixture of micro-organisms according to Jones (1973). Initial total substrate concentration in carbon: C_{SO} = 0.234 g/l; system yield coefficient in dry mass produced per unit mass of carbon consumed, Y = 0.38 g/g

Organism	C_{SO} (g/l)	Y_{max} (g/g)	μ_{max} (h^{-1})	K_S g/l
A	36	0.27	0.90	0.015
B	42	0.47	0.78	0.017
C	18	0.46	0.36	0.019
D	39	0.49	0.17	0.009
E	34	0.11	0.39	0.007
F	18	0.46	0.17	0.006
G	5	0.41	0.64	0.017
H	13	0.49	0.50	0.003
I	6	0.38	0.62	0.017
J	23	0.41	0.63	0.009

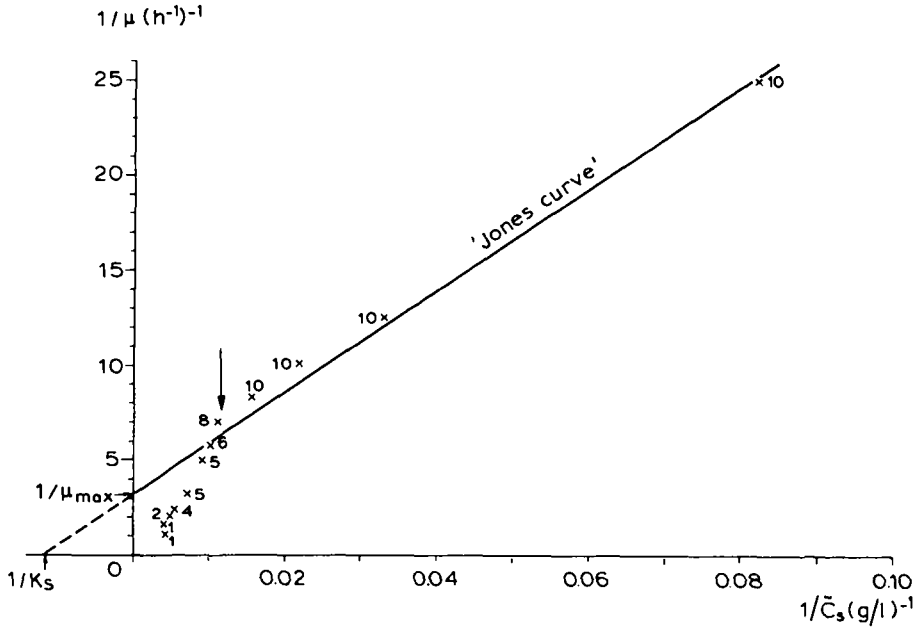


Fig. 10. Lineweaver-Burk plot of the reciprocal of specific growth rate against the reciprocal of concentration of substrate, The curve was calculated with data about the theoretical culture of ten species of micro-organisms as summarized in Table 3. Numbers indicate bacterial species remaining in the culture. The solid line is as calculated by Jones (1973).

by Gaudy et al. (1967), Ramanathan & Gaudy (1969) and Gosh & Pohland (1972). Gosh & Pohland approximated their results by arbitrarily distribution of the culture in slow and fast growers, each with specific values of the Monod coefficients.

This departure from linearity is also what we observed from our own experiments with activated sludge in the chemostat with recycling of biomass (Fig. 11). Linear regression analysis of the data in the linear part of the Lineweaver-Burk plot where $\mu < 0.100\ h^{-1}$ resulted in $K_S = 0.141$ g/l, $\mu_{max} = 0.133\ h^{-1}$, number of data points 19; coefficient of determination 0.95 and standard error of estimate 0.08. However specific growth rates in the region where $\mu = 0.189\ h^{-1}$ at $\tilde{C}_S = 0.423$ g/l could be measured.

The plausible assumption of a shift from slow to fast growers would not be the only explanation of the results. In the region of the specific growth rates of $0.189\ h^{-1}$, the dispersion of the culture increased. Sladká & Zahrádka (1971) found a correlation between the activity and the total free surface of activated sludge. Thus the rate of consumption of substrate might increase by increased dispersion of the activated sludge. Further, wall growth of biomass causes tailing-off from the growth curve at higher specific growth rates as emphasized by Topiwala & Hamer (1971). However, in our experiments, wall growth could be prevented by cleaning the reactor daily (Section 2.6.2). Finally, identical kinetic results were also be found in pure cultures (Herbert et al., 1956; Wargel et al., 1970; Shehata & Marr, 1971). Shehata & Marr explained their results by assuming an enzymic shift from a specific enzyme, for example a permease, with high affinity at low substrate concentrations to a non-specific enzyme with low affinity at higher substrate concentration.

Thus explaining our observations (Fig. 11), the departure from linearity might be ascribed to (1) decreasing diffusion limitations of nutrients by increasingly dispersed

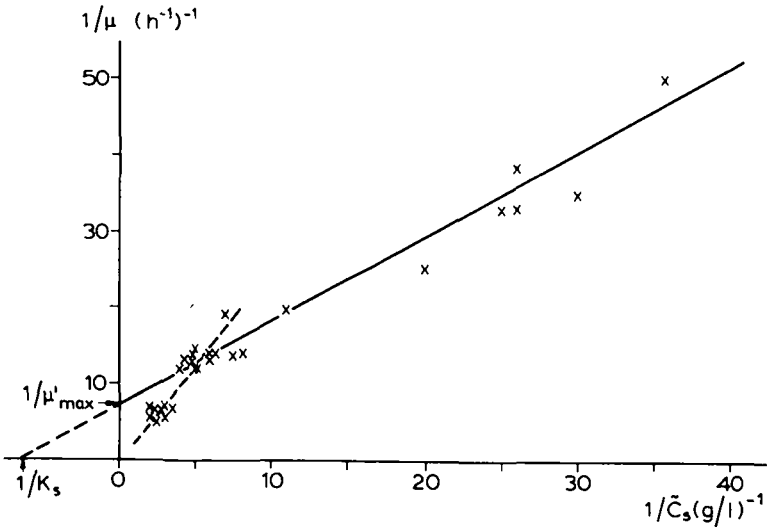


Fig. 11. Lineweaver-Burk plot of the reciprocal of specific growth rate against the reciprocal of concentration of substrate during balanced growth of the activated-sludge culture fed on vanillin. $\mu_{max} = 0.140\ h^{-1}$ and $K_S = 0.155$ g/l.

growth, (2) washing out of slowly growing micro-organisms and relative increase of fast growers with lower substrate affinity, i.e. increase in K_S and μ_{max}, (3) enzymic shifts to non-specific enzymic systems at higher growth rates.

In Figure 12, our experimental data are fitted to the power-like relationship of rate of uptake of substrate by R Component q_{SR}, developed in Section 3.3.2.1 and resulting in the steady-state Equation 71. The calculated values of the kinetic coefficients are k_{SR} = 0.308 (g/g.h.(g/l)^{a_S} and a_S = 0.71, where r^2 = 0.89.
The Monod-like relationship for the consumption of substrate can be derived from Equation 2 by substituting Equation 1 for μ. Then the rate of consumption of substrate by R Compartment will be $q_{SR} = q_{max}.C_S/(K_S + C_S)$.
Fitting our data of the linear part of the Lineweaver-Burk plot (Fig. 11) to the Monod-like relationship we find the following q_{max} = 0.100 g/g.h, K_S = 0.056 g/l, r^2 = 0.71, and the standard error of estimate $s(q_{SR}/C_S)$ = 0.03.
The calculated curve is also presented in Figure 12.

Obviously the power-like relationship (Eqn 71) is far better in fitting also the 'tailing-off' phenomenon of the mixed culture at higher growth rates than the Monod-like relationship. Further data near wash-out are especially relevant for verification of the usefulness of a Monod-like relationship and precise estimates of the model coefficients, as also statistically proved by Johnson & Berthouex (1975). The far-reaching conclusions of Grady & Williams (1975) and Benefield & Randall (1977) about the influence of concentration of incoming substrate on the kinetic coefficients are doubtful because they performed their experiments in the flat regions of the growth curves.

4.2.2.2 Influence of incoming substrate concentration, C_{SO} on the coefficients μ_{max} and K_S

Classical dispersed-growth Monod-type kinetics predict that the concentration of substrate in the effluent from a reactor, C_S, depends only upon the specific growth rate

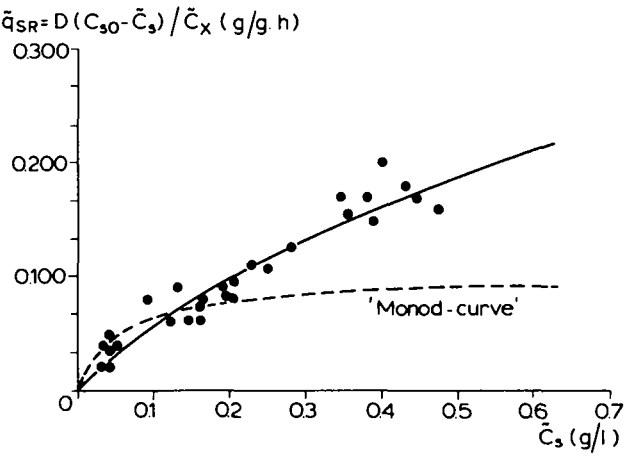


Fig. 12. Rate of consumption of substrate by vanillin-grown activated sludge at different steady-state concentrations of substrate. C_{SO} = 0.631 g/l.

of the micro-organisms in the reactor (Herbert et al., 1956; Lawrence & McCarty, 1970; Grady et al., 1972; Goodman & Englande, 1974).
The only variable that would be expected to change would be the biomass present in the chemostat, reacting on actual amounts of substrate.

Recently Grady & Williams (1975), Grau et al. (1975), Benefield & Randall (1977) and Daigger & Grady (1977) presented data which indicate that in mixed cultures growing on multicomponent substrates, the apparent kinetic coefficients may be affected by C_{SO}. Using unspecific parameters such as TOC or COD, Grau et al. (1975) argued that, if the amount of intermediates with low rates of breakdown were to build up in the medium by increasing the initial substrate concentration, the saturation coefficient, K_S would increase too. Grady & Williams (1975) interpreted their experimental data by an empirical modification of the linear approximation of the Lawrence & McCarty model (Section 1.1.2). Benefield & Randall (1977), following the propositions of Grau et al. (1975) accepted also on empirical grounds, an n^{th}-order rate equation for which a rationale is given in Section 3.3.2.1.

However, Howell (1976) proved mathematically that if wall growth was assumed to occur in microbial-kinetic experiments and $C_{SO} >> \overline{C}_S$, the specific growth rate would not depend only on $\overline{C}_S$ but also on the inverse of C_{SO}.
In fact, every mixing effect may influence the basic dependence of μ on $\overline{C}_S$ (Herbert et al., 1956; Tempest, 1970; Topiwala & Hamer, 1971; Pirt, 1975). Further the kinetic experiments of Grady & Williams and the others were carried out in the flat region of the growth curve, so that the estimates of the kinetic coefficients are doubtful (Section 4.2.2.1). Nevertheless it can easily be proved that for mixed bacterial cultures with some species washed out, the concentration of incoming substrate affects the total kinetic coefficients.

If a mixed culture of ten micro-organisms be assumed with defined values of the kinetic coefficients (Table 3), the total concentration of substrate, $\overline{C}_S$ at different specific growth rates, μ was calculated with Equation 1 for $k_d = 0$. Three series were calculated, in which the initial substrate concentrations of individual organisms increased by a factor 2 and 3, respectively. The results are represented in a Lineweaver-Burk plot (Fig. 13).

The following conclusions may be drawn
(1) The increased initial substrate concentrations of individual micro-organisms result in increased critical dilution rates, i.e. values at which the steady-state biomass concentration is zero, as long as the maximum specific growth rate is not approached. Then a species will be washed out at a higher total specific growth rate of the mixed culture and thus affect the total saturation coefficient and total biomass concentration at a given specific growth rate.
(2) There is no reason to believe that the basic assumption of independence of μ and C_{SO} is not valid as long as no microbial species of the mixed culture have been washed out. At the low specific growth rates usual in activated-sludge engineering, the concentration of incoming substrate, C_{SO} will not influence K_S but rather the composition of the substrate.
(3) The Lineweaver-Burk method is not an adequate method of calculating the coefficients

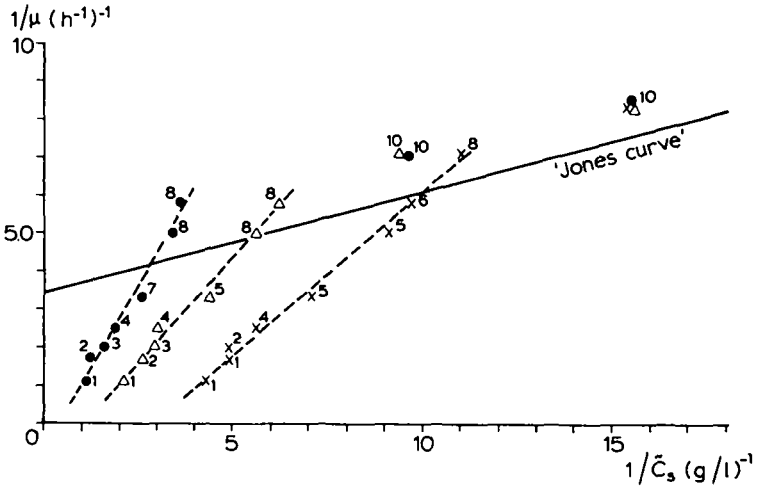


Fig. 13. Lineweaver-Burk plots of data of the theoretical culture of ten species of micro-organisms as summarized in Table 3 at three different concentrations of incoming substrate. × , C_{S0} = 0.234 g/l; Δ , C_{S0} = 0.468 g/l; and ● , C_{S0} = 0.702 g/l. Numbers indicate bacterial species remaining in the culture and the solid line is as calculated by Jones (1973).

of a mixed culture. Figure 13 shows that μ_{max} *and* K_S *cannot be calculated by extrapolation.*

(4) The Monod-derived relationships are not adequate in describing the influence of C_{SO} on μ. The n^{th}-order rate equation is more adequate (Sections 3.3.2.1 and 4.2.2.1; Fig. 12).

In order to avoid complications caused by a changed initial substrate concentration, the concentration of carbon C_{SO} = 0.631 g/l was used in our kinetic experiments.

4.2.2.3 Macromolecular composition of vanillin-grown activated sludge during balanced growth at different growth rates

The composition of macromolecular components, as mass fractions of dry mass, of vanillin-grown activated sludge at different specific growth rates during balanced growth is shown in Figure 14. Laboratory experiments in the chemostat with recycling of biomass (Section 4.2.1) covered the range of μ 0.02 - 0.19 h^{-1}. The series of data points were fitted to straight-line relationships (Eqn 76-78) by using the least-squares method and depicted in Figure 14. The calculated regression coefficients, coefficients of determination and standard errors of estimate are summarized in Table 4.

Figure 14 and Table 4 show that the RNA content of the biomass increased with increasing growth rate but the protein content decreased. Cell DNA did not vary significantly. These results are broadly similar to data of Herbert (1961), Tempest et al., (1965), Maaløe & Kjeldgaard (1966), Morowitz (1968), Dalton & Postgate (1969), Brown & Rose (1969) and Forchhammer & Lindahl (1971), which are summarized in Table 2. For

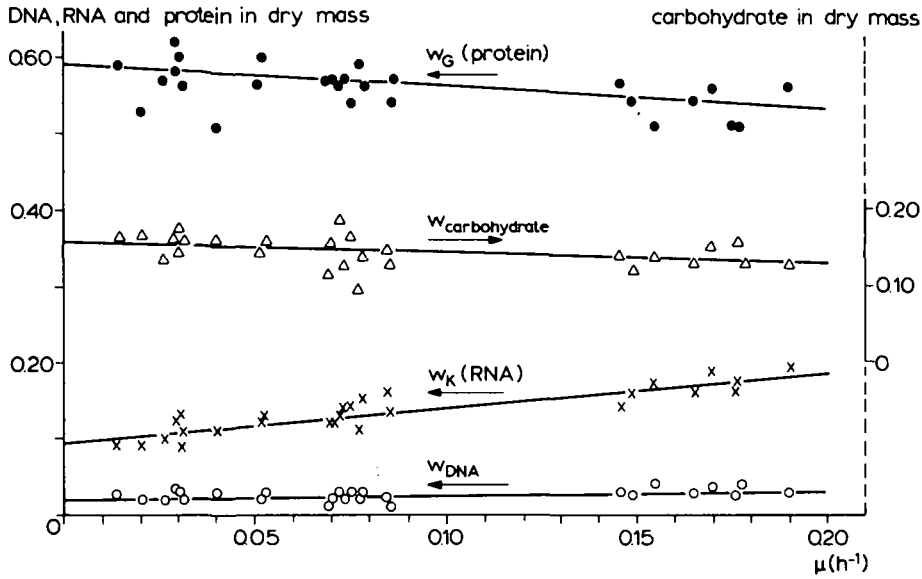


Fig. 14. Mass fractions of DNA, RNA, protein and carbohydrate in dry mass of activated sludge, continuously fed on vanillin at different specific growth rates during steady-state growth.

changes in protein content of activated sludge they resemble those of Weddle & Jenkins (1971). The total carbohydrate content of the activated sludge decreased a little with increasing growth rate using a carbon to nitrogen subtance-mass ratio of C/N = 6.0. However, it can vary significantly when higher carbon to nitrogen ratios and carbohydrates as substrates are used as limiting nutrients (Weddle & Jenkins, 1971; Dawes & Senior, 1973) instead of, for example aromatics.

Table 4. Regression coefficients ω_{io}, $\partial\omega_i/\partial\mu$; coefficients of determindation r^2; and standard errors of estimates $s(\omega/\mu)$ of straight-line fits by the least squares method of the series of data points of the different macromolecular components i, in the activated sludge at different specific growth rates.

	DNA	RNA	Protein	Carbohydrate
$\omega_{io}(\mu = 0)$	0.02	0.10	0.59	0.16
$\partial\omega_i/\partial\mu$	0.056	0.459	-0.298	-0.120
r^2	0.15	0.81	0.38	0.14
$s(\omega/\mu)$	0.008	0.013	0.022	0.017

4.2.2.4 Linear law of substrate consumption and biomass growth in relation to the concept of maintenance

Viable and non-viable micro-organisms require a certain amount of energy for maintenance processes like all ordered open systems. Pirt (1965) related substrate consumption and biomass growth to the concept of maintenance, and assumed that during growth consumption of the energy source was partly growth-dependent and partly growth-independent. Substituting Equation 1 for μ in Equation 2 (Pirt, 1965), we obtain

$$q_S = \frac{1}{Y_{max}} . \mu + m_S \qquad (93)$$

where $m_S = k_d/Y_{max}$, defined as the maintenance coefficient, i.e. consumption of substrate for maintenance purposes divided by biomass and by time.

Specific maintenance functions so far recognized (Stouthamer & Bettenhausen, 1973) are:

1) turnover of cell materials
2) osmotic work to maintain concentration gradients of intracellular metabolites and right ionic composition between the cell and its exterior
3) cell motility
4) maintenance of membrane energization, driving force of transport processes (van Verseveld & Stouthamer, 1978)
5) maintenance of a pool of energy-rich compounds for the creation of entropy in the system, because micro-organisms are open systems, i.e. represent thermodynamic states of non-equilibrium and thus tend to a state of maximum entropy or disorder (Morowitz, 1969; Roels & Kossen, 1978)
6) maintenance of growth-structure, for example flocculent growth of activated sludge.

The linear law (Eqn 93) has as yet been extensively used in the study of the metabolism of micro-organisms (Righelato et al., 1968; van Uden, 1969; Nagai & Aiba, 1972; Stouthamer & Bettenhausen, 1973; Hempfling & Mainzer, 1975; Gons, 1977; Verstraete, 1977; Roels & Kossen, 1978; van Verseveld & Stouthamer 1978). It has also been frequently used in the study of substrate consumption by activated sludge (Lawrence & McCarty, 1970; Gosh & Pohland, 1972; Jones, 1973) and oxygen consumption of activated sludge in relation to sludge growth (Benefield et al., 1976).

However in some experiments, no straight line was obtained by plotting q_S against μ, which was shown to be due to an influence of μ on the fermentation pattern and ATP yield of the organism (Stouthamer & Bettenhausen, 1973). Therefore Stouthamer & Bettenhausen used the concept of Y^{ATP} (Bauchop & Elsden, 1960) and reformulated the Pirt equation (Eqn 93) as follows

$$q_{ATP} = \frac{1}{Y^{ATP}_{max}} . \mu + m_e \qquad (94)$$

where q_{ATP} is the specific rate of ATP production (amount of substance of ATP divided by

dry mass and time); $1/Y_{max}^{ATP}$ is the production of dry mass divided by amount of substance of ATP corrected for energy of maintenance and m_e is the substance consumption of ATP divided by dry mass and time for maintenance purposes.

Now the 'linear' equation of the three-compartment model will be derived and discussed in relation to the Pirt equation (Eqn 93) and the equation of Stouthamer & Bettenhausen (Eqn 94). The starting point is the equation of the rate of biomass production (Eqn 91). If steady state is assumed, ($\partial C_X/\partial t = 0$; $\partial \omega_K/\partial t = 0$ and $\partial \omega_G/\partial t = 0$), Equation 91 can be simplified by substituting from Equation 82, the explicitly written term, $\{m_K.\tilde{\omega}_K + k_{NK}.\tilde{\omega}_K + (r_X.\tilde{\omega}_K/C_X\}$ for the terms between the first braces and from Equation 84, $\{m_G.\tilde{\omega}_G + k_{NG}.\tilde{\omega}_G + (r_X.\tilde{\omega}_G)/C_X\}$ for the terms between the second braces. Then if all terms are divided by C_X we obtain

$$0 = Y_{SR}.k_{SR}.\tilde{C}_S^{a_S} + \{m_K + k_{NK} + (r_X.\tilde{\omega}_K)/C_X\}\ \frac{(Y_{RK} - 1)}{Y_{RK}}$$

$$+ \{m_G + k_{NG} + (r_X.\tilde{\omega}_G)/C_X\}\ \frac{(Y_{RG} - 1)}{Y_{RG}} - \omega_D.D \qquad (95)$$

In balanced growth, $(r_X.\tilde{\omega}_K)/C_X\} = \mu.\tilde{\omega}_K$; $\mu = \omega_D.D$ and $\tilde{q}_{SR} = k_{SR}.\tilde{C}_S^{a_S}$ (Eqn 71). Then Equation 95 can be reformulated to the 'linear' law of the three-component model if $\tilde{q}_{SR}$ is is written explicitly and the right of the new equation worked out in a growth-dependent part and a maintenance/decay-dependent one. The 'linear' law of the three compartment model can be written as

$$\tilde{q}_{SR} = \frac{1}{Y_{SR}}\left[1 + \frac{(1 - Y_{RK})}{Y_{RK}}.\tilde{\omega}_K + \frac{(1 - Y_{RG})}{Y_{RG}}.\tilde{\omega}_G\right]\mu +$$

$$+ \frac{1}{Y_{SR}}\left[\frac{(1 - Y_{RK})}{Y_{RK}}(m_K + k_{NK})\tilde{\omega}_K + \frac{(1 - Y_{RG})}{Y_{RG}}(m_G + k_{NG})\tilde{\omega}_G\right] \qquad (96)$$

In a biochemically well-regulated biosystem, the production of ATP equals the consumption of ATP. In such a balanced system the consumption of ATP can be written as

$$r_{ATP} = \frac{1}{Y_{SR}^{ATP}} \cdot Y_{SR} \cdot r_{SR} + \frac{1}{Y_{RK}^{ATP}} \cdot Y_{RK} \cdot r_{RK} + \frac{1}{Y_{RG}^{ATP}} \cdot Y_{RG} \cdot r_{RG} \qquad (97)$$

where r_{ATP} is the rate of consumption of ATP per unit volume (mol/l.h), $Y_{SR} \cdot r_{SR}$ is the production of R Compartment per unit volume per unit time, $Y_{RK} \cdot r_{RK}$ is the production of K Compartment per unit volume per unit time, $Y_{RG} \cdot r_{RG}$ is the production of G Compartment per unit volume per unit time, Y_{SR}^{ATP} is the molar growth yield of R Compartment for ATP in g R per mol ATP, Y_{RK}^{ATP} is the molar growth yield of K Compartment for ATP in g K per mol ATP and Y_{RG}^{ATP} is the molar growth yield of G Compartment for ATP in g G per mol ATP. Equation 97 can be solved if Equation 96 is substituted for r_{SR}/C_X;

$(m_K + k_{NK} + \mu)\tilde{\omega}_K/Y_{RK}$ is substituted for r_{RK}/C_X and $(m_G + k_{NG} + \mu)\tilde{\omega}_G/Y_{RG}$ is substituted for r_{RG}/C_X. The final result becomes

$$\tilde{q}_{ATP} = \frac{1}{Y_{SR}^{ATP}}\left[1 + \left\{\frac{(Y_{SR}^{ATP} - Y_{RK}^{ATP})}{Y_{RK}^{ATP}} + \frac{1}{Y_{RK}}\right\}\tilde{\omega}_K + \left\{\frac{(Y_{SR}^{ATP} - Y_{RG}^{ATP})}{Y_{RG}^{ATP}} + \frac{1}{Y_{RG}}\right\}\tilde{\omega}_G\right]\mu +$$

$$+ \frac{1}{Y_{SR}^{ATP}}\left[\left\{\frac{(Y_{SR}^{ATP} - Y_{RK}^{ATP})}{Y_{RK}} + \frac{1}{Y_{RK}}\right\}(m_K + k_{NK})\tilde{\omega}_K +\right.$$

$$\left. + \left\{\frac{(Y_{SR}^{ATP} - Y_{RG}^{ATP})}{Y_{RG}^{ATP}} + \frac{1}{Y_{RG}}\right\}(m_G + k_{NG})\tilde{\omega}_G\right] \qquad (97a)$$

in which $\tilde{q}_{ATP}$ is defined as the rate of ATP consumption per unit dry mass (mol ATP/g.h). Comparing Equation 97a and the linear equation of Stouthamer & Bettenhausen (Eqn 94) we can conclude that

$$\frac{1}{Y_{max}^{ATP}} \equiv \frac{1}{Y_{SR}^{ATP}}\left[1 + \left\{\frac{(Y_{SR}^{ATP} - Y_{RK}^{ATP})}{Y_{RK}^{ATP}} + \frac{1}{Y_{RK}}\right\}\tilde{\omega}_K + \left\{\frac{(Y_{SR}^{ATP} - Y_{RG}^{ATP})}{Y_{RG}^{ATP}} + \frac{1}{Y_{RG}}\right\}\tilde{\omega}_G\right] \qquad (98)$$

$$m_e \equiv \frac{1}{Y_{SR}^{ATP}}\left[\left\{\frac{(Y_{SR}^{ATP} - Y_{RK}^{ATP})}{Y_{RK}^{ATP}} + \frac{1}{Y_{RK}}\right\}(m_K + k_{NK})\tilde{\omega}_K +\right.$$

$$\left. + \left\{\frac{(Y_{SR}^{ATP} - Y_{RG}^{ATP})}{Y_{RG}^{ATP}} + \frac{1}{Y_{RG}}\right\}(m_G + k_{NG})\tilde{\omega}_G\right] \qquad (99)$$

From equations 98 and 99 both coefficients Y_{max}^{ATP} and m_e, of the linear equation of Stouthamer & Bettenhausen (1973) and also of the Pirt equation, are predicted by the three-compartment model to be functions of mass fractions of RNA and protein, which are influenced by growth rate (Tables 2 and 4) and thus by the growth-supporting ability of the environment. However, the influence of $\tilde{\omega}_K$ and $\tilde{\omega}_G$ on the theoretical Y_{max}^{ATP} values is small, which can be explained as follows.

From Table 5 (Stouthamer, 1977), ATP requirements for synthesis of RNA (K Component) and protein (G Component) are almost equal. Then the plausible assumption can be made that in general under aerobic conditions, ATP requirement may be proportional to the requirement of carbon. Mathematically

$$Y_i^{ATP} \cdot q_i^{ATP} = Y_i \cdot q_i \qquad (100)$$

Table 5. Utilization of ATP for the formation of cellular components in a glucose-inorganic salts medium (Stouthamer, 1977).

Macromolecule	ATP requirement relative to mass of product (mmol/g)
RNA	37.32
Protein	39.11
Polysaccharides	12.36
Lipid	1.48
DNA	33.00
R Component (polysaccharides, lipid and DNA)	46.84

From Equation 100, if Y_{RK}^{ATP} equals Y_{RG}^{ATP} (Table 5) then Y_{RK} equals Y_{RG}. Subsequently no great error will be made if the turnover rate of K Component and G Component, m_K and m_G, are assumed to be equal (Mandelstam, 1960). Finally in general, whole cells are measured and the decay rates k_{NK} and k_{NG} may be expected to equal to the overall decay rate of the whole cell k_N.

With these assumptions, Equations 96 and 97 can be simplified, respectively, to

$$\tilde{q}_{ATP} = \frac{1}{Y_{SR}^{ATP}} \left[1 + \left\{ \frac{(Y_{SR}^{ATP} - Y_{RK}^{ATP})}{Y_{RK}^{ATP}} + \frac{1}{Y_{RK}} \right\} (\tilde{\omega}_K + \tilde{\omega}_G) \right] \mu +$$

$$+ \frac{1}{Y_{SR}^{ATP}} \left[\left\{ \frac{(Y_{SR}^{ATP} - Y_{RK}^{ATP})}{Y_{RK}^{ATP}} + \frac{1}{Y_{RK}} \right\} (m_K + k_{NK}) (\tilde{\omega}_K + \tilde{\omega}_G) \right] \qquad (101)$$

$$\tilde{q}_{SR} = \frac{1}{Y_{SR}} \left[1 + \frac{(1 - Y_{RK})}{Y_{RK}} (\tilde{\omega}_K + \tilde{\omega}_G) \right] \mu +$$

$$+ \frac{1}{Y_{SR}} \left[\frac{(1 - Y_{RK})}{Y_{RK}} (m_K + k_N) (\tilde{\omega}_K + \tilde{\omega}_G) \right] \qquad (102)$$

in which $Y_{RK}^{ATP} = Y_{RG}^{ATP}$; $Y_{RK} = Y_{RG}$; $m_K = m_G$ and $k_{NK} = k_{NG} = k_N$.
From Table 2 and Table 4, the sum of $\tilde{\omega}_K$ and $\tilde{\omega}_G$ may generally be assumed to be constant and thus the influence of the change of the composition of microbial cells on Y_{ATP}^{max} and m_e (also on Y_{max} and m_S) should be very small, as concluded by Stouthamer (1977).

If large amounts of storage materials in cells, such as lipids, polysaccharides or both, are formed, for example, by a high carbon to nitrogen ratio (Watlers et al., 1968; Dawes & Senior, 1973), then the production of R Component per mol ATP, Y_{SR}^{ATP} or

Y_{SR} is increasing. This results in an increase in m_e (Eqn 99) or m_S. Qualitatively, the ATP requirement for the formation of lipids or polysaccharides is much smaller than for K Component (RNA) or G Component (protein) which can be concluded from Table 5.

The 'linear' equation of the three-compartment model in reduced form (Eqn 102) and the Pirt equation (Eqn 93) can be solved by plotting $\tilde{q}_{SR}$ against μ. The results are shown in Figure 15. A straight-line relationship has been found and the best-fit was calculated by the least-squares method. To calculate Y_{SR} and $Y_{RK} = Y_{RG}$, values of $m_K = m_G$ and $k_{NK} = k_{NG} = k_N$ had to be assumed (Table 6). The calculated results are summarized in Table 6 too.

It may be concluded from Table 6 that the value of m_S obtained for vanillin-grown activated sludge agrees well with data summarized by Verstraete (1977, Table III. 13) for mixed cultures and Verstraete (1977, Table III.10) for pure cultures. Assuming that the mass fraction of carbon in the cells is 50%, the dimensionless values of Y_{max} 0.50 and Y_{SR} 0.69, 0.65 and 0.62 are also plausible results (Verstraete, 1977; Table III.10). From Table 6, the parameters $m_K (= m_G)$ and k_N are relatively insensitive in influencing Y_{SR} and $Y_{RK} = Y_{RG}$. Increasing the specific maintenance rates, m_K and m_G, an increased part of R Component will be converted to G Component and K Component, which results in a decreasing Y_{SR} and increasing Y_{RK} and Y_{RG}.

4.2.2.5 Viability

When micro-organisms are grown at low specific growth rates, cells may be incapable of division but can replenish maintenance energy requirements or may die as a result of a toxic chemical, starvation or 'mistakes' in autosynthesis.

Experiments of Postgate & Hunter (1962), Tempest et al. (1967) and Sinclair & Topiwala (1970) with *Aerobacter aerogenes*, and Weddle & Jenkins (1971) and Walker &

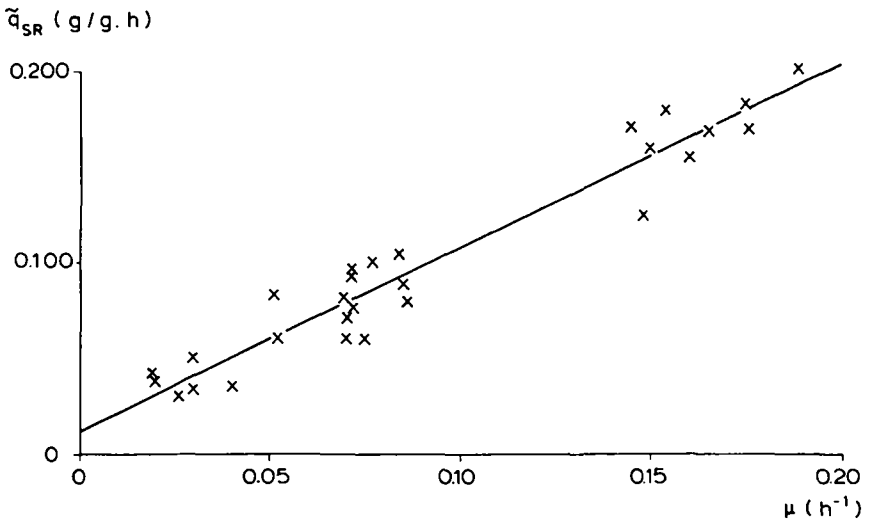


Fig. 15. Rate of uptake of substrate by R Compartment of vanillin-grown activated sludge against specific growth rate at different steady states.

Table 6. Calculated coefficients of the linear law of the three-compartment model (Eqn 102) and of the linear law of Pirt (1965). The sum of mass fractions of K Component and G Component in the range of growth rates could be estimated from $(\omega_K + \omega_G) = 0.70$, deviating 0.01 (Table 4). The coefficients $m_K = m_G$ and $k_{NK} = k_{NG} = k_N$ were assumed to be as indicated but of the magnitude estimated by Mandelstam (1960) and Weddle & Jenkins (1971) respectively. The values of the regression coefficients were $r^2 = 0.94$ and $s(\tilde{q}_{SR}/\mu) = 0.014$.

m_K (h^{-1})	k_N (h^{-1})	$(\tilde{\omega}_K + \tilde{\omega}_G)$	Y_{SR} (g/g)	Y_{RK} (g/g)	m_S (g/g.h)	Y_{max} (g/g)
0.04	0.01	0.70	1.37	0.68	0.012	1.03
0.05	0.01	0.70	1.29	0.73		
0.06	0.01	0.70	1.25	0.77		

Davies (1977) with activated sludge, showed that at very low growth rates ($\mu < 0.05\ h^{-1}$), the viability, i.e. capability of growth, falls. The concept of viability was incorporated in kinetic models of pure cultures by Powell (1967) and Sinclair & Topiwala (1971). Recently Grady & Roper (1974) added to the unified model of Lawrence & McCarty (1970) by incorporating a first-order cell death rate (Sinclair & Topiwala, 1971) and by assuming that both viable and non-viable cells are lost from the system by decay.

In the three-compartment model, first-order kinetics of rate of loss of viability or death rate of cells is also assumed; state equations and steady-state equations are formulated (Eqn 85-90). In steady state, the total non-viable or dead mass fraction, $\tilde{\omega}_N$, may be the summation of the partial mass fractions: $\tilde{\omega}_{NR}$, $\tilde{\omega}_{NK}$ and $\tilde{\omega}_{NG}$ (Eqn 88-90). If it is assumed that whole cells become non-viable or dead, $k_{NR} = k_{NK} = k_{NG}$ and we obtain

$$\tilde{\omega}_N = k_N (\tilde{w}_R + \tilde{w}_K + \tilde{w}_G) \frac{1}{\mu} \tag{103}$$

where k_N is the total specific non-viable or death rate, and there

$$\tilde{\omega}_R + \tilde{w}_K + \tilde{w}_G + \tilde{w}_N = 1 \tag{104}$$

we may substitute for $\tilde{\omega}_R$, Equation 104 into Equation 103 and obtain

$$\tilde{\omega}_N = k_N (1 - \tilde{\omega}_N) \frac{1}{\mu} \tag{105}$$

If $\tilde{\omega}_N$ is the mass fraction of non-viable or dead biomass, $(1 - \tilde{\omega}_N)$ can be defined as the viable-mass fraction.

Reformulating Equation 105, we find

$$(1 - \tilde{\omega}_N) = \frac{\mu}{k_N + \mu} \qquad (106)$$

The specific non-viable or death rate, k_N, may be calculated from a double reciprocal plot $1/(1 - \tilde{\omega}_N)$ against $1/\mu$. Our model is fitted to viability data of Postgate & Hunter (1962) with *Aerobacter aerogenes* and of Weddle & Jenkins (1971) with activated sludge. The plot is given in Figure 16 and it may be concluded that the three-compartment model is found to be capable of simulating the changes in viability within a continuous culture of micro-organisms. Now it may be concluded too that the calculated coefficients ω_{KO} and ω_{GO} are extrapolated values because the viable-mass fractions in total dry mass $\tilde{\omega}_K$ and $\tilde{\omega}_G$ will decrease sharply at low specific growth rates. However, the relationship is linear if the fractions are calculated in terms of viable dry mass.

Practically, activated sludge systems operate at very low growth rates (<< 0.01 h^{-1}). Figure 16 shows that in such systems only a small mass fraction of the organisms is viable. Increasing μ by a factor 2 or 3, for example from 0.005 to 0.015 h^{-1}, the viability increases by a factor 2 (k_N = 0.01 h^{-1}) and a more efficient system in substrate consumption is obtained. The idea can well be used, in the kinetic improvement of a two-stage activated-sludge system (Chap. 5).

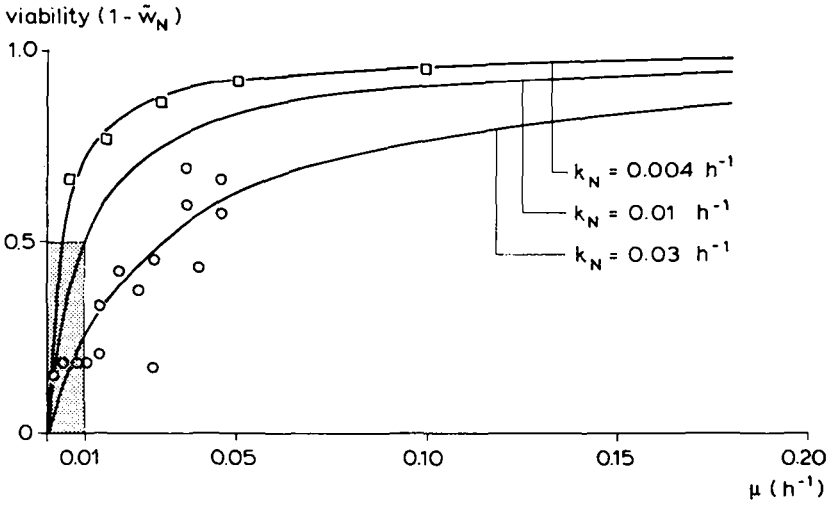


Fig. 16. Total mass fraction of viable mass ($1 - \tilde{\omega}_N$) against specific growth rate. Two curves were calculated from experimental data of Postgate & Hunter (1962) (□) and of Weddle & Jenkins (1971) (o). The third curve was calculated from k_N = 0.01 h^{-1}. Shadowed area indicates operational conditions and resulting viability, normal for activated-sludge plants.

4.2.2.6 Steady-state growth curve of the activated sludge fed on vanillin

In Appendix C, a method is given for calculation of the Taylor-series constants of the balance and steady-state equations (Eqns 82, 84, 91 and 92). The constants were calculated on the data of the coefficients summarized in Table 4 and Table 6, first line. The calculated values are $(\tilde{f}_R \cdot \alpha_R + \tilde{f}_K \cdot \alpha_K) = 0.181$, $(\frac{1}{2} \tilde{f}_{RR} \cdot \alpha_R^2 + \tilde{f}_{RK} \cdot \alpha_R \cdot \alpha_K + \frac{1}{2} \tilde{f}_{KK} \cdot \alpha_K^2) = 0.68$, $(\tilde{f}^*_K \cdot \alpha_K + \tilde{f}^*_G \cdot \alpha_G) = 0.890$ and $(\frac{1}{2} \tilde{f}^*_{KK} \cdot \alpha_K^2 + \tilde{f}^*_{KG} \cdot \alpha_K \cdot \alpha_G + \frac{1}{2} \tilde{f}^*_{GG} \cdot \alpha_G^2) = -0.438$.
From Section 4.2.2.1 $k_{SR} = 0.308$ g/g.h.(g/l)$^{\alpha_S}$ and $\alpha_S = 0.71$.
By substitution of the calculated constants and coefficients into the steady-state equations for substrate and biomass (Eqns 72 and 92), the growth curve of the activated sludge fed on vanillin could be simulated. The calculated curves are shown in Figure 17 and the experimental data are plotted too. The three-compartment model gives a reasonable good fit of the experimental data.

4.3 BATCH AND TRANSIENT KINETICS OF THE ACTIVATED SLUDGE FED ON VANILLIN

4.3.1 Procedures

The batch experiment with activated sludge, used the equipment described in Section 2.6.1. Activated sludge was taken from the chemostat fed on vanillin-basal medium (Section 4.2.1) where $\mu = 0.030\ h^{-1}$, $D = 0.100\ h^{-1}$, $\tilde{C}_S = 0.015$ g/l and $\tilde{C}_X = 1.5$

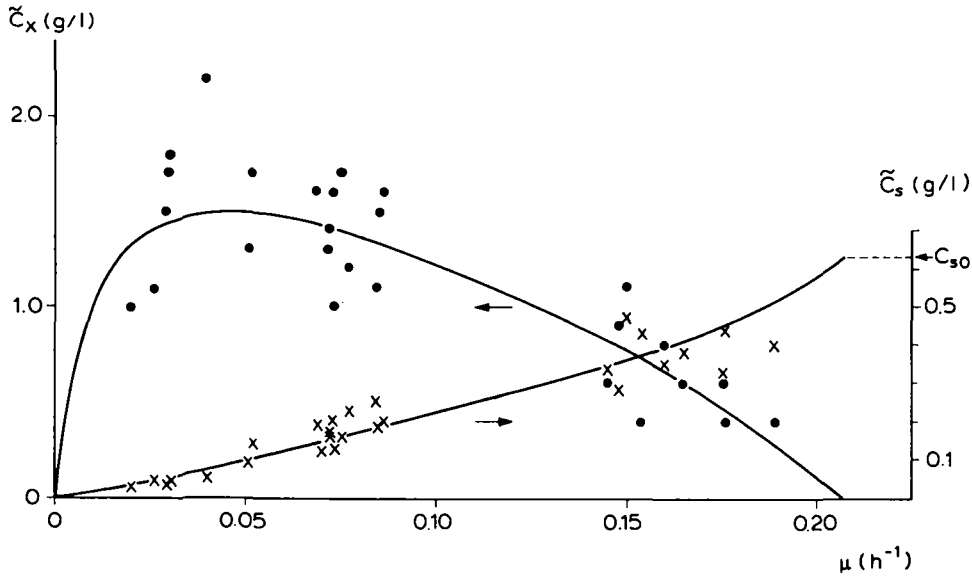


Fig. 17. Steady-state data of limiting substrate $\tilde{C}_S$ and biomass C_X of the activated sludge fed on vanillin in the chemostat with recycling of biomass against the specific growth rate μ. X, total organic carbon; ● , dry mass. The solid lines are the curves predicted by the three-compartment model.

g/l. At the start C_X = 1.0 g/l.

One of the transient experiments with activated sludge fed on vanillin is given here and was with the equipment described in Section 2.6.2. Procedures of maintaining steady-states are given in Section 4.2.1. The initial steady-state conditions were D = 0.065 h^{-1}, ω_D = 0.31, μ = 0.020 h^{-1}, $\tilde{C}_S$ = 0.010 g/l, $\tilde{C}_X$ = 1.0 g/l and V = 4 l. In the experiment shown, the chemostat steady-state was perturbed by a step increase of D from 0.065 to 0.267 h^{-1}. The flow rate of discharge of sludge was proportionally increased to maintain ω_D = 0.31. The perturbation was followed over a period of 38 h and a final μ = 0.083 h^{-1} was measured. Samples of 30 ml or 50 ml of the contents of the reactor were cooled immediately in ice and analysed for total dry mass, RNA, protein and the culture liquor for total organic carbon (Sections 2.4 and 4.2.1).

The balance equations of C_X, ω_K, ω_G, ω_R and C_S were solved on the DEC SYSTEM - 1090 TIMESHARING of the Agricultural University, Wageningen, using the International Mathematical & Statistical Libraries - program DVOGER. The subroutine DVOGER is given in Appendix A.

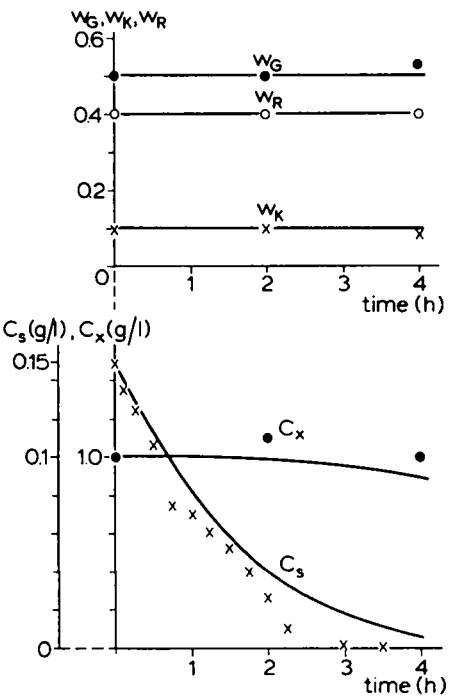


Fig. 18. Consumption of vanillin in the batch system with activated sludge, obtained from the culture with vanillin in the chemostat with recycling of biomass. At the start, C_{SO} = 0.147 g/l measured in terms of total organic carbon, $\tilde{C}_X$ = 1.0 g/l in dry mass, $\tilde{\omega}_G$ = 0.50, $\tilde{\omega}_K$ = 0.10 and $\tilde{\omega}_R$ = 0.40. The curves are simulations of the three-compartment model using values of the coefficients and the constants as determined in the continuous culture experiments.

4.3.2 Simulation of the batch and transient experiments

The dynamic behaviour of the three-compartment model was on the steady-state values of the coefficients (k_{SR} = 0.308 g/g.h.(g/l)^{a_S}, a_S = 0.71, Table 4 and Table 6 first row) and on the Taylor-series constants given in Section 4.2.2.6. Results of the experiments and the simulations are shown in Figures 18 and 19. In the batch experiment and transient experiment, the three-compartment model was quantitatively accurate in simulating the dynamic responses of the biosystem.

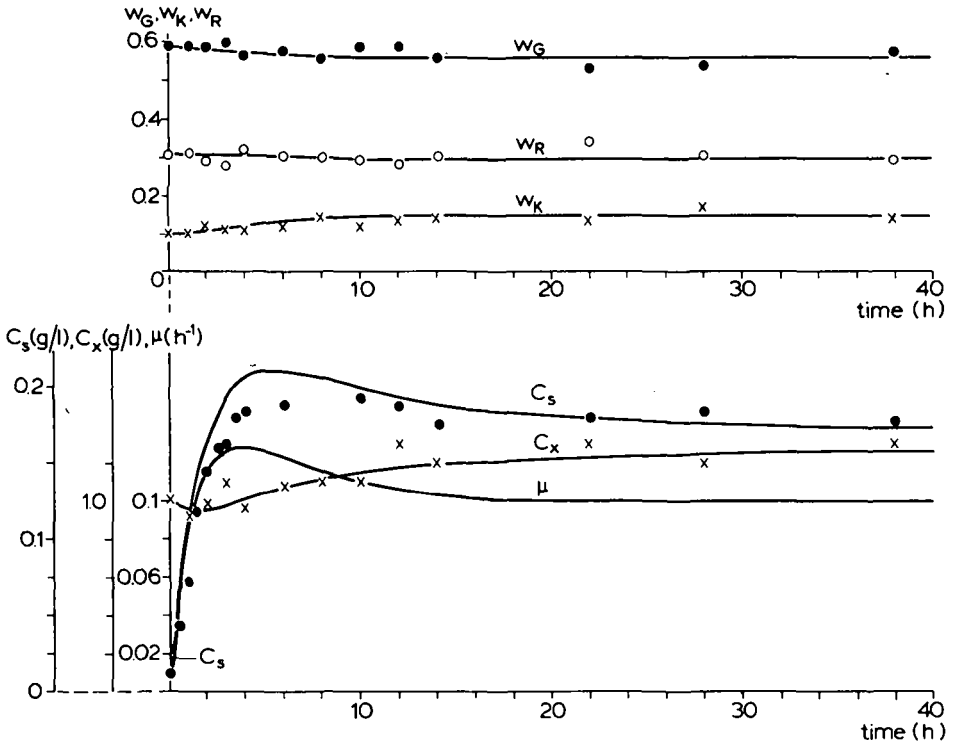


Fig. 19. Response of specific growth rate μ, biomass C_X, limiting substrate C_S, and mass fractions of G, R and K Compartment to an increase of the hydraulic dilution rate D from 0.065 to 0.267 h^{-1}. The specific growth rate increased from 0.020 to 0.083 h^{-1} during the experiment.

5 Kinetics and biochemical aspects of oxidation of vanillin and mixtures of vanillin and acetate in the two-stage activated-sludge chemostat with recycling of biomass

5.1 INTRODUCTION

Because of the need for treatment of specific wastes and because of the high quality required of effluents, two-stage activated-sludge plants will be used more and more in the future (Section 1.2). However, less fundamental work has been done on improving the system and on the influence of the intermediate substrate on the behaviour of the second stage. Existing two-stage plants differ considerably in design of the reducing capacity of the first stage and in volume ratios of the first and second stage (Table 1).

The purpose of Section 5.2 is to formulate a mathematical model for kinetic optimalization of the dimensioning of the two-stage process. The model was tested, feeding a two-stage activated-sludge system on vanillin (Section 5.3.2.1). In Section 5.3.2.2, the biochemical and physiological aspects of the second-stage are discussed. The production of vanillin-grown activated sludge with optimum kinetic dimensioning of both systems has been compared in Section 5.3.2.3. Section 5.3.2.4 discuss the viability of biomass in a system with one and two stages.

Results of a mixture of vanillin and acetate are summarized in Section 5.4. They are also compared with those in the one-stage system.

5.2 MODELLING OPTIMUM KINETICS OF A TWO-STAGE PROCESS

The state equation of the rate of substrate consumption (Eqn 70) of the three-compartment model (Chap. 3) will be the starting point of this kinetic study.

A completely mixed two-stage reactor with separate recycling of biomass for each stage, is schematically represented in Figure 20. The state equation of the limiting substrate concentration in the first-stage reactor, C_{S1}, if Equation 70 is used, may be written as

$$\frac{dC_{S1}}{dt} = -k_{SR1}.C_{S1}^{a_{S1}}.C_{X1} + D_1\,(C_{S0} - C_{S1}) \qquad (103)$$

where the subscript 1 denotes the first-stage reactor and $D_1 = \phi_0/V_1$.
In the second stage, the flow rate ϕ_0 is reduced by the flow rate of discharge of biomass out of the first-stage reactor, $w_1.\phi_0$ (Fig. 20). Therefore the flow rate of the second-stage reactor have to be $(1 - w_1)\phi_0$ and the state equation for it becomes

$$\frac{dC_{S2}}{dt} = -k_{SR2}.C_{S2}^{a_{S2}}.C_{X2} + (1 - w_1)D_2(C_{S1} - C_{S2}) \qquad (104)$$

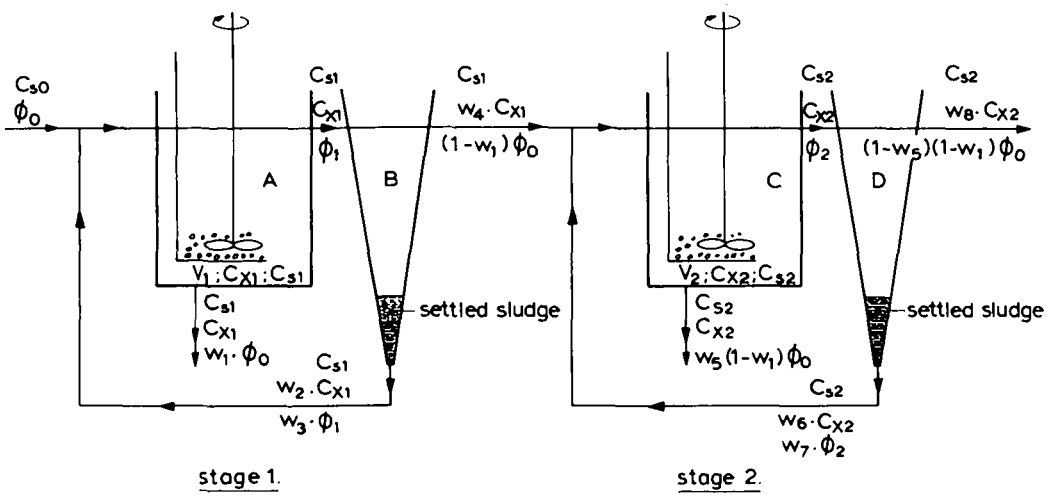


Fig. 20. Two-stage chemostat with recycling of biomass. A, reactor of the first stage; B, separator of the first stage; C, reactor of the second stage; D, separator of the second stage; V_1 and V_2, working volumes of the two reactors; C_{X1}, and C_{X2} concentrations of biomass in both reactors; C_{S0}, concentration of incoming substrate; C_{S1}, concentration of intermediate substrate; C_{S2}, concentration of substrate of the second stage; ϕ_0, flow rate of incoming substrate; ϕ_1, flow rate of substrate out of the first-stage reactor; ϕ_2, flow rate of substrate out of the second-stage reactor; w_1, w_2, w_3, w_4, w_5, w_6 and w_7, mass fractions.

in which the concentration of incoming substrate of the second-stage will be the concentration of effluent out of the separator of the first stage. The mean hydraulic retention time in a chemostat, t, is the reciprocal of the dilution rate, D. So for the two-stage system, they are given by $t_1 = 1/D_1$ and $t_2 = 1/D_2$. If steady state is assumed in both stages, then Equations 103 and 104 can be written out for t_1 and t_2, respectively, as

$$t_1 = \frac{(C_{S0} - \tilde{C}_{S1})}{k_{SR1} \tilde{C}_{S1}^{a_{S1}} \cdot \tilde{C}_{X1}} \tag{105}$$

and

$$t_2 = \frac{(1 - w_1)\ (\tilde{C}_{S1} - \tilde{C}_{S2})}{k_{SR2} \cdot \tilde{C}_{S2}^{a_{S2}} \cdot \tilde{C}_{X2}} \tag{106}$$

In activated-sludge plants, biomass is discharged from the concentrated stream of settled activated sludge which is fed back to the reactor. Then the fraction of substrate $w_1(\tilde{C}_{S1} - \tilde{C}_{S2})$, which is not reduced to the required effluent concentration, $\tilde{C}_{S2}$, may be ignored and Equation 106 reduced to

$$t_2 = \frac{(\partial_{S1} - \partial_{S2})}{k_{SR2}.\partial_{S2}^{a_{S2}}.\partial_{X2}} \tag{107}$$

In equation 107, it is assumed that all incoming substrate with concentration, ∂_{S0}, will be reduced to a final concentration of effluent that is ∂_{S2}. Only then can a one-stage and two-stage system be compared adequately for the kinetics of substrate consumption (Section 5.3).

The total hydraulic retention time of substrate in the two-stage system assuming the separators to be biologically inert systems, can be defined as

$$t_{tot} = t_1 + t_2 \tag{108}$$

The total hydraulic retention time, t_{tot}, becomes a function of the intermediate substrate concentration, ∂_{S1} if one assumes constant physico-chemical conditions, fixed biomass concentration, constant incoming substrate concentration and a defined fractional reduction of substrate ∂_{S2}. If the coefficients of the second stage depend on the composition and concentration of intermediate substrate, ∂_{S1}, then they have to be defined as function of ∂_{S1} too.

$$t_{tot} = f(\partial_{S1})_{C_{S0}};\ \partial_{S2};\ \partial_{X1};\ \partial_{X2};\ T;\ pH \tag{109}$$

The minimum total hydraulic retention time of t_{tot} may be found by setting the derivative with respect to ∂_{S1} of Equation 109 at zero.

$$\frac{\partial t_{tot}}{\partial \partial_{S1}} = \frac{\partial (t_1 + t_2)}{\partial \partial_{S1}} = 0 \tag{110}$$

The partial differential Equation 110 can be solved and t_{tot}, t_1, t_2 and ∂_{S1} calculated. The hydraulic retention time of the one-stage can also be determined if ∂_{S1} be assumed to be already reduced to ∂_{S2} in the first stage. For these calculations, a computer program in Fortran was written (App. B). In this way, the rates of consumption of vanillin and mixtures of vanillin and acetate, in the system with one stage and two stages, were tested and compared.

5.3 OPTIMUM KINETICS OF THE TWO-STAGE ACTIVATED-SLUDGE SYSTEM FED ON VANILLIN

5.3.1 Procedures

The joining together of two chemostat units in series (Section 2.6.2) gave the two-stage system in which the experiments were carried out. Both stages were inoculated with activated sludge adapted to the vanillin-basal medium (Section 2.2). Incoming sub-

strate of the second stage consisted of pumped effluent of the first-stage separator, without any addition of nutrients. The oxygen concentration was maintained at 0.003 g/l or more (Section 2.5.6) and pH 7.2, by adding NaOH 3.0 mol/l automatically (Section 2.5.7). Depending on the degree of metabolism of vanillin in the first stage, HCl 3.0 mol/l must be added to the second stage. Maintenance of temperature at 20°C, cleaning of equipment and approached continuous discharge of biomass are described in Section 2.6.2.

The flow rates of recycling of biomass, $\omega_3.\phi_1$ and $\omega_7.\phi_2$, were always equal to the flow rates of incoming substrate, ϕ_0 = 1.548 l/h and $(1 - \omega_1)\phi_0$ = 0.438 l/h, respectively (Fig. 20). Biomass hardly accumulated in the separators and all biomass could be assumed to be in both reactors.

The analytical procedures were the same as in Section 4.2.1.

As stated before, kinetic coefficients may be affected by concentration and composition of incoming substrate (Sections 1.2 and 4.2.2.2). Then, in agreement with operational procedures of Lohmann (1975, 1977) only good estimates of the kinetic coefficients of the second stage could be made if the intermediate substrate was held constant in composition and concentration. Unfortunately this was overlooked by Illić (1977) in two-stage activated-sludge experiments on laboratory scale with pentaerythrite as model of incoming substrate.

Two series of experiments were done with the following operational procedures. (1) In the first set of experiments, the operational conditions of the first stage were set at: C_{SO} = 0.631 g/l; V_1 = 3 l; D_1 = 0.271 h^{-1} and ω_{D1} = 0.31. This resulted in the average value of q_{SR1} = 0.093 g/g.h, deviating 0.01; μ_1 = 0.084 h^{-1}, deviating 0.010; $\tilde{C}_{X1}$ = 1.3 g/l. The mean concentration of intermediate substrate was, $\tilde{C}_{S1}$ = 0.183 g/l, deviating 0.027 and assumed to be constant in composition (Fig. 21).

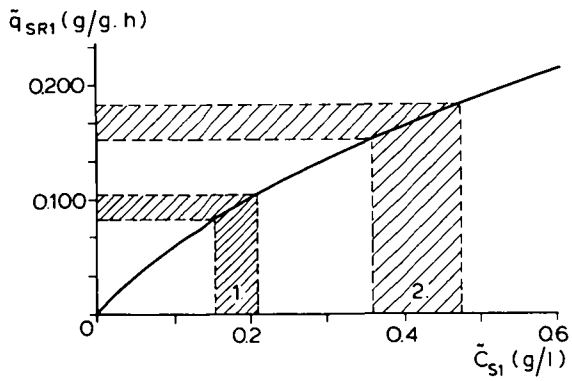


Fig. 21. Rate of uptake of substrate by R Component in the first-stage reactor against steady-state concentration of substrate of the reactor. Shadowed areas indicate operational conditions of the first-stage reactor during the two-stage experiments. Numbers in the graph refer to two series of experiments: 1, in which the second stage was fed on an average concentration of intermediate substrate of $\tilde{C}_{S1}$ = 0.183 g/l, deviating 0.027; 2, in which the second stage was fed on an average concentration of intermediate substrate of $\tilde{C}_{S1}$ = 0.414 g/l, deviating 0.058.

Stellingen

1. De toepassing van de zgn. Monod-kinetiek op mengkulturen van micro-organismen is twijfelachtig.

Dit proefschrift.

2. Het gestructureerde model van Brown en Fitzpatrick houdt geen rekening met de verdunning van de biotische componenten in het systeem door groei van de biomassa.

D.E. Brown & S.W. Fitzpatrick, 1979. A structured model for the kinetics of fungal amylase production. Biotechnol. Letters. 1: 3-8.

3. In de door Dahlem afgeleide formule voor de berekening van de optimale werkingsgraad van de eerste trap van een tweetraps-actiefslib-proces wordt geen rekening gehouden met de concentraties van actiefslib in beide trappen noch met de invloed van het intermediaire substraat op de kinetische coëffiënten van de tweede trap.

H. Dahlem, 1978. Biologische Mehrstufigkeit zur Behandlung Organisch Verschmutzter Industrieller Abwässer. E.A.S. 4th European Sewage and Refuse Symposium. Munnich. p. 180-205.

4. Het valt voor industrieën te overwegen om de kinetische voordelen van een tweetraps-actiefslib-proces te benutten door reeds een voorzuivering van afvalwater toe te passen in een hoogbelaste actiefslibinstallatie alvorens dit wordt geloosd op een centrale rioolwaterzuiveringsinstallatie.

5. De methode van ATP bepaling in actiefslib als maat voor de metabolische activiteit van de biomassa is dubieus omdat veelal de experimentele condities niet éénduidig zijn te definiëren.

6. Onderzoek naar de verandering van het substraatverbruik van micro-organismen voor cellulair onderhoud bij zeer lage specifieke groeisnelheden dient te gebeuren op basis van het gehalte aan levende cellen in de biomassa.

W. Verstraete, 1977. Fundamentele studie van de opbouw- en omzettingsprocessen in microbiële gemeenschappen. Proefschrift tot het verkrijgen van de graad van Geaggregeerde voor het Hoger Onderwijs. Rijksuniversiteit Gent. Gent. p. 218-219.

7. De door Yagi uitgevoerde metingen van de operationele stabiliteit van geïmmobiliseerd hydrogenase zijn nauwelijks relevant omdat de activiteit van het enzym wordt weergegeven in µmol H_2 geproduceerd per minuut per ml oplossing.

T. Yagi, 1977. Use of an enzymic electric cell and immobilized hydrogenase in the study of the biophotolyses of water to produce hydrogen. Biol. Solar Energy Conversion. Acad. Press. p. 61-68.

8. Het is een hardnekkig misverstand dat de remming van NH_4^+ op het proces van de N_2-fixatie in *Azotobacter* wordt veroorzaakt door een verlaagde ATP/ADP ratio (Kleiner).

D. Kleiner, 1975. Ammonium uptake by nitrogen fixing bacteria. I. Azotobacter vinelandii. Arch. Microbiol. 104: 163-169.

9. Het begrip 'the molecular logic of the living state' (Lehninger), en het veelvuldig in de literatuur gebruikte begrip 'Nature' dragen een magisch-religieus karakter.

A.L. Lehninger, 1975. Introduction: The molecular logic of living organisms. In: Biochemistry. Worth Publishers Inc.

10. De oorsprong van Einstein's relativiteitstheorie kan niet alleen worden verklaard uit de neo-kantiaanse filosofie van de relatieve natuur-orde (Aalders) maar moet ook gezocht worden in het natuurwetenschappelijke werk van H.A. Lorentz (1853-1928).

W. Aalders, 1977. Theocratie of ideologie. Voorhoeve. Den Haag. p. 244.

11. Promovendi bevestigen de keuze van sommige overheidsinstanties om academici en artiesten in dezelfde afdeling van beroepsgroepen in te delen.

Gewestelijk Arbeidsbureau Ede.

Proefschrift van A. Harder
Structured model of bacterial growth and tests with activated sludge in a one-stage and two-stage chemostat

The hydraulic dilution rate of the second stage was set at D_2 = 0.219 h^{-1}, deviating 0.004 and the volume of the reactor V_2 = 2 l. The specific growth rate of the second stage corrected for incoming biomass of the first stage, μ_2, ranged from 0.01 - 0.12 h^{-1}, by increasing the flow rate of discharge of biomass, i.e. ω_5 (1 - ω_1) Q_0 (see Fig. 20).

(2) The second series of measurements dealt with the operational conditions of the first stage set at: C_{S0} = 0.631 g/l; V_1 = 3 l; D_1 = 0.516 h^{-1} and ω_{D1} = 0.31. The average value of q_{SR1} = 0.166 g/g.h, deviating 0.016; μ_1 = 0.160 h^{-1}, deviating 0.016; $\hat{C}_{X1}$ = 0.7 g/l; The mean concentration of intermediate substrate was, $\hat{C}_{S1}$ = 0.414 g/l, deviating 0.058 (Fig. 21).

The hydraulic dilution rate of the second stage was set at D_2 = 0.278 h^{-1}, deviating 0.060 and V_2 = 2 l. Here the corrected specific growth rate, μ_2, ranged from 0.03 to 0.13 h^{-1}.

In the second stage, it was easier to keep the hydraulic dilution rate, D_2, constant rather than the hold-up factor, ω_{D2}, because of the limited amount of liquid out of the first stage pumped into the second one. By varying the flow rate of discharge of biomass after an interval greater than three or five times the specific growth rate, reliable estimates of steady states of the second stage could be made during a period of one or two weeks.

However the steady states maintained in this stage were not as stable as those from the one-stage experiments (Chap. 4) and the variations with time were fairly large, from 10 to 20% deviation.

5.3.2 Results and discussion

5.3.2.1 Optimal kinetic dimensioning of the two-stage system fed on vanillin

Figure 21 shows that stable conditions could be maintained in the upswing of the substrate-utilization curve. The slow increase of $\hat{C}_{S1}$, instead of instantaneous increase of $\hat{C}_{S1}$ near washout, could be expected in mixed cultures as already discussed in Sections 4.2.2.1 and 4.2.2.2. It allowed creation of two levels of concentration of intermediate substrate by which the second stage could be fed.

The average data of the second-stage measurements were fitted to Equation 71 and are presented in Figure 22. The calculated values of the coefficients at both concentrations of intermediate substrate are summarized in Table 7. The coefficients of the one-stage experiments (Section 4.2.2.1) are summarized too, because those values equal the coefficients of the second stage if $\hat{C}_{S1}$ = C_{S0}, that is no substrate utilization in the first stage at all.

From Table 7, the kinetic coefficients decrease with decreasing concentration of intermediate substrate. These results are in agreement with the conclusion drawn from the calculations of the coefficients of the theoretical mixed culture (Table 3) in response to changed concentrations of incoming substrate (Section 4.2.2.2). Likewise Lohman (1975, 1977) observed a decrease in the kinetic coefficients of the second stage, an activated-sludge reactor, if the incoming substrate was partially (50%) purified in

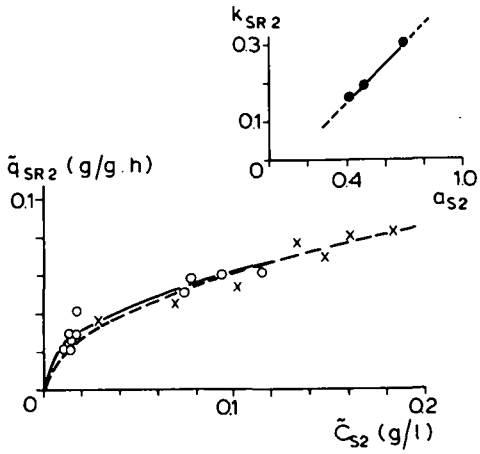


Fig. 22. Rate of uptake of substrate by R Component in the second-stage reactor against steady-state concentration of substrate in the reactor. O , average data of the second stage fed on $\tilde{C}_{S1}$ = 0.183 g/l; ✖ , average data of the second stage fed on $\tilde{C}_{S1}$ = 0.414 g/l. The inset graph represents rate coefficient of consumption of substrate in the second stage against formal order of reaction in the second stage.

a trickling fitter. Lohmann assumed an increase in less biodegradable organic compounds which may be present in the intermediate substrate with increasing purification efficiency of the first stage.

The insetgraph in Figure 22 shows that the kinetic coefficients decrease simultaneously, as already observed by Chudoba et al. (1973) analysing data of Peil & Gaudy (1971) and of Gosh & Pohland (1972). Population shifts were assumed to occur.

In summary our results (Table 7) can well be explained on the influence of de-

Table 7. Kinetic coefficients, k_{SR2} and a_{S2} at the two intermediate-substrate concentrations, $\tilde{C}_{S1}$. Kinetic coefficients of the one-stage experiments (Section 4.2.2.1) are summarized too, because they equal the second-stage coefficients if $\tilde{C}_{S1} = C_{SO}$.

$\tilde{C}_{S1}$ (g/l)	k_{SR2} g/g.h.(g/l)$^{a_{S2}}$	a_{S2}	r^2
0.183	0.158	0.42	0.88
0.414	0.187	0.49	0.92
0.631	0.308	0.71	0.89

creasing concentration of incoming substrate on the composition of the activated-sludge population (Section 4.2.2.2). It is difficult to discuss the influence of the composition of intermediate substrate, because vanillin and vanillic acid were detected but these aromatics contributed only for 45-46% of total organic carbon measured (Section 6.2.2.2; Table 15).

To calculate minima of the total hydraulic retention time, t_{tot}, as a function of concentration of intermediate substrate, the partial differential Equation 110 needs to be solved. However the kinetic coefficients of the second stage are dependent upon the intermediate substrate concentration and have to be defined as functions of it. Empirical exponential relationships were assumed, which resulted in the following equations which are shown in Figure 23 and given in Equations 111 and 112.

$$k_{SR2} = 0.114\ e^{1.474\ \gamma_{S1}} \tag{111}$$

$$a_{S2} = 0.325\ e^{1.182\ \gamma_{S1}} \tag{112}$$

where the coefficients of determination were 0.91 and 0.95, respectively.
Substituting the two empirical relationships for k_{SR2} and a_{S2} in Equation 110, the hydraulic retention time of the second stage, t_2, could be solved at each value of γ_{S1}.
The following assumptions were made for comparison of a one-stage system with a two-stage system

(1) The concentration of incoming substrate in carbon is C_{S0} = 0.631 g/l

(2) The total working volumes of both systems are similar, written as

$$V = V_1 + V_2 \tag{113}$$

where V is the volume of the one-stage system; V_1 and V_2 are the volumes of the first and second stages of the two-stage system, respectively. In this way, the two systems were compared on the basis of flow rates of substrate

(3) Total amounts of biomass in steady state are similar in both systems. This can be defined as

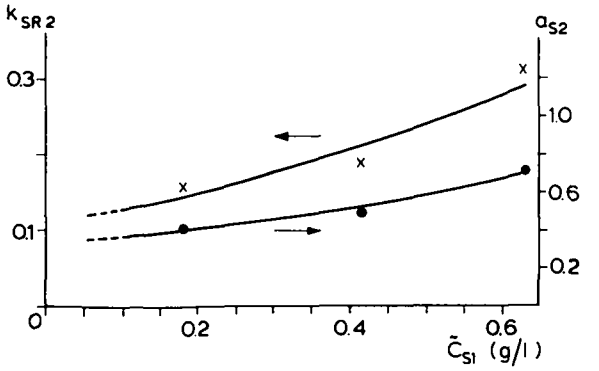


Fig. 23. Rate coefficient of consumption of substrate in the second stage and formal order of reaction in the second stage against concentration of intermediate substrate.

$$V.C_X = V_1.C_{X1} + V_2.C_{X2} \qquad (114)$$

In the first series of calculations, the concentration of biomass in each reactor be chosen to be $\mathcal{C}_X = \mathcal{C}_{X1} = \mathcal{C}_{X2} = 1.0$ g/l and the mass fraction of viable be unity

(4) In the calculations, the experimentally determined kinetic coefficients of the first stage are listed in Table 7, 3rd row. The second-stage ones, which are dependen on $\mathcal{C}_{S1}$, are calculated from Equations 111 and 112

(5) Total fractional reduction of incoming substrate, η_{tot}, is required to be in the two systems of, respectively, 0.80; 0.85; 0.90; 0.95 and 0.98, where $\eta_{tot} = (C_{S0} - C_{S2})/C_{S0}$.

A Fortran computer program (App. B) was written for solving the partial differential Equation 110 on the DEC SYSTEM-1090 TIMESHARING of the Agricultural University, Wageningen.

Results are summarized in Table 8 and two representative curves of t_{tot} against $\mathcal{C}_{S1}$ are presented in Figure 24. The two-stage system is advantageous to the one-stage system in rate of vanillin consumption. For example, (Table 8), the flow rate of substrate in the two-stage system can be a factor 2.9 of that in the one-stage system if a fractional reduction of $\eta_{tot} = 0.95$ be assumed. Kormanik (1972) obtained similar results in a theoretical approach to kinetic optimalization of an aerobic lagoon and facultatively aerobic lagoon in series. Economically (Table 8), at the higher the required total fractional reduction in substrate, the higher the kinetic advantage of the two-stage system (Fig. 25). Increase in η_{tot} requires an increase in the fractional reduction in substrate

Table 8. Summary of optimum kinetic calculations of the one-stage and two-stage systems at increasing purification efficiencies η.
Symbols: t, the hydraulic retention time of the one-stage system; t_1 and t_2, the hydraulic retention time of the first- and second stage, respectively; t_{tot}^{min}, minimum of the total hydraulic retention time in the two-stage system; at the optimum concentration of intermediate substrate, opt $\mathcal{C}_{S1}$; V_1 and V_2, volume of the fist and second stage, respectively; η_1, purification efficiency of the first stage.

η	t_{tot}^{min} (h)	t_1 (h)	t_2 (h)	opt $\mathcal{C}_{S1}$ (g/l)	η_1	V_1/V_2	t(one-stage) (h)
0.80	3.2	1.7	1.5	0.310	0.51	1.1	5.1
0.85	3.7	1.9	1.8	0.284	0.55	1.1	6.7
0.90	4.4	2.3	2.1	0.252	0.60	1.1	9.4
0.95	5.5	3.0	2.5	0.207	0.67	1.2	16.1
0.968	6.2	3.5	2.7	0.187	0.70	1.3	23.0

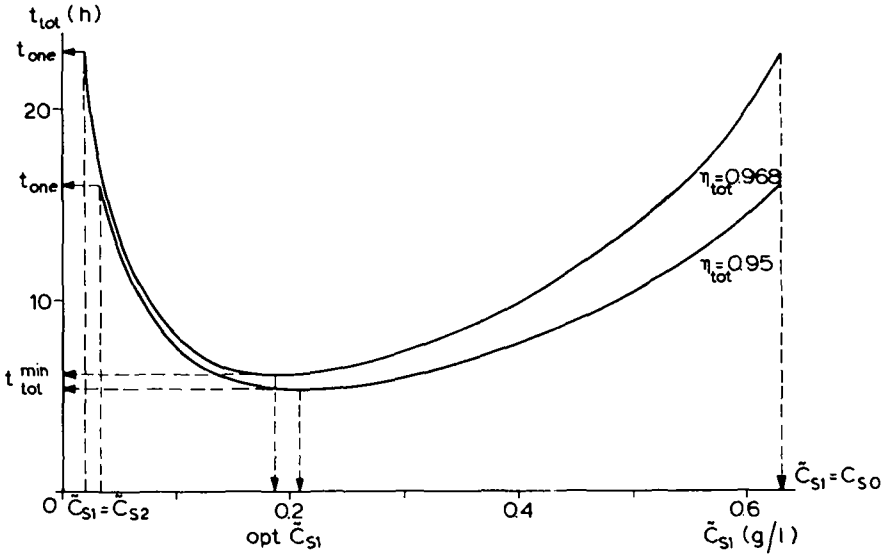


Fig. 24. Calculated curves of total hydraulic retention time of the two-stage system against concentration of intermediate substrate at two total fractional reductions of incoming-substrate concentration (η_{tot}). t^{min}_{tot}, minimum total hydraulic retention time; $\tilde{C}_{S1}$, concentration of intermediate substrate; C_{S2}, concentration of substrate in the second stage; opt C_{S1}, optimum concentration of intermediate substrate; C_{S0}, concentration of incoming substrate; t_{one}, hydraulic retention time of the single-stage system.

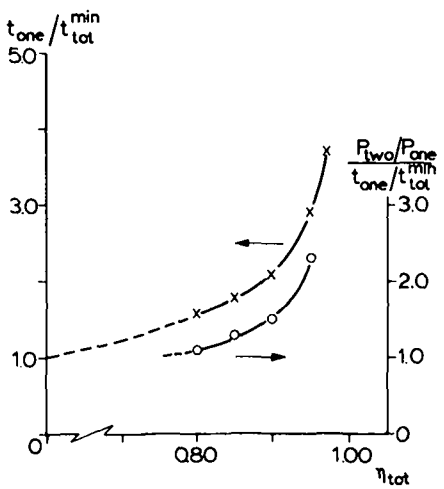


Fig. 25. Ratio of hydraulic retention time of the one-stage system and total hydraulic retention time of the two-stage system against total fractional reduction of incoming substrate (x) and ratio of production of biomass in the two-stage system and production in the one-stage system against total fractional reduction of incoming substrate (o).

at the first stage, η_1, from 0.51 to 0.73. In practice such a reduction of incoming substrate at the first stage can be achieved (Table 1). Questionable may be the possibility to maintain an optimum concentration of intermediate substrate by fluctuation in concentration of incoming substrate. However, for example, at a 50% deviation of optimum concentration of intermediate substrate, opt $\hat{\gamma}_{S1}$, (Fig 24), there is still great kinetic advantage in the two-stage system.

Qualitatively, this advantage of a two-stage system can be explained on the high rate of substrate consumption, achieved by high concentrations of substrate in the first stage (Fig. 12). In a conventional one-stage plant however, the rate of substrate consumption depends on the low concentration of effluent required. In the one-stage system the purification efficiency is limited by the concentration of substrate required for maintenance purposes. The finite fractional reduction in substrate in the one-stage system of our experiments is $\eta = 0.968$ (Table 8). At the corresponding concentration of substrate, $\hat{\gamma}_S = 0.020$ g/l, the rate of consumption of substrate equals the rate of consumption of substrate for maintenance, that is $\tilde{q}_{SR} = m_S = 0.012$ g/g.h. Below that concentration of substrate, the microbial population consumes itself.

In another series of calculations, the partial concentration of biomass in the first and second stages are assumed to be different. Further similar assumptions were made as mentioned above, assuming a total fractional reduction of $\eta_{tot} = 0.95$. The results are summarized in Table 9.

In general, under optimum conditions $V_1/V_2 > 1.0$, which is in agreement with the conclusions of Schellart (1975) in a numerical approach to the optimum ratio of the effective reactor volumes in a single-stream once-through dual-stage system. However, only if $\tilde{C}_{X1}/\tilde{C}_{X2} < 0.4$, did the volume ratio, V_1/V_2 decrease below unity (Table 9). Thus the actual volume ratio depends upon the mass ratio of biomass in both stages (Tables 8 and 9). Nevertheless the optimum mass ratio of biomass with the greatest kinetic advantage lies between $0.66 < \tilde{C}_{X1}/\tilde{C}_{X2} < 1.5$ if $\eta_{tot} = 0.95$ (Table 9). However, for practical applications, the mass ratio of biomass of both stages would fluctuate by a factor 4, resulting in only a slight decrease in the kinetic advantage: $t(\text{one stage})/t_{tot}^{min}$ decreases from 2.9 to 2.2 (Table 9).

From a kinetic viewpoint, these conclusions contradict the dimensioning of most activated-sludge plants, of which first stages are smaller than second ones (Table 1).

5.3.2.2 Biochemical approach of changing yield and maintenance coefficients of the second stage

Both series of data of the second-stage experiments (Section 5.3.2.1) are fitted to the simplified law of the three-compartment model (Eqn 102), written as

$$\tilde{q}_{SR2} = \frac{1}{Y_{max\ 2}} \mu_2 + m_{S2} \qquad (115)$$

where $Y_{max\ 2}$ is defined as

Table 9. Results of the second series of calculations of optimum dimensioning of a two-stage activated-sludge system where $\eta_{tot} = 0.95$ and $\gamma_{X1} \neq \gamma_{X2}$. Symbols: $\bar{c}_X$, $\bar{c}_{X1}$, $\bar{c}_{X2}$ are biomass concentrations; τ, t_{tot}^{min}, t_1 and t_2 are hydraulic retention times and opt γ_{S1} is the optimum concentration of the intermediate substrate.

$\bar{c}_{X1}$ (g/l)	$\bar{c}_{X2}$ (g/l)	t_{tot}^{min} (h)	t_1 (h)	t_2 (h)	opt γ_{S1} (g/l)	V_1/V_2	η_1	t(one-stage) (h)	$\bar{c}_X$ (g/l)	$\frac{t(\text{one-stage})}{t_{tot}^{min}}$
1.0	1.0	5.5	3.0	2.5	0.207	1.2	0.67	16.1	1.0	2.9
1.5	1.0	4.3	2.5	1.8	0.171	1.4	0.73	12.5	1.3	2.9
2.0	1.0	3.7	2.2	1.5	0.147	1.5	0.77	10.0	1.6	2.7
2.5	1.0	3.2	2.0	1.2	0.129	1.7	0.80	8.3	2.0	2.6
4.0	1.0	2.3	1.5	0.8	0.105	1.9	0.83	5.5	3.0	2.4
1.0	1.5	4.5	2.4	2.1	0.250	1.1	0.60	13.0	1.2	2.9
1.0	2.0	4.0	2.0	2.0	0.286	1.0	0.55	10.7	1.5	2.7
1.0	2.5	3.5	1.7	1.8	0.316	0.9	0.50	9.0	1.8	2.6
1.0	4.0	2.7	1.1	1.6	0.389	0.7	0.38	5.8	2.8	2.2

$$Y_{max\ 2} = Y_{SR2}/\{1 + \frac{(1 - Y_{RK2})}{Y_{RK2}} (m_{K2} + k_{N2})(\tilde{\omega}_{K2} + \tilde{\omega}_{G2})\} \quad (116)$$

and m_{S2} as

$$m_{S2} = \frac{1}{Y_{SR2}} \{\frac{(1 - Y_{RK2})}{Y_{RK2}} (m_{K2} + k_{N2})(\tilde{\omega}_{K2} + \tilde{\omega}_{G2})\} \quad (117)$$

Data of $\tilde{q}_{SR2}$ against μ_2 if $\tilde{C}_{S1}$ = 0.414 g/l and if $\tilde{C}_{S1}$ = 0.133 g/l are shown in Figure 26. The assumed straight-line relationships were calculated by the least-squares method and summarized in Table 10: Y_{max2} increased and m_{S2} decreased. Gaudy & Srinivasaraghavan (1974) found also a decreasing value of m_S if the concentration of incoming substrate decreased but also a decreasing Y_{max} was measured. Ecologically this phenomenon was viewed as doubtful (Verstraete, 1977), because to survive in a poor growth-supporting environment it might be expected that bacteria optimalize the efficiency of substrate consumption but minimalize substrate consumption for maintenance purposes. Our results are in agreement with this suggestion (Table 10). In addition it was found too (Section 5.3.2.1) that the affinity for substrate increased with decreasing concentration of intermediate substrate (Table 7). If it is assumed that the decrease of concentration and the change in composition of the intermediate substrate induced a decreasing ability of the environment of the second-stage reactor to support growth, the following ecological principle may be formulated. A decreasing ability selects for a population of micro-organisms characterized by a relatively high maximum yield coefficient, a low maintenance coefficient and a high affinity for substrate.

An increase of Y_{max2} defined in Equation 116, simultaneously with a decrease of m_{S2} (Eqn 117) resulted too if the biochemical data of the second-stage experiments are implicated. These data are presented in Figures 27 and 28. Calculated different coefficients are listed in Table 11. From Figure 27 and Table 11, curves of the mass fractions of DNA and RNA against μ_2 at $\tilde{C}_{S1}$ = 0.183 g/l and at $\tilde{C}_{S1}$ = 0.414 g/l, respectively, equal those of the first-stage ones (Fig. 14; Table 4). However the mass fraction of protein

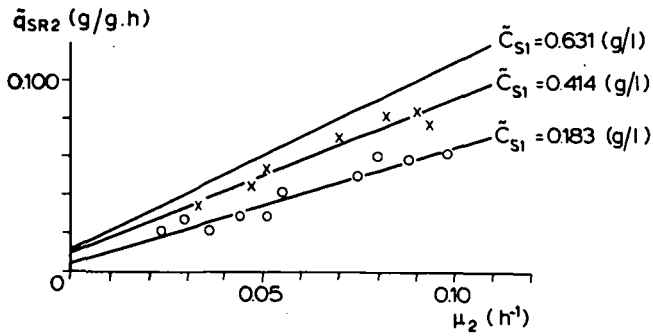


Fig. 26. Rate of uptake of substrate by R Compartment in the second stage against specific growth rate in the second stage at different concentrations of intermediate substrate. x , $\tilde{C}_{S1}$ = 0.414 g/l; o , $\tilde{C}_{S1}$ = 0.183 g/l.

Table 10. Calculated values by the least squares method of $\tilde{q}_{SR2}$ against μ_2 (Eqn 115) if $\tilde{c}_{S1}$ = 0.183 g/l and $\tilde{c}_{S1}$ = 0.414 g/l. First-stage values are also given which are calculated data of the second stage if C_{S1} = 0.631 g/l.

$\tilde{c}_{S1}$ (g/l)	$Y_{max\ 2}$ (g/g)	m_{S2} (g/g.h)	r^2	$s(\tilde{q}_{SR2}, \mu_2)$
0.183	1.64	0.004	0.92	0.005
0.414	1.25	0.010	0.94	0.005
0.631	1.03	0.012	0.94	0.014

of the second stage, $\tilde{w}_{G2}$, fed on $\tilde{c}_{S1}$ = 0.183 g/l, was found to be significantly lower than that of the second stage fed on $\tilde{c}_{S1}$ = 0.414 g/l or of the first stage (Fig. 14; Table 4), for the range of specific growth rates measured. On the other hand, enhancement was observed of the mass fraction of R Component, $\tilde{w}_{R2}$, in the second stage fed on $\tilde{c}_{S1}$ = 0.183 g/l from 0.30 to 0.38. (Fig. 27; Table 11). The relatively higher content of carbohydrate in this microbial culture (Fig. 28; Table 11) indicated a shift from G Component to R Component. In a poor growth-supporting environment, that may be advantageous

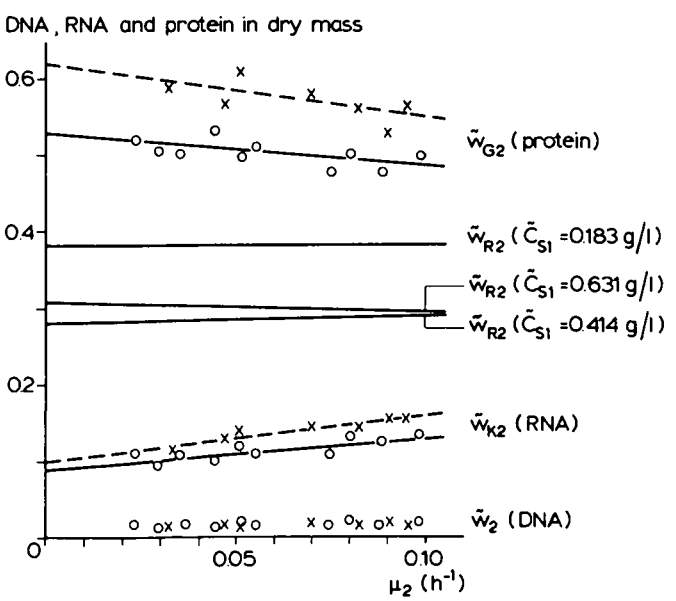


Fig. 27. Mass fractions of DNA, RNA, protein and R Component in dry mass of activated sludge in the second stage against specific growth rate of the second stage during balanced growth. o , C_{S1} = 0.183 g/l; x , C_{S1} = 0.414 g/l.

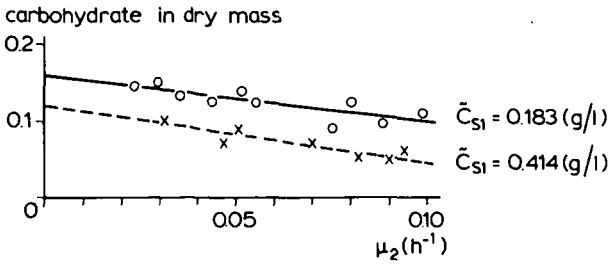


Fig. 28. Mass fraction of carbohydrate in the second stage against specific growth rate of the second stage. **o** , $\tilde{C}_{S1}$ = 0.183 g/l; **x** , $\tilde{C}_{S1}$ = 0.414 g/l.

energetically, because formation of polysaccharides needs less ATP than formation of DNA, RNA and proteins (Table 5).

The decrease of $(\tilde{\omega}_{K2} + \tilde{\omega}_{G2})$ and the increase of $\tilde{\omega}_{R2}$, inducing an increase of Y_{SR2}, result in an increase of Y_{max2} and a decrease of m_{S2} if substituted into Equations 116 and 117, respectively.

5.3.2.3 *Production of biomass in the two-stage system*

Waste of activated sludge is used only as fertilizer after a process of composting or otherwise, only if it contains not toxic concentrations of heavy metals, some organic compounds or pathogenic micro-organisms. Therefore to reduce the cost of sludge disposal, one must remove as much substrate as possible with minimum production of biomass. In this section, the production of biomass in the two-stage process will be com-

Table 11. Regression coefficients ω_{i0}, $\partial\omega_i/\partial\mu$; coefficients of determination r^2; and standard errors of estimates $s(\omega/\mu)$ of straight-line fits by the least squares method of the series of data points of the different macromolecular components i, in the activated sludge of the second stage at different specific growth rates. The second-stage chemostats were fed on concentrations of intermediate substrate of, respectively, $\tilde{C}_{S1}$ = 0.183 g/l and $\tilde{C}_{S1}$ = 0.414 g/l.

	$\tilde{C}_{S1}$					
	0.183 g/l			0.414 g/l		
	RNA	protein	carbohydrate	RNA	protein	carbohydrate
ω_{i0} (μ=0)	0.09	0.53	0.158	0.103	0.62	0.12
$\partial\omega_i/\partial\mu$	0.416	-0.401	-0.588	0.564	-0.734	-0.706
r^2	0.63	0.38	0.63	0.89	0.49	0.78
$s(\omega/\mu)$	0.009	0.014	0.013	0.005	0.020	0.010

pared with that in the one-stage process with optimum kinetics assumed.

Production of biomass in a chemostat may be defined as

$$P = \mu.\tilde{C}_X.V \tag{118}$$

where P is the rate of production of dry mass (g/h). If the simplified linear-law of the three-compartment model (Eqn 115) is substituted for μ into Equation 118, we obtain

$$P = Y_{max}(\tilde{q}_{SR} - m_S)\tilde{C}_X.V \tag{119}$$

Equation 119 has been solved for the steady states of the system with one and two stages (Tables 8 and 9). The Y_{max} and m_S of the single-stage reactor and the first stage of the dual-stage system are Y_{max} = 1.03 g/g and m_S = 0.012 g/g.h (Table 6). However both coefficients of the second stage varied with the concentration of intermediate substrate (Section 5.3.2.2). Therefore at each optimum concentration of intermediate substrate (Tables 8 and 9), Y_{max2} and m_{S2} can be read from Figure 29 if calculated values (Table 10) are plotted against $\tilde{C}_{S1}$.

Results of our calculations are summarized in Table 12. In each reactor, production of biomass decreases with increasing fractional reduction of incoming substrate. Physiologically this phenomenon can be explained from a relative increase in substrate consumed for maintenance with decreasing consumption of substrate (Eqn 119). So in the one-stage system, production of biomass becomes zero if the rate of consumption of substrate equals the rate of substrate consumption for maintenance.

The two systems can be compared only with respect to biomass production if equal amounts of substrate are consumed that is the flow rates of both systems are equal.

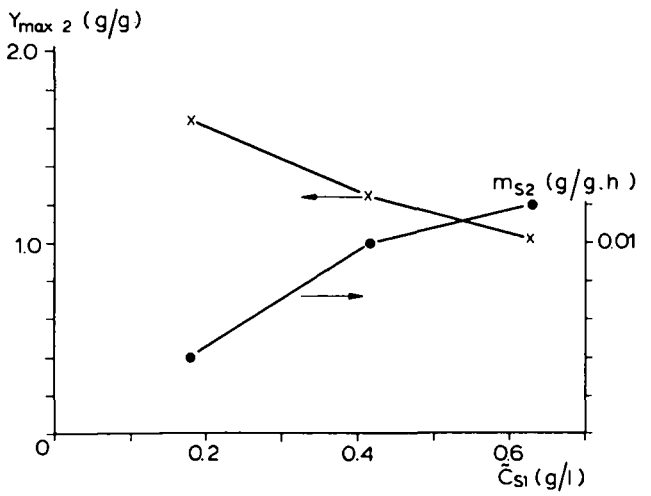


Fig. 29. Maximum yield coefficient of the second stage (x) and rate of consumption of substrate for maintenance in the second stage (●) against concentration of intermediate substrate.

Table 12. Calculated production of biomass in a one-stage and two-stage activated-sludge process with optimum kinetics as summarized in Table 8. P_1, P_2 and P are rates of production of biomass (g/h) in the first stage, the second stage and one stage, respectively. Production of biomass in both systems have been compared for equal amounts of substrate consumed. Therefor P(one-stage)/P(two-stage) was divided by t(one-stage)/t_{tot}^{min}

η	opt $\tilde{C}_{S1}$ (g/l)	$\frac{t(\text{one-stage})}{t_{tot}^{min}}$	P_1 (g/h)	P_2 (g/h)	P(one-stage) (g/h)	$\frac{P(\text{two-stage})/P(\text{one-stage})}{t(\text{one-stage})/t_{tot}^{min}}$
0.80	0.310	1.6	0.065	0.042	0.060	1.1
0.85	0.284	1.8	0.062	0.037	0.042	1.3
0.90	0.252	2.1	0.057	0.033	0.029	1.5
0.95	0.207	2.9	0.049	0.025	0.011	2.3
0.968	0.187	3.7	0.046	0.018	0.000	---

Therefore the ratio of biomass production in the one-stage and two-stage system, P (two stage)/P (one stage), have to be divided by the ratio of hydraulic retention time, t (one stage)/t_{tot}^{min} which equals ϕ (two stage)/ϕ (one stage), because $V = V_1 + V_2$ (Section 5.3.2.1).

With optimum kinetics, the two-stage system always produces more biomass than the one-stage system (Table 12, last column). The higher the total fractional reduction, the higher the difference as shown in Figure 25. Especially, this can be explained from the higher rate of consumption of substrate in the first stage of the dual-stage system than the single reactor and thus $\tilde{q}_{SR} >> m_S$ (Eqn 119). Moreover as pointed out in Section 5.3.2.2, with decreasing concentration of intermediate substrate, the maintenance coefficient decreased (Fig. 29) resulting in a higher production of biomass.

No better results were obtained if the partial concentration of biomass in both stages was assumed to be different (Table 9). If the ratio of biomass, C_{X1}/C_{X2}, increased from 1 to 4, the ratio of production of biomass in the one-stage and two-stage systems, corrected for equal amounts of substrate consumed, decreased from 2.3 to 1.9. And if C_{X1}/C_{X2} decreased from 1 to 0.25, the biomass-production ratio remained equal.

Stover & Kincannon (1976) reported also higher production of sludge in a two-stage activated-sludge system than in a one-stage for a range of specific growth rates. So, if biomass is difficult to dispose of, the one-stage system might offer advantages over the two-stage system. However one of the possibilities in diminishing biomass production is to operate the second stage below the rate of substrate consumption for maintenance: $\tilde{q}_{SR} < m_S$ (Eqn 119). Then discharge of activated sludge of the first stage can be pumped into the second stage for maintenance of an active and viable population of micro-organisms at that stage (Klapproth, 1976). From the viewpoint of extended aeration, i.e. $\tilde{q}_{SR} < m_S$, the reportedly smaller production of biomass in a two-stage system than a one-stage process by Dixit & Patterson (1975) and Wu & Kao (1976) might be explained.

5.3.2.4 Viability of biomass in the two systems

It has so far been assumed (Sections 5.3.2.1 and 5.3.2.2) that all cells present in the activated sludge are capable of division, i.e the fractional viability, $(1 - \tilde{w}_N)$ was unity. However, viability depends on growth rate (Section 4.2.2.4; Fig. 20). The state of cells in the one-stage and two-stage systems will be discussed now. With the concept of viability, one can calculate concentrations of total mass, viable plus non-viable or dead mass, needed to obtain, for instance, concentrations of viable mass in the reactors of both systems of 1.0 g/l.

If a fractional reduction of $\eta = 0.95$ is assumed, the concentrations of substrate in the reactors can be read from Table 8; in the one-stage reactor, $\tilde{C}_S = 0.032$ g/l and in the two-stage reactors, $\tilde{C}_{S1} = 0.207$ g/l and $\tilde{C}_{S2} = 0.032$ g/l. Then the rates of consumption of substrate in the single reactor and first stage of the double reactor can be read from Figure 12 $\tilde{q}_{SR} = 0.02$ g/g.h and $\tilde{q}_{SR1} = 0.100$ g/g.h. From Figure 22, $\tilde{q}_{SR2}$ in the second-stage reactor can be obtained: q_{SR2} 0.034 g/g.h. This corresponds to specific growth rates of μ(one stage) = 0.008 h^{-1}, $\mu_1 = 0.09$ h^{-1} (Fig. 15) and, in the second stage, $\mu_2 = 0.04$ h^{-1} (Fig. 26).

At similar concentrations of substrate, a higher specific growth rate can be obtained in the second-stage reactor than in the one-stage reactor because of the decreased formal order of reaction (Table 8). This results in a more active and viable system at low concentrations of effluent.

The fractional viability of the one-stage reactor ($\mu = 0.008$ h^{-1}) is 0.21; of the first-stage reactor ($\mu_1 = 0.09$ h^{-1}) 0.75 and of the second-stage reactor ($\mu_2 = 0.04$ h^{-1}) 0.57, if $k_N = 0.03$ h^{-1} (Fig. 20). Then, if viable-mass concentrations of 1.0 g/l are required, the concentrations of solids (viable plus non-viable or dead mass) are respectively $\tilde{C}_X$(total mass) = 4.8 g/l, $\tilde{C}_{X1}$(total mass) = 1.3 g/l, $\tilde{C}_{X2}$(total mass) = 1.7 g/l. The working volume of the one-stage reactor is assumed to be $V = 1$ l. Then from Equation 114 and $V_1/V_2 = 1.2$ (Table 8), the volumes of the two-stage reactors will be: $V_1 = 0.55$ l and $V_2 = 0.45$ l. Then the total amounts of solids in the reactors will be: 4.8 g in the one-stage reactor; 0.7 g in the first-stage reactor and 0.8 g in the second-stage reactor.

The amount of non-viable or dead mass in the one-stage system is thus a factor 7.6 times higher as in the two-stage system, to achieve equal amounts of viable mass. In terms of elimination of substrate by consumption, a more active and viable mass can be obtained in the two-stage system. However especially in the one-stage system, elimination of substrate by adsorption on the greater amount of solids might be a large contribution to elimination of substrate.

5.4 OPTIMUM KINETICS OF THE TWO-STAGE ACTIVATED-SLUDGE SYSTEM FED ON VANILLIN AND ACETATE; INFLUENCE OF THE FORMAL ORDER OF REACTION

5.4.1 Introduction

Heinicke (1967) reported that in a two-stage activated-sludge plant, preferred carbon sources like glucose stimulated oxidation of sodium formate and trioxane. In

batch experiments, acetate, commonly consumed by micro-organisms (W. Harder, 1973), affected the rate of consumption of vanillin (Section 6.2.2.3). In microbial cells, acetate may directly be bound to coenzyme A and directly used in the tricarboxylic acid cycle. It could easily be assayed as described in Section 2.5.4. Therefore this organic compound was used as a second substrate in the breakdown of vanillin. The influence of the formal order of reaction on the kinetics of the two-stage system was studied too.

5.4.2 Procedures

Operational procedures were similar to those described in Sections 2.6.2. However the vanillin-basal medium was modified as described in Section 2.3. The carbon ratio of vanillin to acetate was held at 4.
The analytical procedures were the same as described in Section 4.2.1. In addition acetate (Section 2.5.4) and total aromatics (Section 2.5.2) were assayed. The operational conditions were set at V = 4 l, C_{SO} = 0.789 g/l and the hold-up factor, ω_D, could be maintained at 0.36, deviating 0.12. After an interval greater than 5 times the reciprocal of μ, reliable estimates of changes in rate of substrate consumption against $\bar{C}_S$ could be made. In our experiments, the specific growth rate ranged from 0.043 to 0.235 h^{-1} by increasing the dilution rate and the flow of discharge of biomass.

In the two-stage experiments, it was hardly possible to hold the concentration of intermediate substrate constant at different levels as obtained in the vanillin-fed two-stage experiments (Section 5.3.1; Fig. 21). In the one series of experiments, the operational conditions of the first stage were set at: V_1 = 3 l; D_1 = 0.417 h^{-1}; C_{SO} = 0.789 g/l; ω_D = 0.36 and the mean concentration of biomass: $\bar{C}_{X1}$ = 0.8 g/l. This resulted in the mean concentration of intermediate substrate of: $\bar{C}_{S1}$ = 0.390 g/l, deviating 0.102. The operational conditions of the second stage were held on D_2 = 0.187 h^{-1}; V_2 = 3 l. The specific growth rate of the second stage, corrected for incoming biomass of the first stage, ranged from 0.016 to 0.098 h^{-1} by increasing the flow rate of discharge of biomass, i.e ω_5 $(1 - \omega_1)\phi_0$ (Fig. 20).

Similarly to Section 5.3.1, the hydraulic dilution rate, D_2, was kept constant rather than the hold-up factor, ω_{D2}. By varying the flow rate of discharge of sludge, estimates of a steady state could be made during a period of 2 weeks. However the fluctuations in steady-state concentrations of substrate were again fairly lare and the deviation ranged from 20 to 25% in the second stage.

5.4.2 Results and discussion

Figure 30 shows the rate of substrate utilization in terms of carbon (TOC and 280 nm measurements), correction for acetate, against the concentration of substrate in terms of carbon, corrected for acetate. Our experimental data were fitted to Equation 71 and the calculated kinetic coefficients were: k_{SR} = 0.260 g/g.h.$(g/l)^{a_S}$ and a_S = 0.24, where r^2 = 0.89. Comparing this curve with that obtained from the vanillin experiments (Section 4.2.2.1, Fig. 12), the formal order of reaction decreased significantly from 0.71 to 0.24. This causes difficulty in maintaining stable conditions in the upswing of the

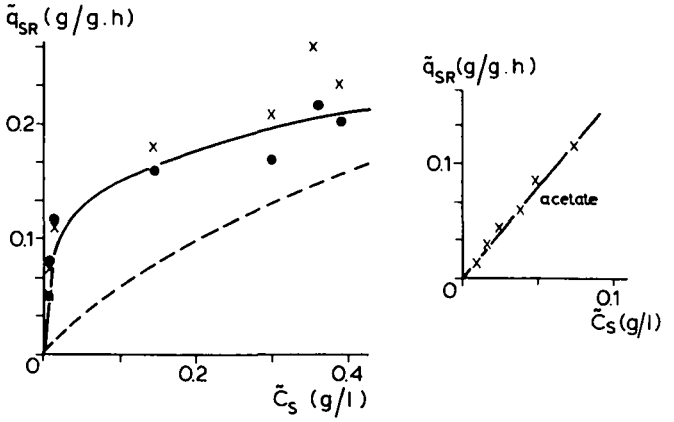


Fig. 30. Rate of uptake of substrate by R Compartment against concentration of substrate if the reactor was fed on vanillin with acetate (—) or vanillin without acetate (– –). ● , average data in terms of total organic carbon; ✖ , average data in terms of total aromatics (λ_{abs} = 280 nm). The calculated line is based on the data in carbon.

substrate utilization curve because then a slight change in q_{SR} might induce a distinct shift in concentration of substrate. A possible explanation of the enhancement of the rate of vanillin consumption at low growth rates if acetate is present, may be that given in Section 6.2.2.3. From Figure 30, the curve in terms of carbon parallels the curve measured in terms of total aromatics (λ_{abs} = 280 nm). This indicates that the rate of breakdown of aromatics may be the rate-limiting step. From the insetgraph in Figure 30 the rate of acetate consumption was found to be proportional to its concentration in the reactor. This confirms the results of studies by Downing (1966) using acetate as sole carbon and energy source.

The average data of the second-stage measurements were fitted to Equation 71 and depicted in Figure 31. The calculated values are: k_{SR2} = 0.174 g/g.h.(g/l)^{a_S}, a_{S2} = 0.187 and r^2 = 0.35. The poor correlation of $\tilde{q}_{SR2}$ against $\tilde{C}_{S2}$ is due to fluctuation in concentra-

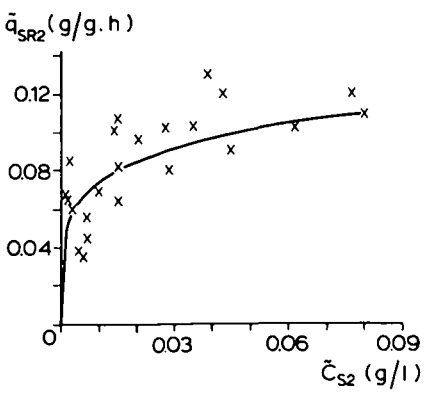


Fig. 31. Rate of uptake of substrate by R Compartment of the second stage of the two-stage system against concentration of substrate in the reactor.

tion of intermediate substrate, 0.402 g/l, deviating 0.152 and low formal order of reaction. Nevertheless reliable calculations could be made to optimilize vanillin decomposition in the dual-stage process in the presence of acetate, because the total rate of substrate utilization was mainly determined by that in the first (Section 5.3.2.1).

Optimum kinetics of the one-stage and two-stage system, fed on vanillin or vanillin if acetate was present were calculated, based on the assumptions in Section 5.3.2.1. The total fractional reduction was assumed to be, $\eta_{tot} = 0.95$. Results are summarized in Table 13 and also those of the vanillin-fed system are given. From Table 13, the enhancement of the rate of vanillin consumption at low concentrations of substrate in the reactor, if acetate were present, resulted in a lowering of the kinetic advantage of the two-stage system by a factor 2. This can be explained by the fact that in the one-stage reactor, for example at a required fractional reduction, $\eta = 0.95$ and $\tilde{C}_S = 0.032$ g/l, the rate of consumption of carbon, $\tilde{q}_{SR}$, increased by a factor 4.8 times that in the presence of acetate (Fig. 30). This reduced the kinetic advantage of maintaining a high q_{SR} in the first stage of the dual-stage process.

In practice, one must bear in mind the stimulatory effects of, for instance, glucose or acetate if the kinetic advantage of a two-stage system are to be pursued in breakdown of organic compounds that are difficult to break down biologically.

Table 13. Summary of the optimum kinetic calculation of the one and two-stage system fed on vanillin or vanillin in the presence of acetate if the fractional reduction of incoming-substrate concentration was, $\eta = 0.95$. Definitions of symbols are given in Table 8.

Substrate	t_{tot}^{min} (h)	t_1 (h)	t_2 (h)	opt $\tilde{C}_{S1}$ (g/l)	V_1/V_2	η_1	t(one) (h)	t_{one}/t_{tot}^{min}
vanillin	5.5	3.0	2.5	0.207	1.2	0.67	16.1	2.9
vanillin if acetate present	3.5	2.5	1.0	0.165	2.5	0.73	4.7	1.3

6 Microbiology of activated sludge fed on vanillin or vanillin and acetate

6.1 INTRODUCTION

In Chapter 1 and 4, the significance was emphasized of microbial interactions and population shifts on the kinetics of the activated-sludge process. A study of the microbial composition, the numbers of bacteria and their physiology is necessary for a closer understanding of the biological process. Recently Hughes & Stafford (1976), Taber (1976) and Verstraete (1977) reviewed waste-water micro-organisms and population dynamics. These studies revealed a delicately balanced population of bacteria, each interacting with and influencing the other members of the population.

The purpose of this chapter is to give information on the microbial changes in the mixed cultures, the predominating types and their activities during continuous growth on vanillin or vanillin and acetate. Evidence was presented for a correlation of the results obtained in the kinetic experiments with those of the microbial observations (Section 6.2). The floc-forming ability of isolated cultures was tested and the flocs were examined for the presence of cellulose fibrils (Section 6.3).

6.2 CHARACTERIZATION, COUNTS AND ACTIVITIES OF ACTIVATED-SLUDGE BACTERIA

6.2.1 Procedures

The activated-sludge chemostat with recycling of biomass (Section 2.6.2) was inoculated with activated sludge (Section 2.1) and adapted to the vanillin-basal medium (Section 2.2) or sodium acetate-vanillin-basal medium (Section 2.3). The experimental conditions were pH 7.2. temperature 20°C and oxygen concentration 0.003 g/l or more (Section 4.2.1).

The activated-sludge population grown on vanillin was characterized at a low and high specific growth rate of, respectively, $\mu = 0.032\ h^{-1}$, deviating 0.002 and $\mu = 0.112\ h^{-1}$, deviating 0.017. The hydraulic dilution rates were, respectively, $D = 0.010\ h^{-1}$ and $D = 0.361\ h^{-1}$ resulting in a hold-up factor of $\omega_D = 0.31$. The volume of the reactor was 4 l. The concentration of incoming substrate of vanillin in carbon was 0.631 g/l. The vanillin-acetate growing culture was maintained at $\mu = 0.109\ h^{-1}$, deviating 0.022 and $D = 0.303\ h^{-1}$, with a hold-up factor of $\omega_D = 0.36$. The volume of the reactor was 4 l. The concentration of incoming substrate of vanillin in carbon was held on 0.631 g/l and sodium acetate trihydrate added in the concentration of carbon of 0.158 g/l. The carbon to introgen ratio was held on 6.0.

The following media were used:

(1) Aromatic medium contained: vanillin, vanillic acid or protocatechuic acid 1.0 g;

$(NH_4)NO_3$ 0.5 g; $MgSO_4.7H_2O$ 0.1 g; K_2HPO_4 1.0 g; KH_2PO_4 0.5 g; a trace of yeast extract (Difco); and 1.0 l of tap water; pH 7.0. The aromatics were separately sterilized through a Seitz-filter.

(2) Glucose-nutrient-broth medium contained: glucose 5.0 g; nutrient broth 8.0 g; tryptone 8.0 g; yeast extract (Difco) 4.0 g; and 1.0 l of tap water; pH 7.0.

(3) Casitone-glycerol medium consisted of the following ingredients: casitone (Difco) 1.0 g; glycerol 2.0 g; yeast extract (Difco) 0.35 g; and 1.0 l of tap water; pH 6.9.

For plate counts, agar 12 g/l was added. In all experiments, the carbon source was the growth-limiting nutrient.

Samples of 10 ml of activated sludge, grown under the steady-state conditions as indicated, were collected in sterilized bottles and the sludge flocs were dispersed with a M.S.E. Sonifier (20 kHz, 40 W, 0.5 min, 0^oC), in order to liberate the bacteria from the interior of the floc. The dispersed flocs were diluted by a factor 10^{-4} - 10^{-6} in sterilized water to provide a microbial concentration within the usual ranges for counting. Duplicate samples of 0.1 ml were spread on sets of 5 previously dried agar plates, containing glucose-nutrients broth or casitone-glycerol medium. After 14 days incubation at 20^oC, colonies were counted and isolated. Pure cultures were characterized by the scheme of Schmidt-Lorenz (1965) and Bergey's manual (1974) and maintained on casitone-glycerol slants.

The pure cultures of bacteria were grown in shake cultures at 30^oC (200 ml in a 1 litre Erlenmeyer) on aromatic medium. Total concentration of aromatics (Section 2.5.2) and total dry mass (Section 2.4.1) were measured during growth. Simultaneously the growth of a culture was also tested on glucose-nutrient broth.

Batch experiments with activated sludge, used the equipment described in Section 2.6.1. Sludge was taken from the chemostat fed on vanillin-basal medium (Section 4.2.1) where $\mu = 0.030\ h^{-1}$; $D = 0.100\ h^{-1}$; $C_S = 0.015$ g/l and $C_X = 1.5$ g/l, or taken from the chemostat fed on vanillin-acetate-basal medium (Section 5.4.2) where $\mu = 0.043\ h^{-1}$; $D = 0.126\ h^{-1}$; $\tilde{C}_S = 0.006$ g/l and $\tilde{C}_X = 2.5$ g/l.

6.2.2 Results and discussion

6.2.2.1 Characterization of bacteria in the chemostat fed on vanillin or vanillin and acetate

In both activated-sludge cultures, most of the isolates were Gram-negative and non-fermentative, rod forming bacteria. They were identified as *Pseudomonas; Flavobacter / Xanthomonas; Alcaligenes; Moraxella* and *Acinetobacter* (Table 14). Two strains were Gram-positive and belonged to the genus *Corynebacterium*. They gave yellow colonies on agar plates and the pleomorphic rods did not transform into coccoids in old cultures like Arthrobacter-type species (Veldkamp et al., 1963; van Gils, 1964; Bergey's manual, 1974; Crombach, 1974). Various species of protozoa as rotifers and ciliates were observed, along with stalked types like *Epistylis* and *Vorticella*. Isolation of pure cultures of bacteria was hampered by formation of extracellular slime layers on the agar plates which resulted in a fraction of unidentified species in every sample.

Table 14. Aerobic heterotrophic bacteria present in the continuous culture of activated sludge fed on vanillin or vanillin and acetate and counted on glucose-nutrient broth agar. Data are given in percentage of the total number of bacteria counted.

Genus	Carbon source		
	vanillin		vanillin + acetate
	$\mu = 0.032\ h^{-1}$	$\mu = 0.112\ h^{-1}$	$\mu = 0.109\ h^{-1}$
Pseudomonas	0.8	4.9	26.0
Flavobacter Xanthomonas	0.7	0.5	7.6
Alcaligenes	7.7	45.3	28.5
Acinetobacter	41.5	14.3	18.2
Moraxella	5.1	23.3	1.1
Corynebacterium	28.6	0.5	-
Unidentified	15.6	11.2	18.6
Total number of bacteria per g dry mass	1.6×10^{12}	0.3×10^{12}	0.75×10^{12}

The Gram-negative aerobic heterotrophic bacteria as predominant species were also found by: McKinney & Horwood (1952), Porges (1960), McKinney (1962), Adamse (1966), Prakasam & Dondero (1967a, 1967b), Unz & Dondero (1970), Benedict & Carlson (1971) and Liac & Dawson (1975). The Gram-positive *Corynebacterium* was isolated from sludges by treating wastes with a high proportion of carbohydrate such as confectionery and fruit-juice wastes (Takii, 1977). Gram-positive bacteria were also isolated by Adamse (1966) from activated sludge fed on dairy waste but identified as species of *Arthrobacter*.

Thus despite feeding on the specific organic compound vanillin, a great variety of bacteria and protozoa were cultured, resulting in an optimally functioning activated sludge. Orshanskaya et al. (1975) obtained similar results by feeding an activated-sludge culture on sulphanilic acid. In this experiments however *Pseudomonas* was found to be the predominant bacterium.

6.2.2.2 Counts and activities of the bacteria grown on vanillin

There is no equivocal medium that can support all the heterotrophic bacteria present in activated sludge. In the experiments of Banks & Walker (1977), a casitone-glycerol agar proved to be the most generally useful medium. The glucose-nutrient broth agar and casitone-glycerol agar used here for counting the total aerobic heterotrophs

gave quite similar results. The activated sludge dealt with here contained about 10^{11}-10^{12} viable cells per g dry mass (Table 14) which agreed with bacterial counts reported in the literature (van Gils, 1964; Weddle & Jenkins, 1971; Benedict & Carlson, 1971; Walker & Davies, 1977 and Takii, 1977).

The populations of *Acinetobacter*, *Moraxella* and *Corynebacterium* were predominant (75%) at $\mu = 0.032\ h^{-1}$ (Table 14). In a well aerated environment, Baumann et al. (1968) observed ready and abundant growth of *Acinetobacter* species with minimum nutritional conditions. So these organisms may rely on the action of bacteria that produce low-molecular intermediates such as succinate, acetate and ethanol from organic substrates. These intermediates may be produced by the second predominant bacterium, that is *Corynebacterium*, a facultative anaerobe. Otherwise *Corynebacterium* can accumulate polysaccharides (Takii, 1977). Dias & Bhat (1964) and Pipes (1966) suggested that the ability to store polysaccharides or lipids may be an important factor in survival in an environment where food supply is limited. A first approach to modelling of this hypothesis is given in Section 5.3.2.3. Perhaps also of ecological significance is the ability of *Acinetobacter* to store large amounts of polyphosphate (Fuhs & Min Chen, 1975; Yall et al., 1975).

The increase in the specific growth rate from 0.032 to 0.112 h^{-1} resulted in a significant population shift to *Pseudomonas* (from 0.8 to 4.9 %); *Alcaligenes* (from 7.7 to 45.3 %) and *Moraxella* (from 5.1 to 23.3 %). Both populations of *Acinetobacter* and *Corynebacterium* decreased sharply, from 41.5 to 14.3 % and from 28.6 to 0.5 % respectively (Table 14). Simultaneous decrease of *Acinetobacter* and *Corynebacterium* might support our suggestion that *Corynebacterium* promotes the growth of *Acinetobacter* in the activated sludge.

Activities of a number of bacteria grown on vanillin are summarized in Table 15. Only the minor population of *Pseudomonas* was responsible for the breakdown of vanillin to protocatechuic acid (Section 4.1; Scheme 2). Most of the other strains could utilize

Table 15. Growth of a number of isolated strains of bacteria on vanillin, vanillic acid, protocatechuic acid and glucose-nutrient broth. Symbols: growth, +; no growth, -; poor growth, ±.

	Number of strains	Vanillin	Vanillic acid	Protocatechuic acid	Glucose-nutrient broth
Pseudomonas	9	+	+	+	+
Flavobacter / Xanthomonas	11	-	-	-	+
Alcaligenes	13	-	-	+	+
Acinetobacter	3	-	-	±	+
Moraxella	5	-	-	+	+
Moraxella	4	-	-	-	+
Corynebacterium	2	-	-	±	+

protocatechuic acid as carbon and energy source in shake cultures (Table 15). Stanier et al. (1977, Table 19.3) listed *Pseudomonas, Alcaligenes, Acinetobacter* and *Moraxella* as capable of dissimilating aromatic compounds through the β-ketoadipate pathway. In crude extracts of the activated sludge, only the activity of EC 1.13.11.3-protocatechuate 3,4-dioxygenase was found (Section 4.1). This enzyme catalyzed the ortho cleavage of protocatechuic acid, resulting in the β-ketoadipate pathway for break down of vanillin. So the observed biochemical and microbial results are in agreement with the data of Stanier et al.

The formation of extracellular products by micro-organisms that promote the growth of other species, so-called commensalism, is often observed in mixed-culture populations (Meers, 1973; Fredrickson, 1977). The low percentage of *Pseudomonas* responsible for break down of vanillin to protocatechuic acid and otherwise the large percentage of bacteria that could grow on protocatechuic acid supported the more detailed analysis of the effluents of the chemostats and media of the batch cultures (Section 2.5.3). Results of these effluent analyses, at two specific growth rates of the vanillin-fed chemostat, on vanillin, vanillic acid and protocatechuic acid are given in Table 16. The intermediate, vanillic acid, was present in the medium but protocatechuic acid was not detected. This might be explained by the large number and wide range of bacteria that could utilize protocatechuic acid (Table 15). Low-molecular compounds like acetic acid (Section 2.5.4) could not be detected. Nevertheless, if the concentrations of vanillin and vanillic acid were calculated in terms of carbon, only 45-46% of total organic carbon (TOC) consisted of vanillin and vanillic acid in the media at both growth rates (Table 16).

Similar observations were made in batch experiments with activated sludge (Sections 2.6.1 and 6.2.1). Figure 32 presents the results of one of these experiments. The initial concentration of vanillin in carbon was 0.147 g/l. and the concentration of biomass was 1.0 g/l. During consumption of vanillin, the medium was analysed for total organic carbon (Section 2.5.1), total aromatics (Section 2.5.2), and vanillin vanillic acid and protocatechuic acid respectively (Section 2.5.3). From Figure 32, the consumption of vanillin in time, resulted in the formation of vanillic acid in the medium. This is reflected in a bend in the curve of the consumption of vanillin measured in terms of total organic carbon.

Table 16. Analysis of the effluents of the first stage of the two-stage activated-sludge system fed on vanillin (Sections 2.5.3 and 5.3.2.1). Vanillin, vanillic acid were identified by comparison mass spectra with authentic compounds (Section 2.5.3) m/e of trimethylsilyl derivatives of vanillin and vanillic acid were respectively: 224 and 312.

μ (h^{-1})	$\tilde{C}_{S1}$ (g/l)	Vanillin (g/l)	Vanillic acid (g/l)	Protocatechuic acid (g/l)	Fraction of total carbon
0.075	0.183	0.010	0.150	< 0.005	0.45
0.173	0.414	0.122	0.230	< 0.005	0.46

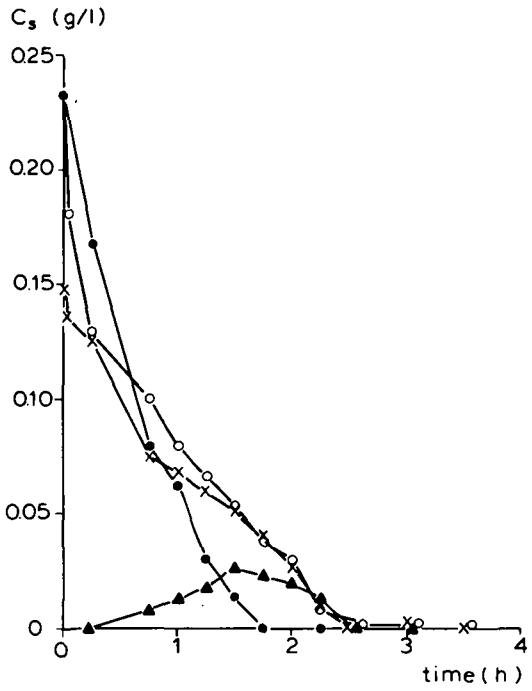


Fig. 32. Batch removal of vanillin with activated sludge taken from the chemostat fed on vanillin-basal medium (Section 4.2.1) where $\mu = 0.030\ h^{-1}$. The initial concentration of vanillin in carbon was, $C_{S0} = 0.147$ g/l. ● , vanillin (as indicated); × , total organic carbon; ▲ , vanillic acid (as indicated); o , total aromatics.

6.2.2.3 Counts and activities of activated-sludge bacteria grown on vanillin in the presence of acetate

In Table 14, the results are presented of counts of bacteria continuously grown on vanillin and acetate ($\mu = 0.10\ g\ h^{-1}$). Addition of acetate resulted in a variety of species, more or less similar to the vanillin-fed cultures. However the quantitative composition is quite different: *Pseudomonas* increased from 4.9 to 26.0 % of the total number of bacteria per g dry mass. The large increase of *Pseudomonas* which can break down vanillin, suggests a partial explanation of the acetate-stimulated rate of continuously consumption of vanillin (Section 5.4). *Alcaligenes* and *Moraxella* decreased, respectively, from 45.3 to 28.5 % and from 23.3 to 1.1 %. This decrease of both genera which can consume protocatechuic acid, might be explained by the increased population of *Pseudomonas* competiting for its own excretion products, The maintenance of the population of *Acinetobacter* might be supported by acetate in the medium.

An enhancement in the rate of consumption of vanillin could also be obtained with increasing concentration of acetate, in a series of batch experiments. Different concentrations of acetate were added to the vanillin-basal medium and inoculated with continuously-pregrown activated sludge ($\mu = 0.06\ h^{-1}$) adapted to vanillin and acetate. The carbon to nitrogen ratio of the medium was 6.0. Results are shown in Figure 33. The

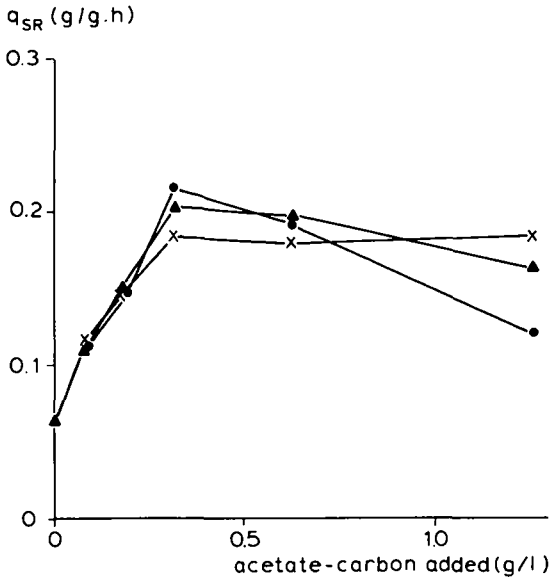


Fig. 33. Initial rates of consumption of vanillin with different concentrations of acetate added. The batch experiments were with activated sludge from the chemostat fed on vanillin-acetate-basal medium (Section 5.4.2) where μ = 0.043 h^{-1}. The initial concentration of vanillin in carbon was, C_{SO} = 0.631 g/l and acetate as indicated. ▲, total organic carbon; × , total aromatics (λ_{abs} = 280 nm); ● , acetate in terms of carbon.

rate of consumption of substrate was measured in terms of total organic carbon (Section 2.5.1), total aromatics (Section 2.5.2) and acetate (Section 2.5.4). The rate of consumption of total aromatics was stimulated in the presence of acetate (Fig. 33). A maximum enhancement was obtained at a mass ratio of vanillin to acetate (in carbon) of 2. The rate of consumption of acetate decreased above a concentration of 0.3 g/l in the medium. However the rate of consumption of total aromatics remained constant.

The enhancement of the rate of consumption of vanillin is poorly explained by an increase of substrate, here acetate, as measured in the batch experiments, because in the continuous culture, no acetate was detected below a specific growth rate of 0.100 h^{-1}.

In batch experiments of Tischler & Eckenfelder (1969) removal rates of mixtures of glucose, aniline and phenol were not affected by addition of acetate. However the rates of consumption of aniline and phenol could be already enhanced by glucose in the medium (Heinicke, 1967).

6.3 Formation and structure of the bacterial flocs

6.3.1 Procedures

Floc-forming ability was tested on the isolated bacterial cultures by inoculating the micro-organisms in tubes containing 6 ml of casitone-glycerol medium (Section 6.2.1).

The tubes were incubated for 2 days on a rotary shaker at 30°C after which floc formation was visually estimated.

Deinema & Zevenhuizen (1971) showed that a number of Gram-negative heterotrophs, isolated from various activated-sludge installations in the Netherlands, were able to grow in flocs by the formation of extracellular cellulose fibrils. Our floc-forming isolates were estimated for the presence of cellulose-fibrils according to the method of Deinema & Zevenhuizen. The settled flocs of the pure cultures were washed twice with distilled water and finally suspended in 5 ml of sodium citrate buffer 0.05 mol/l, pH 4.5. To 1 ml of floc suspension, a solution of 2 mg of cellulase (Maxazym 2000, extract of Trichoderma viride, Gist-Brocades, Delft) in 2 ml of sodium citrate buffer, 0.05 mol/l, pH 4.5, was added. Floc suspensions without cellulase were put up simultaneously and both series incubated at 45°C for 2 h with occasional shaking. In general, cellulase-sensitive flocs were completely dissolved within this time.

6.3.2 Results and discussion

6.3.2.1 Floc structure at different specific growth rates

Samples taken from the vanillin-fed chemostat at $\mu = 0.032\ h^{-1}$ and $\mu = 0.112\ h^{-1}$ and from the vanillin-acetate-fed chemostat at $\mu = 0.109\ h^{-1}$ were examined macroscopically by a phase contrast microscope. Figures 34-36 give the predominant forms of activated-sludge flocs at these respective specific growth rates. Figure 34 shows a finger-like floc with a loose packing of the cells. Figures 35 and 36 give more compact flocs without any definite form. Generally more branched flocs were observed at low growth rates than at higher ones. More disperse growing bacteria were observed at the higher specific growth rates. Increased dispersion might result in an increase of the total free surface of bacteria which may react with substrate in the reactor (Sladká & Zahrádka, 1971). Then, a more active system in substrate consumption may be expected because of decreasingly diffusion limitation of substrate into the flocs. This might be one of the explanations of the delay in washout of the culture, observed in our experiments (Section 4.2.2.1).

The structure of the sludge flocs, present in the vanillin experiment with $\mu = 0.032\ h^{-1}$, was also examined under the E.M. (Figure 37). This electron micrograph shows an irregular network of fibrils in which many different bacterial cells are enclosed.

The photographs (Figures 34-37) show a great similarity to those published by Deinema & Zevenhuizen (1971).

6.3.2.2 Floc structure of the isolated bacteria

The isolated strains (Table 15) were tested for flocculent growth (Section 6.2): 3 strains of *Pseudomonas* and 1 strain of *Acinetobacter* gave a positive result. The other strains were no or only poor floc-formers. It may be concluded that species of *Pseudomonas* and *Acinetobacter* were mainly responsible for floc formation of the activated sludge fed on vanillin or vanillin and acetate. Fuhs & Mi Chen (1975) reported also the ability of *Acinetobacter* to form flocs.

6.3.2.3 Cellulase-sensitivity of the flocs

Incubation of flocs of the activated sludge or the pure cultures by cellulase (Section 6.2) resulted in dispersion of the flocs within 1-2 h. Fibrillar fractions of native exopolymers could be obtained by treatment of activated sludge from vanillin flocs with NaOH 1 mol/l at 100°C for 1 h, to dissolve cellular proteins and nucleic acid. Then the alkali-stable fraction was digested with HCl 0.66 mol/l at 100°C for 2 h to remove non-cellulosic glucans. The residue was made acid-free and after drying, a network of fibrils (about 1% of the dry mass) was observed as shown in Figure 38. These fibrillar network could be dispersed completely within 1 h by incubation with cellulase. So a greater part of the fibrils present in the sludge flocs is build up by cellulose-like exopolymers and plays an essential role in the mechanism of flocculation.

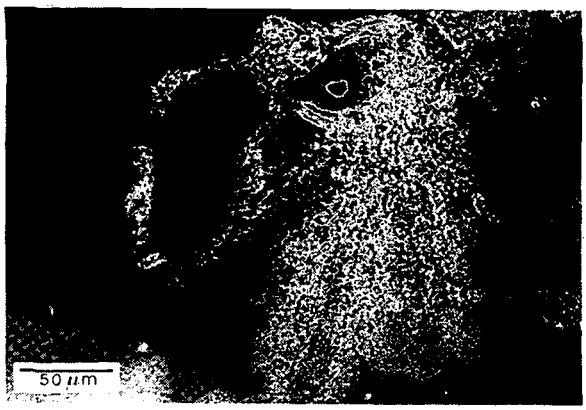


Fig. 34. Floc structure of the activated sludge under the phase-contrast microscope. The sludge was grown in the chemostat fed on vanillin (Section 4.2.1) where $\mu = 0.032\ h^{-1}$.

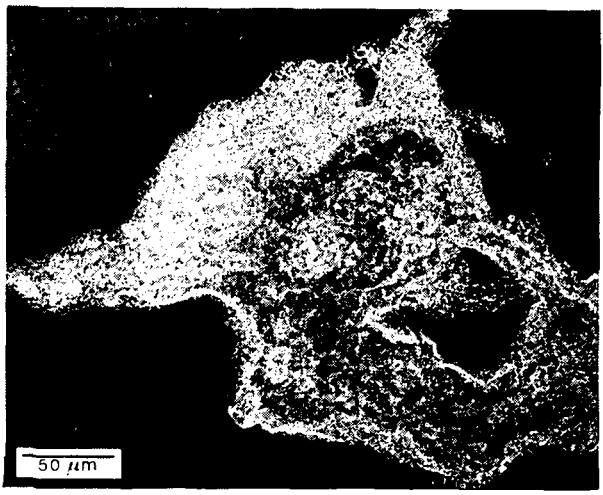


Fig. 35. Floc structure of the sludge under phase-contrast microscope fed on vanillin (Section 4.2.1) where $\mu = 0.112\ h^{-1}$.

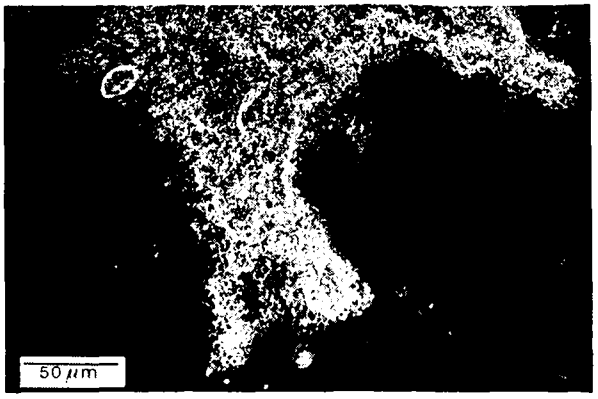


Fig. 36. Floc structure of the activated sludge under phase-contrast microscope. The sludge was grown in the chemostat fed on vanillin and acetate (Section 5.4.2) where $\mu = 0.109\ h^{-1}$.

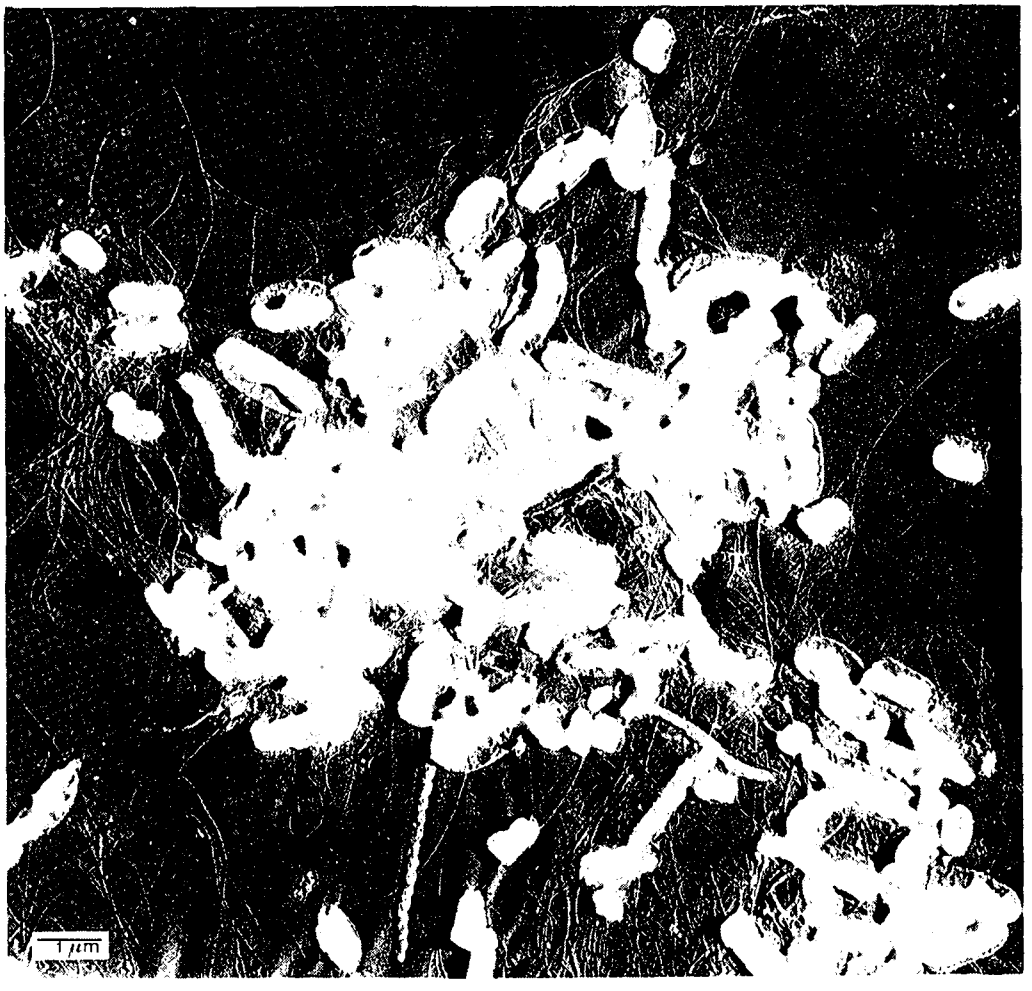


Fig. 37. Electron micrograph of the activated sludge taken from the chemostat fed on vanillin where $\mu = 0.032\ h^{-1}$ (Section 4.2.1). The photograph shows an irregular network of fibrils in which many different bacterial cells are enclosed.

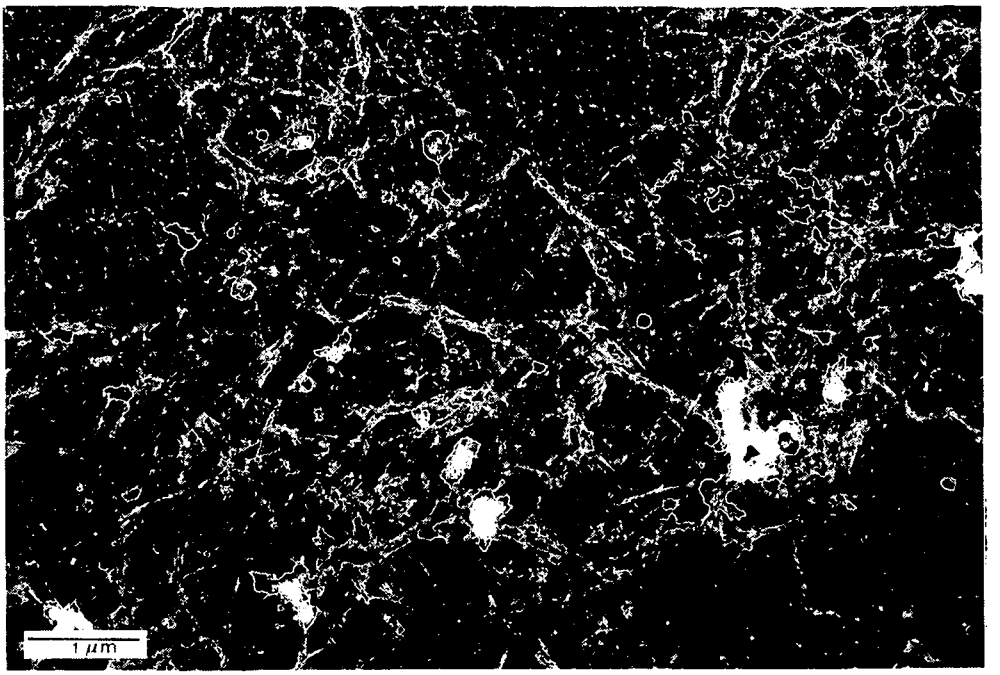


Fig. 38. Electron micrograph of fibrillar fraction of native exopolymers of the sludge fed on vanillin (μ = 0.032 h^{-1}) (Section 4.2.1) after treatment with NaOH 1 mol/l at 100 °C for 1 h and HCl 0.66 mol/l at 100 °C for 2 h.

Summary

On modelling kinetics of a mixed culture of micro-organisms, e.g. activated sludge, several of models have been developed in the last twenty years. These models are based on principles originally formulated for growth of pure cultures of bacteria. Most of these models do not account for cellular composition of bacterial cells and poorly describe the dynamic behaviour of bacterial cultures. The first aim of this study was to develop a kinetic model for a growing culture of micro-organisms that correlated the biochemical structure of cells with quantitative physiological behaviour (Chap. 3).

The model assumed that a cell comprised three basic compartments:
(1) a building-block for synthesis of energy-rich compounds, small metabolites, DNA and storage materials, denoted as R Compartment
(2) the protein-synthesizing machinery, denoted as K Compartment
(3) the pool of enzymes and other proteins, the G Compartment.
This three-compartment model is presented in Figure 5. The R Compartment is produced by uptake from externally available nutrients. The K Compartment, i.e. RNA, is synthesized from the R Compartment. The G Compartment is build up by the R Compartment constituents, but governed by the K Compartment. To explain the concept of maintenance, the K and G Compartments were assumed to turn over to the R Compartment. The phenomenon of viability, i.e. ability to grow, was incorporated in our model by postulating irreversible conversions from R, K and G Compartments to NR, NK and NG Compartments, respectively. NR, NK and NG were defined to be inert fractions of R, K and G.

The mathematical approach of the three-compartment model was based on matrix representation techniques (Scheme 1). The biosystem is defined by the general state vector, subdivided in the state vector of the abiotic phase, i.e. environment of cells, and the state vector of the biotic phase, i.e. interior of cells. The principle of conservation of mass allowed definition of balance equations for abiotic and biotic components in the system. The problem of dilution of intracellular components brought about by expansion (growth) of the biomass was overcome by distinguishing two types of reactions: (1) reactions of the biotic components expressed as the mass fractions of dry mass of cells; (2) reactions of the abiotic components controlled by the mass concentrations of these components in the culture fluid. Then a set of expressions for rates of conversion of components within the system were defined. A chemostat with recycling of biomass was chosen as the biotechnical system. Finally, state and steady-state equations could be formulated for substrate, biomass, R, K, G, NR, NK and NG Components (Section 3.4).

To test the model, meaningful studies could be done with microbial cultures only under rigidly controlled conditions of growth. Therefore an adequate chemostat suited for our research on activated sludge was developed (Section 2.6.2). By stirring and vigorous aerating, activated sludge in the reactor precipitated on the wall just above

the surface of the culture liquor. It dried up and could hardly resuspended. This problem was solved by covering the reactor, so increasing the relative humidity. A second problem was continuous discharge of biomass from the reactor by flocculent growth of the sludge. The only satisfactory solution was to discharge sludge by gravity through a magnetic valve connected to a pulse-pause switch. A continuous way of wasting off biomass could be approached by short pulse-pause time intervals.

A first approach to experimental verification of the three compartment model is given in Chapter 4. Activated sludge was fed on vanillin (3-methoxy-4-hydroxy-benzaldehyde) as carbon and energy source. A well-settling and optimally functioning activated sludge was obtained. The breakdown of vanillin was found to follow the β-ketoadipate pathway (Scheme 2). The constitutive equation of the rate of conversion of substrate to the R Component was defined and rationalized by an n^{th}-order rate equation instead of a commonly used Monod-like relationship (Section 3.3.2.1). Monod kinetics in mixed cultures of bacteria and calculation of its coefficients by the Lineweaver-Burk method were found to be doubtful (Sections 4.2.2.1 and 4.2.2.2).

The macromolecular composition of the activated sludge, continuously fed on vanilline, resembled that from pure cultures reported in the literature. Maximum yield and maintenance coefficients could be calculated from data fitted to the equation of substrate consumption or ATP production and biomass growth in relation to the concept of maintenance (Section 4.2.2.4). So both coefficients depend on the mass fractions of K and G Compartments, yield coefficients of the respective compartments, turnover rates and rates of decay or loss of K and G Compartments. The calculated coefficients agreed with those from the literature or were plausible estimates.

The concept of viability in the three-compartment model was tested by means of data from the literature. Change in viability of a continuously growing culture of bacteria could be simulated well (Section 4.2.2.5).

The model was fitted to data from the batch experiments, the continuous experiments and the transient experiments. The observed kinetics of the activated-sludge cultures, fed on vanillin, could be simulated too (Sections 4.2.2.6 and 4.2.3).

Special attention was paid to the completely mixed two-stage activated-sludge process (Chapter 5). Ways were examined improving the two-stage process and a mathematical model was developed for kinetic optimalization of the process (Section 5.2). With some assumptions, the total hydraulic retention time of the two-stage system could be defined as a function of concentration of intermediate substrate. The minimum was calculated by setting the derivative with respect to concentration of intermediate substrate, $\partial t_{tot}/\partial C_{S1}$, at zero. In two series of experiments two levels of concentrations of intermediate substrate were set in the first stage by which the second stage was fed. The kinetic coefficients of the second stage were affected by concentration and composition of intermediate substrate (Section 5.3.2.1). Empirical relationships were defined and incorporated in the two-stage model.

From our experimental results and calculations, the two-stage process was advantageous to the one-stage process in rate of vanillin consumption (Figure 24). The higher the desired total fractional reduction of incoming substrate, the higher the kinetic advantage (Figure 25). Under optimum conditions, the volume of the first stage was

generally greater than that of the second. This finding conflicts with the proportions of most activated-sludge plants, of which the first stage is smaller than the second stage.

Increase of maximum yield coefficient and simultaneous decrease of maintenance coefficient of the second stage with decreasing concentration of intermediate substrate was predicted by fitting the biochemical data to our model (Section 5.3.2.2). At the lowest concentration of intermediate substrate, a shift from protein to carbohydrate in the bacteria was observed. In the poor environment for growth in the second stage, the shift might be advantageous energetically. Formation of polysaccharides needs less ATP than formation of DNA, RNA or proteins.

The production of biomass in the two-stage system under optimum kinetics was always greater than that of the one-stage system (Section 5.3.2.3). The greater the total fractional reduction, the greater the difference (Figure 25). Biomass production might be diminished however by pumping discharge of activated sludge from the first stage to a second stage whose rate of substrate consumption must be below that for maintenance.

The non-viable or dead biomass in the one-stage system was calculated to be a factor 7.6 times as high as that in the two-stage system to achieve equal amounts of viable biomass (Section 5.3.2.4). Addition of acetate to the vanillin-basal medium enhanced of the rate of vanillin consumption at low concentrations of substrate. The presence of acetate allowed higher rates at low concentrations of substrate (Section 5.4.2), reducing the kinetic advantage of maintaining a higher substrate consumption in the first stage of the two-stage system.

Chapter 5 presents microbial research of the activated sludge fed on vanillin or vanillin and acetate. Species of *Pseudomonas*, *Flavobacter* or *Xanthomonas*, *Alcaligenes*, *Acinetobacter*, *Moraxella* and *Corynebacterium* were isolated. Significant population shifts of the predominant genera were observed if the specific growth rate was increased by a factor 4 or when acetate was added to the vanillin-basal medium. *Pseudomonas* was responsible for the breakdown of vanillin to protocatechuic acid, but was not predominant in the cultures. Addition of acetate increased the relative number of *Pseudomonas* from 4.9 to 26 % of the total number of bacteria. Most of the other isolated types of bacteria were able to consume protocatechuic acid, the metabolic intermediate of vanillin breakdown.

Samenvatting

De laatste twintig jaar werden een aantal modellen ontwikkeld voor de beschrijving van de groei van mengculturen van bacteriën, bijvoorbeeld actief slib. Deze kinetische modellen werden afgeleid van enkele modellen ontwikkeld voor de groei van micro-organismen in reincultuur. In deze modellen ontbreekt veelal de definitie van de cellulaire samenstelling van de bacteriële cellen en bijgevolg wordt het dynamisch gedrag van de bacteriën slecht beschreven. In het eerste deel van deze studie werd een kinetisch model ontwikkeld voor een mengcultuur van micro-organismen, waarin de biochemische structuur van de bacteriële cellen gecorreleerd wordt aan het biologisch gedrag.

Het fundamentele uitgangspunt voor de formulering van het kinetisch model vormde de onderverdeling van een cel in drie compartimenten:
(1) het R-compartiment, waarin energierijke verbindingen en kleine metabolieten gesynthetiseerd worden, het DNA zich bevindt en reserve materialen opgeslagen worden,
(2) het K-compartiment, met het apparaat voor de synthese van eiwit,
(3) het G-compartiment met de enzymen en andere eiwitten van de cel.
Dit drie-compartimenten-model is schematisch weergegeven in figuur 5. Het R-compartiment wordt gevormd door de opname van nutriënten buiten de cel. De K- en C-compartimenten worden gesynthetiseerd uit bouwstenen van het R-compartiment. De synthese van G-compartiment wordt evenwel gereguleerd door het K-compartiment. Het concept van de onderhoudsenergie van een bacteriële cel wordt gedefinieerd door de ontleding van gevormde K- en C-componenten in R-componenten. De vitaliteit van een cel wordt in het model vastgelegd met de aanname van irreversibele omzettingen van R-, K- en G-componenten in NR-, NK- en NG-componenten. NR, NK en NG zijn gedefinieerd als inerte fracties van R, K en G.

De wiskundige uitwerking van het drie-compartimenten-model werd gebaseerd op technieken algemeen toegepast in de matrixalgebra (schema 1). Het biosysteem werd allereerst gedefinieerd door een algemene-toestandsvector. Deze vector werd onderverdeeld in de toestandsvector van de abiotische fase, d.w.z. de omgeving van de bacterie in het systeem, en de toestandsvector van de biotische fase, d.w.z. het inwendige van de bacterie. De wet van behoud van massa leidde tot een stelsel van balans vergelijkingen voor de abiotische en de biotische componenten in het biosysteem. De verdunning van de intracellulaire componenten door expansie (groei) van de biomassa vereisten de definitie van twee typen reacties in het biosysteem: (1) reacties van de biotische componenten, uitgedrukt in massafracties van het drooggewicht van de bacteriën en (2) reacties van de abiotische componenten, bepaald door de concentraties in het medium. Een stelsel van snelheidsvergelijkingen van de omzettingen van de componenten in het systeem, z.g. constitutieve vergelijkingen, kon nu opgesteld worden. Het drie-compartimenten-model werd daarna uitgewerkt voor een chemostaat met terugvoer van biomassa (figuur 7). Dit resulteerde in de formulering van toestands- en evenwichtstoestandsvergelijkingen voor het substraat, de

biomassa, de R-, K-, G-, NR-, NK- en NG-componenten (sectie 3.4).

Een cultuur van micro-organismen kan alleen zinvol bestudeerd worden in continu-cultuur en daarom werd een chemostaat ontwikkeld, die geschikt was voor het onderzoek met actiefslib als mengcultuur (sectie 2.6.2). Afzetting en indroging van slib boven de rand van de vloeistofspiegel in de reactor bemoeilijkte resuspendering in het medium. Dit probleem werd ondervangen door de reactor af te sluiten met een verticaal beweegbare deksel, waardoor de relatieve luchtvochtigheid boven de vloeistof steeg en opgespat slib niet indroogde. De continue verwijdering van biomassa ter compensatie van de groei in het biosysteem, vormde een tweede probleem, dat veroorzaakt werd door de vlokvormige groei van de bacteriën. De enige bevredigende oplossing werd gevonden in de afvoer van slib met vrije val door een magneetklep, die geschakeld was aan een tijdschakelaar.

Hoofdstuk 4 geeft de resultaten van een eerste aanzet tot de experimentele verificatie van het drie-compartimenten-model. Vanilline (3-methoxy-4-hydroxybenzaldehyde) werd gebruikt als koolstof- en energiebron voor de micro-organismen. Een goed bezinkbaar en optimaal functionerend actiefslib werd gekweekt op deze organische verbinding. De afbraak van vanilline verliep via het indermediaire metaboliet β-ketoadipinezuur (schema 2). De constitutieve vergelijking van de omzettingssnelheid van vanilline in R-component werd gedefinieerd en wiskundig gefundeerd met een machtswet relatie, de z.g. n^{de}-orde snelheidsvergelijking, in plaats van de algemeen gebruikte Monod-vergelijking (sectie 3.3.2.1). De Monod-vergelijking en de berekening van haar kinetische coëfficiënten met behulp van de Lineweaver-Burk methode bleken twijfelachtig, indien toegepast op een theoretische mengcultuur van bacteriën of op het actief slib (secties 4.2.2.1 en 4.2.2.2).

De macromoleculaire samenstelling van het actief slib, continu gevoed met vanilline over een traject van groeisnelheden, bleek in overeenstemming met waarden uit de literatuur gemeten aan reinculturen. De maximale-opbrengstcoëfficiënt en de onderhoudscoëfficiënt konden berekend worden met de vergelijking van substraatconsumptie (of ATP-produktie) en biomassagroei, in relatie tot de consumptie van substraat voor cellulair onderhoud (sectie 4.2.2.2). Hieruit kon geconcludeerd worden, dat beide coëfficiënten afhankelijk zijn van de massafracties van de K- en G-compartimenten, de opbrengstcoëfficiënten van de onderscheiden compartimenten, de snelheden waarmee K- en G-componenten weer ontleden in R-componenten en de snelheden waarmee K en G irreversibel omgezet worden in NK en NG. De berekende waarden van de coëfficiënten komen overeen met die uit de literatuur of zijn geloofwaardige schattingen.

Het concept van vitaliteit, als beschreven door het drie-compartimenten-model, werd getoetst met gegevens uit de literatuur. De gemeten verandering in vitaliteit van een continu-cultuur over een traject van groeisnelheden kon adequaat gesimuleerd worden (sectie 4.2.2.5).

Het model werd tenslotte getoest met waarden uit de ladingsgewijze, de continu- en de overgangsexperimenten. De waargenomen kinetiek van het actiefslib wordt goed beschreven door het model (secties 4.2.2.6 en 4.2.3).

In hoofdstuk 5 wordt het onderzoek aan het tweetraps-actiefslib-proces beschreven. De optimalisering van dit proces werd nader onderzocht. Een optimaliseringsmodel werd opgesteld, waarin met enige aannamen, de totale hydraulische verblijftijd van substraat in het tweetraps systeem werd gedefinieerd als functie van de concentratie van het inter-

mediaire substraat. Het minimum van de totale hydraulische verblijftijd kon berekend worden door zijn eerste afgeleide naar de concentratie van het intermediaire substraat, $\partial t_{tot} / \partial C_{SI}$, op nul te stellen. Twee series van experimenten werden uitgevoerd, waarbij in iedere serie de tweede trap van het systeem gevoed werd met een bepaalde concentratie van het intermediaire substraat uit de eerste trap. De kinetische coëfficiënten van de tweede trap bleken afhankelijk van de concentratie en de samenstelling van het intermediaire substraat (sectie 5.3.2.1). Empirische relaties werden geformuleerd en gesubstitueerd in het optimaliseringsmodel van het tweetrapssysteem.

Uit de experimenten en de berekeningen blijkt dat vanilline sneller afgebroken wordt in een tweetraps-actiefslib-systeem dan in een ééntrapssysteem (Figuur 24). Dit voordeel van het tweetrapsproces neemt toe naarmate een verdergaande zuivering van vanilline vereist is (Figuur 25). Het volume van de eerste trap was altijd groter dan het volume van de tweede trap indien onder optimale kinetische condities berekend. Dit resultaat weerspreekt de dimensionering van de meeste tweetraps-actiefslib-installaties, waarvan de eerste trap kleiner is dan de tweede.

De gemeten toename van de maximale-opbrengstcoëfficiënt en tegelijkertijd de afname van de onderhoudscoëfficiënt van de tweede trap (met afnemende concentratie van het intermediaire substraat) konden ook berekend worden door de substitutie van de biochemische gegevens van de tweede trap in het drie-compartimenten-model (sectie 5.3.2.2). Een verschuiving van de massafractie van eiwit naar die van polysacchariden in de bacteriën van de tweede trap werd gemeten, indien de tweede trap werd gevoed met de laagste concentratie van het intermediaire substraat. Deze verschuiving kan energetisch voordelig zijn in dit groei-arm milieu, omdat de vorming van polysacchariden minder ATP behoeft dan de vorming van DNA, RNA of eiwit.

De produktie van actiefslib in het tweetrapsproces bleek onder optimale kinetiek altijd groter dan in het ééntrapsproces (sectie 5.3.2.3). Hoe hoger de vereiste zuivering van vanilline, hoe groter het verschil in beide processen (figuur 25). Deze produktie van biomassa zou wellicht verminderd kunnen worden, indien de tweede trap wordt gevoed met afgevoerd actiefslib uit de eerste trap. In de tweede trap moet de consumptie van substraat dan liggen beneden die van de consumptie van substraat voor onderhoud van de bacteriën.

De vitaliteit van de biomassa in beide systemen werd berekend. De hoeveelheid massa, d.w.z. levende en niet-levende bacteriën, moest in het ééntrapssysteem een factor 7,6 maal hoger liggen dan in het tweetrapssysteem om gelijke hoeveelheden levende cellen te krijgen.

De snelheid van de consumptie van vanilline bij lage concentraties van substraat nam sterk toe in de aanwezigheid van azijnzuur als tweede koolstof en energiebron. Het kinetische voordeel van het handhaven van een hoge snelheid van substraatconsumptie in de eerste trap werd daarmee beperkt omdat nu ook bij lage concentraties van vanilline in aanwezigheid van azijnzuur hoge verbruikssnelheden bereikt konden worden (sectie 5.4.2).

De resultaten van de microbiologische analyses van het actiefslib, gevoed met vanilline of vanilline en azijnzuur zijn samengevat in hoofdstuk 5. *Pseudomonas* species, *Flavobacter* sp. of *Xanthomonas* sp., *Alcaligenes* sp., *Acinetobacter* sp., *Moraxella* sp. en *Corynebacterium* sp. werden rein gekweekt uit het actiefslib. Aanzienlijke verschuivingen

in de samenstelling van de predominante genera werden waargenomen indien de groeisnelheid een factor 4 toenam of azijnzuur aan het medium toegevoegd werd. *Pseudomonas* was verantwoordelijk voor de omzetting van vanilline tot protocatechnaat, maar was niet predominant aanwezig in het actiefslib. De relatieve populatie van *Pseudomonas* nam toe van 4,9 tot 26,0 % van het totale aantal van bacteriën indien azijnzuur toegevoegd werd. Protocatechuaat kon door de meeste andere soorten van bacteriën verbruikt worden.

References

Abson, J.W. & K.H. Todhunter, 1961. Plant for continuous biological treatment of carbonisation effluents. In: Continuous culture of micro-organisms. S.C.I.Monograph. 12: 147-184.

Adamse, A.D., 1966. Bacteriological studies on dairy waste activated sludge. Ph.D. Thesis. Agricultural University. Wageningen.

Baillod, D.R. & W.C. Boyle, 1970. Mass transfer limitations in substrate removal. J. San. Engng. Div. 96: 525-545.

Banks, C.J. & I. Walker, 1977. Sonication of activated sludge flocs and the recovery of their bacteria on solid media. J. gen. Microbiol. 98: 363-368.

Bargmann, R.D., J.M. Betz & W.F. Garber, 1957. Aeration requirements of a high oxygen demand sewage. J. W. P. C. F. 29: 768-790.

Bauchop, T. & S.R. Elsden, 1960. The growth of micro-organisms in relation to their energy supply. J. gen. Microbiol. 23: 457-469.

Bauman, P., M. Doudoroff & R.Y. Stanier, 1968. A study of the Moraxella group. II. Oxidative-negative species (genus Acinetobacter). J. Bacteriol. 95: 1520-1541.

Benedict, R.G. & D.A. Carlson, 1971. Aerobic heterotrophic bacteria in activated sludge. Wat. Res. 5: 1023-1030.

Benefield, L.D., C.W. Randall & P.H. King, 1976. Process control by oxygen uptake and solids analysis. J. W. P. C. F. 47: 2498-2503.

Benefield, L.D. & C.W. Randall, 1977. Evaluation of a comprehensive kinetic model for the activated-sludge process. J. W. P. C. F. 49: 1636-1641.

Bonotan-Dura, F.M. & P.Y. Yang, 1976. The application of constant recycle solids concentration in the activated-sludge process. Biotechnol. Bioengng. 18: 145-165.

Brownes, P.H., 1973. Considerations in the multi-stage activated sludge treatment of industrial wastewater. Wat. Res. 7: 783-791.

Brown, C.M. & A.H. Rose, 1969. Effects of temperature on composition and cell volume of Candida utilis. J. Bacteriol. 97: 261-272.

Buchanan,R.E. & N.E. Gibbons, 1974. Bergey's manual of determinative bacteriology. Eighth edition. Baltimore: Williams and Wilkins Company.

Burton, K., 1956. A study of conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of DNA. Biochem. J. 62: 315-323.

Casteele, K. van de, K.H. de Pooter & C.F. van Sumere, 1976. Gaschromatographic separation and analysis of trimethylsilyl derivatives of some naturally occurring non-volatile phenolic compounds and related substances. J. Chromatography. 121: 49-63.

Chase, L.M., 1977. A dynamic kinetic model of the activated-sludge process. Biotechnol. Bioengng. 19: 1431-1447.

Chi, C.T. & J.A. Howell, 1976. Transient behaviour of a continuous stirred tank biological reactor utilizing phenol as an inhibitory substrate. Biotechnol. Bioengng. 18: 63-80.

Chiu, S.Y., L.E. Erickson, L.T. Fan & J.C. Kao, 1972. Kinetic model identification in mixed populations using continuous culture data. Biotechnol. Bioengng. 14: 207-231.

Chudoba, J., P. Grau & V. Ottová, 1973. Control of activated-sludge filamentous bulking. II. Selection of microorganisms by means of a selector. Wat. Res. 7: 1389-1406.

Crombach, W.H.J., 1974. Genetic morphological and physiological relationships among coryneform bacteria. Ph.D. Thesis. Agricultural University, Wageningen.

Dabes, J.N., R.K. Finn & C.R. Wilke, 1973. Equations of substrate-limited growth: The case for Blackmann kinetics. Biotechnol. Bioengng. 15: 1159-1177.

Dagley, S., 1971. Catabolism of aromatic compounds by micro-organisms. Adv. Microb. Physiol. 6: 1-46.

Daigger, G.T. & C.P.L. Grady, 1977. A model for the bio-oxidation process based on product formation concepts. Wat. Res. 11: 1049-1057.

Dalton, H. & J.R. Postgate, 1969. Growth and physiology of Azotobacter chroococcum in continuous culture. J. gen. Microbiol. 56: 307-319.

Dawes, E.A. & P.J. Senior, 1973. The role and regulation of energy reserve polymers. Adv. Microb. Physiol. 10: 135-266.

Dawes, E.A., M. Midgley & P.H. Whiting, 1976. Control of transport systems for glucose, gluconate and 2-oxo-gluconate and of glucose metabolism in Pseudomonas aeruginosa. In: A.C.R. Dean, D.C. Ellwood, C.G.T. Evans & J. Melling, (Eds.). Continuous culture 6; applications and new fields. Ellis Horwood Ltd. p. 195-207.

Dedem, G. van & M. Moo-Young, 1973. Cell growth and extracellulair enzyme synthesis in fermentations. Biotechnol. Bioengng. 15: 419-439.

Deinema, M.H. & L.P.T.M. Zevenhuizen, 1971. Formation of cellulose fibrils by Gram-negative bacteria and their role in bacterial flocculation. Arch. Mikrobiol. 78: 42-57.

Dias, F.F. & J.V. Bhat, 1964. Microbial ecology of activated sludge. Appl. Microbiol. 12: 412-417.

Dietrich, K., 1968. Das biologisch phasengerechte Mehrstufenverfahren zur Reinigung industrieller und kommunaler Abwässer. Wasser, Luft und Betrieb. 12: 694-701.

Dixit, S.N.S. & J.W. Patterson, 1975. Bicultural, two-stage, high-rate activated-sludge process. Proc. of the 30th Ind. Waste Conference. Purdue University. Ann Arbor. p. 23-47.

Downing, A.L., 1966. Activated sludge plant design problems. Process Biochem. 1: 1-9.

Dubach, M., 1966. The Attisholz system of waste water treatment. Delta Engineering b.v. Rotterdam.

Dubach, M., 1968. Ueber eine Weiterentwicklung der zweistufig-biologischen Abwasserreinigung. Aus: Liebmann, Münchener Beiträge. Band 5.2. R. Oldenbourg. München. p. 319-330.

Eckenfelder, W.W., Jr., 1961. Theory and practice of activated-sludge process modifications. Wat. & Sew. Works. 108: 145-150.

Ekama, G.A. & G.v.R. Marais, 1977. The activated-sludge process. Part II. Dynamic behaviour. Water S.A. (S.Africa). 3: 18-50.

Forchhammer, J. & L. Lindahl, 1971. Growth rate of polypeptide chains as a function of the cell growth rate in a mutant of Escherichia coli 15. J. Molec. Biol. 55: 563-568.

Fredrickson, A.G., R.D. Megee & H.M. Tsuchiya, 1970. Mathematical models for fermentation processes. Adv. Appl. Microbiol. 13: 419-465.

Fredrickson, A.G., 1976. Formulation of structured growth models. Biotechnol. Bioengng. 18: 1481-1486.

Fredrickson, A.G., 1977. Behaviour of mixed cultures of microorganisms. Ann. Rev. Microbiol. 31: 63-87.

Fuhs, G.W. & Min Chen, 1975. Microbiological basis of phosphate removal in the activated-sludge process for the treatment of wastewater. Microbial Ecology 2: 119-138.

Fujisawa, H., 1970. Protocatechuate 3,4-dioxygenase (Pseudomonas). In: S.P. Colowick & N.O. Kaplan. Methods in Enzymology. 17a: p. 526-529.

Gall, L., 1970. Significance of microbial interactions in control of microbial ecosystems. Biotechnol. Bioengng. 12: 333-340.

Gaudy, A.F., M. Ramanathan & B.S. Rao, 1967, Kinetic behaviour of heterogeneous populations in completely mixed reactor. Biotechnol. Bioengng. 9: 387-411.

Gaudy, A.F. & R. Srinivasaraghavan, 1974. Effect of specific growth rate on biomass yield of heterogeneous populations growing in both continuous and batch systems. Biotechn. Bioengng. 16: 423-427.

George, T.K. & A.F. Gaudy, 1973. Response of completely mixed systems to hydraulic shock loads. J. Env. Engng. Div. 99: 593-606.

Ghosh, S. & F.G. Pohland, 1972. Kinetics of assimilation of multiple substrates in dispersed growth systems. Wat. Res. 6: 99-115.

Gils, H.W. van, 1964. Bacteriology of activated sludge. Ph.D. Thesis. IG-TNO Rept. no. 32. Res. Inst. Pub. Health Eng. The Hague. The Netherlands.

Gons, H.J., 1977. On the light-limited growth of Scenedesmus protuberans Fritsch. Ph.D. thesis. University of Amsterdam.

Goodman, B.L. & A.J. Englande, 1974. A unified model of the activated sludge process. J. W. P. C. F. 46: 312-332.

Grady, C.P.L., L.J. Harlow & R.R. Riesing, 1972. Effects of growth rate and influent substrate concentration on effluent quality from chemostats containing bacteria in pure and mixed culture. Biotechnol. Bioengng. 14: 391-410.

Grady, C.P.L. & R.E. Roper, 1974. A model for the bio-oxidation process which incorporates the viability concept. Wat. Res. 8: 471-483.

Grady, C.P.L. & D.R. Williams, 1975. Effects of influent substrate concentration on the kinetics of natural microbial populations in continuous culture. Wat. Res. 9: 171-180.

Grau, P., M. Dohányos & J. Chudoba, 1975. Kinetics of multicomponent substrate removal by activated sludge. Wat. Res. 9: 637-642.

Harder, W., 1973. Microbial metabolism of organic C_1 and C_2 compounds. Ant. v. Leeuwenhoek 39: 650-652.

Heinicke, D., 1967. Möglichkeiten und Grenzen der biologischen Reinigung von Abwässern der chemischen Industrie. Chemie Ing. Techn. 39: 183-192.

Hempfling, W.P. & S.E. Mainzer, 1975. Effects of varying the carbon source limiting growth on yield and maintenance characteristics of Escherichia coli in continuous culture. J. Bacteriol. 123: 1076-1087.

Herbert, D., R. Elsworth & R.C. Telling, 1956. The continuous culture of bacteria; a theoretical and experimental study. J. gen. Microbiol. 14: 601-622.

Herbert, D., 1961. The chemical composition of micro-organisms as a function of their environment. In: G.G. Meynell & H. Gooder (Eds.). Microbial reaction to environment. Soc. for gen. Microbiol. Symp. University Press. London 11: 391-416.

Herbert, D., P.J. Phipps & R.E. Strange, 1971. Chemical analysis of microbial cells. In: J.R. Norris & D.W. Ribbons (Eds.). Methods in Microbiol. Academic Press. London. 5b: 244-252.

Hodge, J.E. & B.T. Hofreiter, 1962. Determination of reducing sugars and carbohydrates. In: Methods in carbohydrate chemistry. Academic Press. London. 1: 380-381.

Howell, J.A., 1976. Effects of influent substrate concentration on the kinetics of natural microbial populations in continuous culture. Wat. Res. 10: 271.

Hughes, D.E. & D.A. Stafford, 1976. The microbiology of the activated-sludge process. Critical Rev. Environ. Control 6: 233-259.

Ierusalimsky, N.D., 1967. Bottle-necks in metabolism as growth rate controlling factors. In: E.O. Powell, C.G.T. Evans, R.E. Strange & D.W. Tempest (Eds.). Microbial physiology and continuous culture. Her Majesty's Stationery Office. London. p. 23-33.

Ilić, P., 1977. Untersuchungen zum Verhalten schwer abbaubarer Modellsubstanzen bei der biologischen Reinigung. Dissertation. Darmstadt.

Imhoff, K., 1955. Two-stage operation of activated sludge plants. Sew. & Ind. Waste. 27: 431-433.

Imhoff, K.R., 1977. Mehrstufige biologische Anlagen. ATV-Fortbildungskurses. Eppingen.

Jannasch, H.W. & R.I. Mateles, 1974. Experimental bacterial ecology studied in continuous culture. Adv. Microb. Physiol. 11: 165-212.

Johnson, D.B. & P.W. Berthouex, 1975. Efficient biokinetic experimental designs. Biotechn. Bioengng. 17: 557-570.

Jones, G.L., 1973. Bacterial growth kinetics: measurements and significance in the activated-sludge process. Wat. Res. 7: 1475-1492.

Jung, H., 1960. Wesen und Wirkung der hochbelasteten biologischen Abwasserreinigung. Vom Wasser 27: 185-197.

Klapproth, H., 1976. Betriebliche Erfahrungen mit zweistufiger biologischer Reinigung von Abwässern aus chemisch-pharmazeutischer Produktion. Wasser Luft und Betrieb 20: 298-302.

Klapwijk, A., 1978. Eliminatie van stikstof uit afvalwater door denitrificatie. Ph.D. Thesis (in dutch). Pudoc. Wageningen.

Klee, O., 1970. Reinigung industrieller Abwässer, Grundlagen und Verfahren. Chemie-Monographien. Frankh'sche Verlagshandlung. Stuttgart. 41-58.

Koch, A.L., 1970. Overall controls on the biosynthesis of ribosomes in growing bacteria; rate of RNA synthesis depends on λ^2. J. Theor. Biol. 28: 203-231.

Koch, A.L., 1971. The adaptive responses of Escherichia coli to a feast and famine existence. Adv. Microb. Physiol. 6: 147-218.

Kormanik, R.A., 1972. Design of two-stage aerated lagoons. J. W. P. C. F. 44: 451-458.

Krishnan, P. & A.F. Gaudy, 1976. Response of activated sludge to quantitative shock loadings. J. W. P. C. F. 48: 906-929.

Krul, J.M., 1977. The relationship between dissimilatory nitrate reduction and oxygen uptake by cells of an Alcaligenes strain in flocs and in suspension and by activated sludge flocs. Wat. Res. 10: 337-341.

Lawrence, A.L. & P.L. McCarty, 1970. Unified basis for biological treatment design and generation. J. San. Engng. Div. 96: 757-778.

Levenspiel, O., 1971. Chemical reaction engineering. Second edition. John Wiley, New York. p. 51.

Liao, C.F.H. & R.N. Dawson, 1975. Microbiology of two-stage kraft waste treatment. J. W. P. C. F. 47: 2384-2396.

Liebmann, H. & K. Offhaus, 1966. Volumetrische BSB-Messungen mit Hilfe des Sapromaten, einem neuen Gerät zur Bestimmung des biochemischen Sauerstoffbedarfs (BSB_5) und der Toxizität. Abwassertechnik 3: 4-6.

Lindner, W., 1957. Das zweistufige Belebungsverfahren in der Abwasserreinigung. Dissertation. Thomas-Verlag. Kempen-Niederrhein.

Lohmann, J.H., 1975. Untersuchungen über die Bemessung von Tropfkörper- und Belebungsverfahren am Beispiel eines hochbelasteten Industrieabwassers. Vom Wasser 44: 273-318.

Lohmann, J.H., 1977. Possibilities of the one- and two-stage biological purification. Progress in Waste Technology 8: 229-234.

Lowry, O.H., N.J. Rosebrough, A.L. Farr & R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. biol. Chem. 193: 265-275.

Luven, J. van, 1972. Orientation report for high-rate two stage activated sludge process. Env. Prot. Service Rep. Series. EPS4-WP72-4. Dep. of the Environment. Ottawa. Ontario. Canada.

Maaløe, O. & N.O. Kjeldgaard, 1966. Control of macromolcular synthesis. A study of DNA, RNA and protein synthesis in bacteria. W.A. Benjamin Inc. New York.

Magara, Y., S. Nambu & K. Utosawa, 1976. Biochemical and physical properties of an activated sludge on settling characteristics. Wat. Res. 10: 71-77.

Mandelstam, J., 1960. The intracellular turnover of protein and nucleic acids and its role in biochemical differentiation. Bacteriol. Rev. 24: 289-308.

Marais, G.v.R. & G.A. Ekama, 1976. The activated sludge process. Part I. Steady state behaviour. Water S.A. (S.Africa). 2: 163-200.

McKinney, R.E., 1962. Mathematics of complete-mixing activated sludge. J. San. Engng. Div. 88: 87-113.

Meers, J.L., 1973. Growth of bacteria in mixed culture. CRC Critical Rev. in Microbiol. 2: 139-184.

Monod, J., 1942. Recherches sur la croissance des cultures bactériennes. Paris. Hermann and Cie.

Morand, J.M., 1964. Ph.D. Thesis. University of Wisconsin.

Morowitz, H.J., 1968. Energy flow in biology: Biological organization as a problem in thermal physics. Academic Press. New York.

Nagai, S. & S. Aiba, 1972. Reassessment of maintenance and energy uncoupling in the growth of Azotobacter vinelandii. J. gen. Microbiol. 73: 531-538.

Neidhardt, F.C., 1963. Effects of environment on the composition of bacterial cells. Ann. Rev. Microbiol. 17: 61-86.

Nierlich, D.P., 1978. Regulation of bacterial growth, RNA and protein synthesis. Ann. Rev. Microbiol. 32: 393-432.

Orshanskaya, F.B., Z.A. Arkad'eva & E.J. Kozlova, 1975. Active-sludge microflora participating in the decomposition of sulfanitic acid. Microbiology 44: 134-136.

Pace, N.R., 1973. The structure and synthesis of the ribosomal ribonucleic acid of prokaryotes. Bacteriol. Rev. 37: 562-603.

Painter, M.J.B. & H.R. Bungay, 1971. The effect of microbial interactions on performance of waste treatment processes. In: Biological Waste Treatment. Chicago. p. 51.

Paul, U., 1969. Die betriebseigene biologische Abwasserreinigungsanlage einer Central-Molkerei. Wasser Luft und Betrieb 13: 89-92.

Peil, K.M. & A.F. Gaudy, 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.

Pipes, W.O., 1966. The ecological approach to the study of activated sludge. Adv. Appl. Microbiol. 8: 77-103.

Pirt, S.J., 1965. The maintenance energy of bacteria in growing cultures. Proc. Royal Soc. London. Series B. 163: 224-231.

Pirt, S.J., 1975. Principles of microbe and cell cultivation. Blackwell Sci. Publ. London.

Postgate, J.R. & J.R. Hunter, 1962. The survival of starved bacteria. J. gen. Microbiol. 29: 233-263.

Powell, E.O., 1967. Growth rate of microorganisms as a function of substrate concentration. In: E.O. Powell, C.G.T. Evans, R.E. Strange & D.W. Tempest (Eds.). Microbial physiology and continuous culture. Her Majesty's Stationery Office. London. p. 34-56.

Powell, E.O., 1969. Transient changes in the growth rate of microorganisms. In: I. Malek, K. Beran, Z. Fencl, V. Munk, J. Ričica & H. Smrčková (Eds.). Continuous cultivation of microorganisms. Acad. Press. New York. p. 275-284.

Porges, N., 1960. Newer aspects of waste treatment. Adv. Appl. Microbiol. 2: 1-30.

Prakasam, T.B.S. & N.C. Dondero, 1967a. Aerobic heterotrophic bacterial populations of sewage and activated sludge. I. Enumeration. Appl. Microbiol. 15: 461-467.

Prakasam, T.B.S. & N.C. Dondero, 1967b. Aerobic heterotrophic bacterial populations of sewage and activated sludge II. Method of characterization of activated sludge bacteria. Appl. Microbiol. 15: 1122-1127.

Ramanathan, M. & A.F. Gaudy, 1969. Effect of high substrate concentration and cell feedback on kinetic behaviour of heterogeneous populations in completely mixed systems. Biotechnol. Bioengng. 11: 207-237.

Ramanathan, M. & A.F. Gaudy, 1971. Steady state model for activated sludge with constant recycle sludge concentration. Biotechnol. Bioengng. 13: 125-145.

Rawlings, D.E. & D.R. Woods, 1977. Bacterial growth kinetics in the completely mixed activated sludge treatment of fellmongery effluent. Wat. Res. 11: 281-288.

Rensink, J.H., 1974. New approach to preventing bulking sludge. J. W. P. C. F. 46: 1888-1894.

Rensink, J.H., J.W. Voetberg & T.S.J. Ywema, 1977. Die Verhinderung der Entwicklung von Blähschlamm bei der vollbiologischen Reinigung von Abwasser der Pommes-frites-Industrie. G. W. F. - Wasser/Abwasser. 118: 75-79.

Righelato, R.C., A.P.J. Trinci, S.J. Pirt & A. Peat, 1968. The influence of maintenance energy and growth rate on the metabolic activity, morphology and conidiation of Penicillium chrysogenum. J. gen. Microbiol. 50: 399-412.

Roels, J.A., 1978. Regulatory mechanisms and the modelling of fermentation processes. Paper presented at the First European Congress on Biotechnol. Interlaken.

Roels, J.A. & N.W.F. Kossen, 1978. On the modelling of microbial metabolism. In: M.J. Bull (Ed.). Progr. Ind. Microbiol. Elsevier Sci. Publ. Co. 14: 95-203.

Savageau, M.A., 1976. Biochemical systems analysis. Addison Wesley Publ. Co. Massachusetts.

Schellart, J.A., 1975. Fungal protein from corn waste effluents. Ph.D. Thesis. Communications Agricultural University Wageningen. The Netherlands.

Schmidt-Lorenz, W., 1965. Methoden zur quantitativen Isolierung und Bestimmung von in Lebensmitteln vorkommenden Bakterien. In: Anreicherungskultur und Mutantenauslese. Symposium Göttingen. Fischer. Stuttgart. 270-325.

Shehata, T.E. & A.G. Marr, 1971. Effect of nutrient concentration on the growth of Escherichia coli. J. Bacteriol. 107: 210-216.

Sinclair, C.G. & H.H. Topiwala, 1970. Model for continuous culture which considers the viability concept. Biotechnol. Bioengng. 12: 1069-1079.

Sladká, A. & V. Zahrádka, 1971. Relationship between activated sludge morphology and activity. Wat. Res. 5: 871-876.

Speece, R.E., R.S. Engelbrecht & D.R. Aukamp, 1973. Cell replication and biomass in the activated-sludge process. Wat. Res. 7: 361-364.

Srinivasaraghavan, R. & A.F. Gaudy, 1975. Operational performance of an activated sludge process with constant sludge feedback. J. W. P. C. F. 47: 1946-1960.

Stall, T.R. & J.H. Sherrard, 1976. Effect of wastewater composition and cell residence time on phosphorus removal in activated sludge. J. W. P. C. F. 48: 307-322.

Stanier, R.Y., E.A. Adelberg & J.L. Ingraham, 1977. General microbiology. Fourth edition. The Macmillan Press Ltd. London.

Stouthamer, A.H. & C. Bettenhaussen, 1973. Utilization of energy for growth and maintenance in continuous and batch cultures of microorganisms. Biochim. Biophys. Acta. 301: 53-70.

Stouthamer, A.H., 1976. Yield studies in micro-organisms. Patterns of progress. Meadowfield Press Ltd.

Stouthamer, A.H., 1977. Energetics of the growth of micro-organisms. In: B.A. Haddock & W.A. Hamilton (Eds.). Soc. for Gen. Microbiol. Cambridge University Press. London. 27: 285-315.

Stover, E.L. & D.F. Kincannon, 1976. One- versus two-stage nitrification in the activated-sludge process. J. W. P. C. F. 48: 645-651.

Taber, W.A., 1976. Wastewater microbiology. Ann. Rev. Microbiol. 30: 263-277.

Takii, S., 1977. Bacterial characteristics of activated sludges treating carbohydrate wastes. Wat. Res. 11: 85-89.

Tempest, D.W. & J.R. Hunter, 1965. The influence of temperature and pH value on the macromolecular composition of magnesium-limited and glycerol-limited Aerobacter aerogenes growing in a chemostat. J. gen. Microbiol. 41: 267-273.

Tempest, D.W. & J.W. Dicks, 1967a. Inter-relationships between potassium, magnesium, phosphorus and ribonucleic acid in the growth of Aerobacter aerogenes in a chemostat. In: E.O. Powell, C.G.T. Evans, R.E. Strange & D.W. Tempest (Eds.). Microbial physiology and continuous culture. Her Majesty's Stationery Office. London. p. 140-154.

Tempest, D.W., D. Herbert & P.J. Phipps, 1967b. Studies on the growth of Aerobacter aerogenes at low dilution rates in a chemostat. In: E.O. Powell, C.G.T. Evans, R.E. Strange & D.W. Tempest. Microbial physiology and continuous culture. Her Majesty's Stationery Office. London. p. 240-254.

Tempest, D.W., 1970. The place of continuous culture in microbiological research. Adv. Microb. Physiol. 4: 223-250.

Tischler, L.F. & W.W. Eckenfelder, 1969. Linear substrate removal in the activated sludge. Adv. Wat. Poll. 4: 361-374.

Toms, A. & J.M. Wood, 1970. The degradation of trans-ferulic acid by Pseudomonas acidovorans. Biochem. 9: 337-343.

Topiwala, H.H. & G. Hamer, 1971. Effect of wall growth in steady-state continuous culture. Biotechnol. Bioengng. 13: 919-922.

Uden, N. van, 1967. Transport-limited growth in the chemostat and its competitive inhibition: a theoretical treatment. Archiv. Mikrobiol. 58: 145-154.

Unz, R.F. & N.C. Dondero, 1970. Nonzoogloeal bacteria in wastewater Zoogloeas. Wat. Res. 4: 575-579.

Veldkamp, H., G. van den Berg & L.P.T.M. Zevenhuizen, 1963. Glutamic acid production by Arthrobacter globiformis. Ant. v. Leeuwenhoek 29: 35-51.

Veldkamp, H. & H.W. Jannasch, 1972. Mixed culture studies with the chemostat. J. appl. Chem. Biotechnol. 22: 105-123.

Veldkamp, H., 1976. Mixed culture studies with the chemostat. In: A.C.R. Dean, D.C. Ellwood, C.G.T. Evans & J. Melling, (Eds.). Continuous culture 6; applications and new fields. Ellis Horwood Ltd. p. 315-328.

Verseveld, H.W. van & A.H. Stouthamer, 1978. Oxidative phosphorylation during autotrophic growth of P. denitrificans. Arch. Microbiol. 118: 21-26.

Verstraete, W., 1977. Fundamentele studie van de opbouw- en omzettingsprocessen in microbiële gemeenschappen. Proefschrift tot het verkrijgen van de graad van Geaggregeerde voor het Hoger Onderwijs. Rijksuniversiteit Gent. Gent.

Walker, J. & M. Davies, 1977. The relationship between viability and respiration rate in the activated sludge process. Wat. Res. 11: 575-578.

Walters, C.F., R.S. Engelbrecht & R.E. Speece, 1968. Microbial substrate storage in activated sludge. J. San. Engng. Div. 94: 257-269.

Wargel, R.J., C.A. Shadur & F.C. Neuhaus, 1970. Mechanism of D-cycloserine action: transport systems for D-alanine, D-cycloserine, L-alanine and glycine. J. Bacteriol 103: 778-788.

Weddle, C.L. & D. Jenkins, 1971. The viability and activity of activated sludge. Wat. Res. 5: 621-640.

Wheelis, M.L., N.J. Palleroni & R.Y. Stanier, 1967. The metabolism of aromatic acids by Pseudomonas testosteroni and P. acidovorans. Arch. Mikrobiol. 59: 302-314.

Williams, F.M., 1967. A model of cell growth dynamics. J. Theoret. Biol. 15: 190-207.

Williams, F.M. 1975. In: B.V. Patten (Ed.). System Analysis and Simulation in Ecology. Acad. Press. 3: 197.

Wu, Y.C. & C.F. Kao, 1976. Activated sludge treatment of yeast industry wastewater. J. W. P. C. F. 48: 2609-2618.

Wuhrmann, K., 1968. Research developments in regard to concept and base values of the activated-sludge system. In: E.F. Gloyna & W.W. Eckenfelder (Eds.). Advances in Water Quality Improvement. p. 143-170.

Yall, I., N.A. Sinclair, F.A. Roinestad & C.F. Russ, 1975. Phosphorus removal from wastewater. S.A. Patent. 75/7917. Appl. 10. December.

Young, T.B., D.F. Bruley & H.R. Bungay, 1970. A dynamic mathematical model of the chemostat. Biotechnol. Bioengng. 12: 747-769.

List of symbols most frequently used

symbol	qualitative description	unit
a_S	= formal order of reaction	1
a_{S1}	= formal order of reaction in the first stage	1
a_{S2}	= formal order of reaction in the second stage	1
C_G	= concentration of G Component	g/l
C_K	= concentration of K Component	g/l
C_R	= concentration of R Component	g/l
C_{NG}	= concentration of inert-G Component	g/l
C_{NK}	= concentration of inert-K Component	g/l
C_{NR}	= concentration of inert-R Component	g/l
C_S	= concentration of substrate in carbon	g/l
C_{S1}	= concentration of substrate in carbon of the first stage	g/l
C_{S2}	= concentration of substrate in carbon of the second stage	g/l
C_{SO}	= concentration of incoming substrate	g/l
C_X	= concentration of biomass in dry mass	g/l
C_{X1}	= concentration of biomass in dry mass of the first stage	g/l
C_{X2}	= concentration of biomass in dry mass of the second stage	g/l
D	= hydraulic dilution rate	h^{-1}
D_1	= hydraulic dilution rate in the first stage	h^{-1}
D_2	= hydraulic dilution rate in the second stage	h^{-1}
k_N	= total rate of loss of G, K and R	h^{-1}
k_{N2}	= total rate of loss of G, K and R in the second stage	h^{-1}
k_{NG}	= rate of loss of G	h^{-1}
k_{NK}	= rate of loss of K	h^{-1}
k_{NR}	= rate of loss of R	h^{-1}
k_{SR}	= rate coefficient of consumption of substrate	$g/g.h.(g/l)^{a_S}$
k_{SR1}	= rate coefficient of consumption of substrate in the first stage	$g/g.h.(g/l)^{a_S}$
k_{SR2}	= rate coefficient of consumption of substrate in the second stage	$g/g.h.(g/l)^{a_S}$
m_e	= consumption of ATP per unit biomass and time	mol/g.h
m_G	= turnover of G Compartment	h^{-1}
m_K	= turnover of K Compartment	h^{-1}
m_{K2}	= turnover of K Compartment in the second stage	h^{-1}
m_S	= rate of consumption of substrate for maintenance	g/g.h
m_{S2}	= rate of consumption of substrate for maintenance in the second stage	g/g.h

P	= rate of production of dry mass	g/h
q_S	= rate of consumption of substrate per unit dry mass	g/g.h
q_{SR}	= rate of uptake of substrate by R Compartment	g/g.h
q_{SR1}	= rate of uptake of substrate by R Compartment in the first stage	g/g.h
q_{SR2}	= rate of uptake of substrate by R Compartment in the second stage	g/g.h
q_{max}	= maximum rate of consumption of substrate per unit dry mass	g/g.h
q_{ATP}	= rate of ATP consumption per unit dry mass	mol/g.h
r_{ATP}	= rate of ATP consumption per unit volume	mol/l.h
r_S	= rate of consumption of substrate	g/l.h
r_{SR}	= rate of conversion of S to R	g/l.h
r_{RK}	= rate of conversion of R to K	g/l.h
r_{RG}	= rate of conversion of R to G	g/l.h
r_{GR}	= rate of turnover of G to R	g/l.h
r_{KR}	= rate of turnover of K to R	g/l.h
r_{NR}	= rate of loss of R to NR	g/l.h
r_{NK}	= rate of loss of K to NK	g/l.h
r_{NG}	= rate of loss of G to NG	g/l.h
r_X	= rate of production of biomass	g/l.h
t	= hydraulic retention time of substrate	h
t_1	= hydraulic retention time of substrate in the first stage	h
t_2	= hydraulic retention time of substrate in the second stage	h
t_{tot}	= total hydraulic retention time ($t_1 + t_2$)	h
t_{tot}^{min}	= mimimum of total hydraulic retention time	h
V	= working volume of reactor	l
V_1	= working volume of reactor of the first stage	l
V_2	= working volume of reactor of the second stage	l
w_G	= mass fraction of G in dry mass	g/g
w_{G2}	= mass fraction of G in dry mass of the second stage	g/g
w_K	= mass fraction of K in dry mass	g/g
w_{K2}	= mass fraction of K in dry mass of the second stage	g/g
w_R	= mass fraction of R in dry mass	g/g
w_{NG}	= mass fraction of NG in dry mass	g/g
w_{NK}	= mass fraction of NK in dry mass	g/g
w_{NR}	= mass fraction of NR on dry mass	g/g
w_N	= total mass fraction of non-viable or dead mass	g/g
w_1	= mass fraction of ϕ_0 discharged from reactor	1
w_2	= mass fraction of C_X fed back into reactor	1
w_3	= mass fraction of ϕ_1 fed back	1
w_4	= mass fraction of C_X leaving the separator	1
w_D	= hold-up factor of biomass	1
w_{GO}	= mass fraction of G at $\mu = 0$	1
w_{KO}	= mass fraction of K at $\mu = 0$	1

ω_{RO}	= mass fraction of R at $\mu = 0$	1
Y_{max}	= maximum yield coefficient	g/g
Y_{max2}	= maximum yield coefficient of the second stage	g/g
Y_{max}^{ATP}	= maximum yield of biomass per mol ATP	g/mol
Y_{RG}	= yield of G Component per unit of R Component converted	g/g
Y_{RG}^{ATP}	= yield of G Component per mol ATP	g/mol
Y_{RK}	= yield of K Component per unit of R Component converted	g/g
Y_{RK2}	= yield of K Component per unit of R Component converted of the second stage	g/g
Y_{RK}^{ATP}	= yield of K Component per mol ATP	g/mol
Y_{SR}	= yield of R Component per unit of substrate converted	g/g
Y_{SR2}	= yield of R Component per unit of substrate converted of the second stage	g/g
Y_{SR}^{ATP}	= yield of R Component per mol ATP	g/mol
α_G	= rate coefficient of net production of G	g/g.h
α_K	= rate coefficient of net production of K	g/g.h
α_R	= rate coefficient of net production of R	g/g.h
μ	= specific growth rate	h^{-1}
μ_{max}	= maximum specific growth rate	h^{-1}
η	= fractional reduction of C_{S0}	1
η_1	= fractional reduction of C_{S0} in the first stage	1
η_2	= fractional reduction of C_{S1} in the second stage	1
η_{tot}	= total fractional reduction, $(\eta_1 + \eta_2)$	1
ϕ_0	= flow rate of incoming substrate	g/l.h
ϕ_1	= outflow rate of substrate	g/l.h
ϕ_G	= outflow rate of G Component in ω_G	g/l.h
ϕ_K	= outflow rate of K Component in ω_K	g/l.h
ϕ_R	= outflow rate of R Component in ω_R	g/l.h
ϕ_{NG}	= outflow rate of NG Component in ω_{NG}	g/l.h
ϕ_{NK}	= outflow rate of NK Component in ω_{NK}	g/l.h
ϕ_{NR}	= outflow rate of NR Component in ω_{NR}	g/l.h
ϕ_X	= outflow rate of biomass in dry mass	g/l.h
ϕ_S	= total flow rate of substrate	g/l.h

abbreviations for chemical substances

ATP	= adenosine triphosphate
DNA	= deoxyribonucleic acid
G	= compartment of enzymes and other proteins
K	= compartment of RNA
NG	= inert-G Component
NK	= inert-K Component
NR	= inert-R Component

R = compartment of DNA, small metabolites, storage materials

RNA = ribonucleic acid

S = growth-limiting nutrient

X = biomass in dry mass

Appendices

APPENDIX A

```
C*************************************************************************
C
C  PROGRAM FOR THE CALCULATION OF THE BALANCE-EQUATIONS OF THE THREE
C  COMPARTMENT MODEL OF BACTERIAL GROWTH
C
C  LIST OF SYMBOLS                                              UNIT
C
C       AS      : FORMAL ORDER OF REACTION                      1
C       D       : HYDRAULIC DILUTION RATE                       1/H
C       KN      : TOTAL RATE OF LOSS OF G,K AND R               1/H
C       KSR     : RATE COEFFICIENT OF CONSUMPTION OF
C                 SUBSTRATE                                     G/G.H.(G/L)**AS.
C       MG      : TURNOVER OF G-COMPARTMENT                     1/H
C       MK      : TURNOVER OF K-COMPARTMENT                     1/H
C       RX      : RATE OF PRODUCTION OF BIOMASS                 G/L.H
C       WD      : HOLDUP FACTOR                                 1
C       Y,10    : MASS FRACTION OF K AT MU = 0                  G/G
C       Y,20    : MASS FRACTION OF G AT MU = 0                  G/G
C       Y,30    : MASS FRACTION OF R AT MU = 0                  G/G
C       Y50     : CONCENTRATION OF INCOMING SUBSTRATE           G/L
C       Y(I,1)  : MASS FRACTION OF K                            G/G
C       Y(I,2)  : MASS FRACTION OF G                            G/G
C       Y(I,3)  : MASS FRACTION OF R                            G/G
C       Y(I,4)  : CONCENTRATION OF BIOMASS (CX)                 G/L
C       Y(I,5)  : CONCENTRATION OF SUBSRTATE                    G/L
C       Y(I,6)  : MASS FRACTION OF WN                           G/G
C       DY(J)   : DERIVATIVES OF Y(I,J) WITH RESPECT TO
C                 THE TIME T
C       PW(M,M): STORAGE OF THE JACOBIAN MATRIX
C       YMAX(J): N-VECTOR CONTAINING THE MAXIMUM
C                 ABSOLUTE VALUES OF EACH COMPONENT
C                 OF Y CALCULATED SO FAR.
C                 YMAX(J) IS SET TO 1 FOR J = 1,N
C       YRG     : YIELD OF G PER UNIT R CONVERTED               G/G
C       YRK     : YIELD OF K PER UNIT R CONVERTED               G/G
C       YSR     : YIELD OF R PER UNIT S CONVERTED               G/G
C       ALPHAK  : RATE COEFFICIENT OF NETT PROD. OF K           G/G.H
C       ALPHAG  : RATE COEFFICIENT OF NETT PROD. OF G           G/G.H
C       BETA1   : TAYLOR-SERIES CONST. OF THE K BALANCE         G/G
C       BETA2   : TAYLOR-SERIES CONST. OF THE K BALANCE         G/G.H
C       BETA3   : TAYLOR-SERIES CONST. OF THE G BALANCE         G/G
C       BETA4   : TAYLOR-SERIES CONST. OF THE G BALANCE         G/G.H
C       MU      : THE SPECIFIC GROWTH RATE                      1/H
C
C  THE PROGRAM USES AN IMSL(INTERN. MATH. & STAT. LIB.,INC,TEXAS)-
C  SUBROUTINE "DVODGER" FOR SOLVING THE BALANCE-EQUATIONS USING THE
C  GEAR'S METHOD BY A PREDICTOR-CORRECTOR PROCEDURE( GEAR C.W.,
C  "THE AUTOMATIC INTEGRATION OF ORDINARY DIFF.-EQATIONS",
C  COMM. A.C.M. 14(MARCH 1971) PAGE 176)
C
C  ARGUMENTS OF THE SUBROUTINE "DVODGER" (SEE ALSO THE IMSL-MANUAL)
C
C       DFUN    : USERS SUPPLIED FUNCTION SUBROUTINE
```

```
C       Y(I,J) : TWO DIM. ARRAY OF THE INDEPENDENT VARIABLES
C                I = 1,2....,MAXDER; J = 1,2,.....N
C       T      : INDEPENDENT VARIABLE VARING FROM 0 TO TMAX
C       N      : NUMBER OF ORDINARY DIFFERENTIAL EQUATIONS
C       MTH    : METHOD PARAMETER
C                MTH = 0  THE APPROXIMATION WIHTOUT JACOBIAN MATRIX
C                MTH = 1  THE APPROXIMATION WITH JACOBIAN MATRIX
C                         SUPPLIED BY THE USER IN DFUN WITH IND = 1
C                MTH = 2  THE APPROXIMATION WITH JACOBIAN MATRIX
C                         EVALUATED NUMERICALLY
C       MAXDER : THE MAXIMUM ORDER IN THE APPROXIMATION
C       JSTART : JSTART = 0 : INITIAZING THE INTEGRATION
C                JSTART = 1 : CONTINUING THE INTEGRATION FROM LAST STEP
C                JSTART =-1 : CONTINUING THE INTEGRATION WITH NEW STEP
C       H      : STEPSIZE TO BE ATTEMPED ON THE NEXT STEP
C       HMIN   : SMALLEST STEPSIZE ALLOWED IN THE INTEGRATION
C       HMAX   : LARGEST STEPSIZE ALLOWED IN THE INTEGRATION
C       EPS    : MAXIMUM ERROR CRITERIUM -- ERROR/YMAX(J).LT.EPS --
C       ERROR  : N-VECTOR CONTAINING THE ONE-STEP ERROR IN EACH COMP.
C       WORK   : WORK AREA DIMENSION 17*N FOR MTH=0 AND N*(N+17) MTH#0
C       IER    : ERROR PARAMETER:
C                IER = 33 THE REQUESTED ERROR IS NOT ACHIEVED UNLESS
C                         H = HMIN
C                IER = 34 NO CORRECTOR CONVERGENCE ACHIEVED WITH H.GT.
C                         HMIN
C                IER = 35 THE REQESTED ERROR IS TOO SMALL
C                IER = 36 (WARNING) MAXDER MAY BE TOO LARGE MAXDER IS
C                         ADJUSTED AUTOMATICALLY
C
C*********************************************************************
        DIMENSION Y(8,6),YMAX(6),ERROR(6),WORK(138)
        COMMON/A/ KSR,MK,MG,AS,KN,WD,MUI
        COMMON/Y/ YSR,YRK,YRG,Y10,Y20,Y30,Y50
        COMMON/DD/ D,ALPHAK,ALPHAG,RK
        COMMON/STEADY/ BETA1,BETA2,BETA3,BETA4
        REAL KSR,MK,MG,MU,KN
        EXTERNAL DFUN
C
150     READ(1,1)N,KSR,MK,MG,AS,KN,D,WD,MUI,TMAX
        IF(N.LT.0) GO TO 200
        READ(1,2) YSR,YRK,YRG,Y10,Y20,Y30,Y50
        READ(1,2) (YMAX(I),I=1,N)
        READ(1,2) ALPHAK,ALPHAG
        READ(1,1) MAXDER,H,HMIN,HMAX
C
C**** READ IN STARTING VALUES FOR WK, WG, WR, CX, CS AND WN
C
        READ(1,2) (Y(1,J),J=1,N)
        READ(1,2) (ERROR(I),I=1,N), EPS
C
        NN = N
        IF(N.LT.6) NN=6
        BETA1 = (Y10 + ALPHAK * (MK + KN))/YRK
        BETA3 = (Y20 + ALPHAG * (MG +  KN))/YRG
        BETA2 = ALPHAK/YRK
        BETA4 = ALPHAG/YRG
C
        WRITE(5,1000) BETA1,BETA2,BETA3,BETA4
1000    FORMAT(/,' BETA1 =',F7.4,/' BETA2 =',F7.4,/' BETA3 =',F7.4,/
       1' BETA4 =',F7.4,/)
        MTH = 2
        JSTART = 0
        T = 0.0
        TOLD = T
C
C
```

```
      CALL  DVODGER( DFUN, Y, T, N, MTH, MAXDER, JSTART, H, HMIN, HMAX, EPS,
     1              YMAX, ERFOR, WORK, IER1)
C
      YSUM = Y(1,1) + Y(1,2) + Y(1,3) + Y(1,6)
      STEP = T - TOLD
      TOLD = T
      MU = RX/Y(1,4)
      WRITE(5,3)
      WRITE(5,4)  T, STEP, (Y(1,J), J=1,NN), YSUM, MU
C
      JSTART = 1
C
C
50    CALL  DVODGER( DFUN, Y, T, N, MTH, MAXDER, JSTART, H, HMIN, HMAX,
     1              EPS, YMAX, ERFOR, WORK, IER2)
C
      YSUM = Y(1,1) + Y(1,2) + Y(1,3) + Y(1,6)
      STEP = T - TOLD
      TOLD = T
      MU = RX/Y(1,4)
C
      WRITE(5,4)  T, STEP, (Y(1,J), J=1,NN), YSUM, MU
C
      IF(T.GT.TMAX)  GO TO  100
      GO TO  50
100   CONTINUE
      GO  TO  150
200   CALL  EXIT
C
1     FORMAT(I,9F)
2     FORMAT(8F)
3     FORMAT(2X,' TYD',4X,' STEP',4X,' K',5X,' G',5X,' R',5X,' X',5X,
     1 ' S',5X,' NW',3X,' YSUM',2X,' MU',/)
4     FORMAT(F8.4, F8.5, 8F7.3)
      END
C
      SUBROUTINE DFUN(YP, TP, M, DY, IND, PW)
      DIMENSION YP(8,6), PW(M,M)
      DIMENSION Y(6), DY(M)
      COMMON/A/KSR,MK,MG,AS,KN,WL,MUI
      COMMON/Y/YSR,YRK,YRG,Y10,Y20,Y30,Y50
      COMMON/DD/ D, ALPHAK, ALPHAG, RX
      COMMON/STEADY/ BETA1, BETA2, BETA3, BETA4
      REAL KSR,MK,MG,MU,KN
C
9     CONTINUE
      DO  10 I = 1,M
10    Y(I) = YP(1,I)
      IF(TP.EQ.0.0)  GO TO  4
      DD = D
      GO TO  5
4     DD = .03/WL
5     CONTINUE
C
      DY(5) = -KSR*Y(4)*Y(5)**AS + DD*(Y50 - Y(5))
C
      DY(4) = YSR * KSR * Y(4) * Y(5)**AS - WL * DD * Y(4)
      DY(4) = DY(4) + ((MK+KN)*Y10/YRK+BETA1*(Y(1)-Y10)/ALPHAK +
     1 BETA2*(Y(1) - Y10)**2/ALPHAK/ALPHAK)*(YRK-1)*Y(4)
      DY(4) = DY(4) + ((MG+KN)*Y20/YRG+BETA3*(Y(2)-Y20)/ALPHAG +
     1 BETA4*(Y(2)-Y20)**2/ALPHAG/ALPHAG)*(YRG-1.)*Y(4)
C
      IF(TP)  1, 1, 2
1     RX = WL * DD * Y(4)
      GO TO  3
```

```
2       RX = DY(4) + WD * DD * Y(4)
C
3       MU = RX/Y(4)
        DY(1)=(MK+KN)*Y10 +YRK*BETA1*MU+YRK*BETA2*MU*MU
        1 -(MK+KN)*Y(1) - RX*Y(1)/Y(4)
C
        DY(2)=(MG+KN)*Y20+ YRG*BETA3*MU+YRG*BETA4*MU*MU
        1 -(MG + KN)*Y(2) - RX*Y(2)/Y(4)
C
        DY(3) = -DY(2) - DY(1)
C
C
D       DY(6) = KN*(Y(1) + Y(2) + Y(3)) - RX*Y(6)/Y(4)
C
11      CONTINUE
        RETURN
        END
```

APPENDIX B

```
C       ******************************************************************
C       PROGRAM TWO-STAGES
C
C
C           THIS PROGRAM CALCULATES THE MINIMUM HYDRAULIC RETENTION
C       TIME OF SUBSTRATE IN THE FIRST STAGE AND THE SECOND STAGE
C       OF THE TWO-STAGE SYSTEM WITH RECYCLING OF BIOMASS.
C       THE ADJUSTED SUBSTRATE CONCENTRATION IN THE FIRST STAGE
C       IS ALSO CALCULATED.
C           THE HYDRAULIC RETENTION TIME IN THE ONE-STAGE SYSTEM
C       IS ALSO BE DETERMINED ASSUMING COMPLETE CONSUMPTION OF
C       SUBSTRATE IN THE FIRST STAGE.
C
C
C       LIST OF SYMBOLS
C
C       AS1     : FORMAL ORDER OF REACTION IN THE FIRST STAGE
C       AS2     : FORMAL ORDER OF REACTION IN THE SECOND STAGE
C       AS2.1   : CONSTANT IN THE EXPONENTIAL FUNCTION WHICH
C                 RELATES AS2 TO CS1
C       AS2.2   : CONSTANT IN THE EXPONENTIAL FUNTION WHICH
C                 RELATES AS2 TO CS1
C       CS0     : CONCENTRATION OF INCOMING SUBSTRATE
C       CS1     : CONCENTRATION OF SUBSTRATE IN THE FIRST STAGE
C       CS2     : CONCENTRATION OF SUBSTRATE IN THE SECOND STAGE
C       CX      : CONCENTRATION OF BIOMASS IN THE SINGLE STAGE
C       CX1     : CONCENTRATION OF BIOMASS IN THE FIRST STAGE
C       CX2     : CONCENTRATION OF BIOMASS IN THE SECOND STAGE
C       KSR1    : RATE COEFFICIENT OF CONSUMPTION OF SUBSTRATE
C                 IN THE FIRST STAGE
C       KSR2    : RATE COEFFICIENT OF CONSUMPTION OF SUBSTRATE
C                 IN THE SECOND STAGE
C       KSR2.1  : CONSTANT IN THE EXPONENTIAL FUNCTION WHICH
C                 RELATES KSR2 TO CS1
C       KSR2.2  : CONSTANT IN THE EXPONENTIAL FUNCTION WHICH
C                 RELATES KSR2 TO CS1
C       T       : TOTAL HYDRAULIC RETENTION TIME IN THE TWO-STAGE SYSTEM
C       T1      : HYDRAULIC RETENTION TIME IN THE FIRST STAGE
C       T2      : HYDRAULIC RETENTION TIME IN THE SECOND STAGE
C       T3      : HYDRAULIC RETENTION TIME IN THE ONE-STAGE SYSTEM
C       V       : VOLUME OF THE ONE-STAGE SYSTEM
C       V1      : VOLUME OF THE FIRST STAGE
C       V2      : VOLUME OF THE SECOND STAGE
```

```
C       W1. CS1 : FRACTION OF SUBSTRATE DISCHARGED FROM THE
C                 FIRST STAGE
C
C
C       THE FOLLOWING ASSUMPTIONS ARE MADE:
C
C       V = V1 + V2
C       CX. V = CX1. V1 + CX2. V2
C       V. CX = V1. CX1 + V2. CX2
C
C
C
C       ***********************************************************************
C
        DIMENSION C(100), D(100), E(71)
        REAL KSR1, KSR2, KSR2.1, KSR2.2
18      TYPE 2
        ACCEPT 1, CX1, CX2, CS0, CS2, KSR1, AS1, KSR2.1, KSR2.2, AS2.1, AS2.2, C1,
       1V1
 1      FORMAT (14F)
        TYPE 3
        ACCEPT 1, CS1MIN, CS1MAX
 2      FORMAT (' PARAMETERS ?')
 3      FORMAT (' MINIMUM AND MAXIMUM OF CS1 ?')
        WRITE (5,7) CX1, CX2, CS0, CS2, KSR1, AS1, KSR2.1, KSR2.2, AS2.1, AS2.2,
       1C1, V1
        TYPE 8
        DELTA=(CS1MAX-CS1MIN)/99.
        CS1=CS1MIN-DELTA
        TYPE 13
        DO 4 I=1, 100
          CS1=CS1+DELTA
          KSR2=KSR2.1*EXP(KSR2.2*CS1)
          AS2=AS2.1*EXP(AS2.2*CS1)
          D(I)=CS1
          C(I)=(CS0-CS1)/(KSR1*((CS1/CS0)**AS1)*CX1)
       1       +(1-C1)*(CS1-CS2)/(KSR2*((CS2/CS1)**AS2)*CX2)
          IF (I.EQ.1) TYPE 12, C(I), CS1
          IF (I.EQ.20*(I/20)) TYPE 12, C(I), CS1
 4      CONTINUE
        AMIN=10.**20
        AMAX=-AMIN
        DO 5 I=1, 100
          IF (C(I).GT.AMAX) AMAX=C(I)
          IF (C(I).LT.AMIN) AMIN=C(I)
          IF (AMIN.EQ.C(I)) K=I
 5      CONTINUE
        RANGE=AMAX-AMIN
        WRITE (5,6) CS1MIN, CS1MAX, AMIN, AMAX
 6      FORMAT (5X,' MIN CS1=', F10.3,/,5X,' MAX CS1=', F10.3,/,
       15X,' MIN T  =', F10.3,/,5X,' MAX T  =', F10.3,/)
 7      FORMAT (/,5X,' PARAMETERS:',/,5X,' CX1    =', F10.3,/,5X,' CX2
       1=', F10.3,/,5X,' CS0    =', F10.3,/,5X,' CS2    =', F10.3,/,
       25X,' KSR1   =', F10.3,/,5X,' AS1    =', F10.3,/,
       35X,' KSR2.1 =', F10.3,/,5X,' KSR2.2 =', F10.3,/,
       45X,' AS2.1  =', F10.3,/,5X,' AS2.2  =', F10.3,/,
       55X,' C1     =', F10.3,/,5X,' V1     =', F10.3,/)
 8      FORMAT (/,71(1H*))
        CS1=D(K)
        T1=(CS0-CS1)/(KSR1*((CS1/CS0)**AS1)*CX1)
        T2=C(K)-T1
        WRITE (5,9) T1, T2, C(K), D(K)
 9      FORMAT (' THE HYDRAULIC RETENTION TIME IN THE FIRST-
       1STAGE    : ', ' T1  =', F9.3,
       2/,' THE HYDRAULIC RETENTION TIME IN THE SECOND-STAGE
```

```
      3     : ', ' T2  = ', F9.3,
      4/, ' THE HYDRAULIC RETENTION TIME IN THE TWO STAGES
      5     : ', ' T   = ', F9.3,/,
      6' THE SUBSTRATE CONCENTRATION IN THE FIRST-STAGE
      7' CS1 = ', F9.3)
      CX=(V1*CX1+V1*(T2/T1)*CX2)/(V1+V1*(T2/T1))
      T3=(CS0-CS2)/(KSR1*((CS2/CS0)**AS1)*CX)
      WRITE (5,11) T3
11    FORMAT (' THE HYDRAULIC RETENTION TIME IN THE ONE-STAGE SYSTEM :
      1', ' T3 = ', F9.3)
      WRITE (5,8)
12    FORMAT (' T  = ', F7.3, '   CS1  = ', F7.3,/)
13    FORMAT (' INTERMEDIATE CALCULATIONS OF T AND CS1',/)
      TYPE 16
16    FORMAT (' POS. NUMBER GO ON')
      ACCEPT 17,L
17    FORMAT (I1)
      IF (L.LT.0) STOP
      GO TO 18
      END
```

APPENDIX C

Estimation of the Taylor-series constants

The balance equation of ω_K in the reactor with steady-state assumptions made can be written as (Eqn 82).

$$\frac{\partial \omega_K}{\partial t} = \frac{1}{C_X}\left[Y_{RK}\left\{\frac{(m_K + k_{NK})\omega_{KO}}{Y_{RK}} + \beta_1.\mu + \beta_2.\mu^2\right\}C_X + \right.$$

$$\left. - m_K.\omega_K.C_X - k_{NK}.\omega_K.C_X - r_X.\omega_K\right] \qquad (C.1)$$

where $\beta_1 = \tilde{f}_R.\alpha_R + \tilde{f}_K.\alpha_K$ and $\beta_2 = \frac{1}{2}.\tilde{f}_{RR}.\alpha_R^2 + \tilde{f}_{KR}.\alpha_R.\alpha_K + \frac{1}{2}.\tilde{f}_{KK}.\alpha_K^2$

In steady state ($\partial\omega_K/\partial t = 0$ and $r_X.\omega_K/C_X = \mu.\omega_K$) Equation C.1 can be written after rearrangement as

$$0 = -(m_K + k_{NK})(\tilde{\omega}_K - \omega_{KO}) + Y_{RK}.\beta_1.\mu + Y_{RK}.\beta_2.\mu^2 - \mu.\tilde{\omega}_K \qquad (C.2)$$

From Equation 77 we have $\tilde{\omega}_K - \omega_{KO} = \alpha_K.\mu$ and substituting $\alpha_K.\mu$ for $(\tilde{\omega}_K - \omega_{KO})$ into Equation C.2 we obtain

$$0 = -(m_K + k_{NK}).\alpha_K.\mu + Y_{RK}.\beta_1.\mu + Y_{RK}.\beta_2.\mu^2 - \mu.\tilde{\omega}_K \qquad (C.3)$$

If all the terms of Equation C.3 are divided by μ and $\tilde{\omega}_K$ is written explicitly, we have

$$\tilde{\omega}_K = Y_{RK}.\beta_1 - (m_K + k_{NK})\alpha_K + Y_{RK}.\beta_2.\mu \qquad (C.4)$$

Equation C.4 and Equation 77 ($\tilde{\omega}_K = \omega_{KO} + \alpha_K \cdot u$) must be identical and therefore β_1 and β_2 can be solved as

$$\beta_1 = \frac{(m_K + k_{NK})\alpha_K}{Y_{RK}} \tag{C.5}$$

and

$$\beta_2 = \frac{\alpha_K}{Y_{RK}} \tag{C.6}$$

The same mathematical procedure can be followed for solving the Taylor-series constants of the balance equation of ω_G if we start with Equation 84. The final results are

$$\beta_3 = \frac{(m_G + k_{NG})\alpha_G}{Y_{RG}} \tag{C.7}$$

and

$$\beta_4 = \frac{\alpha_G}{Y_{RG}} \tag{C.8}$$