*Wat. Sci. Tech.* Vol. 23, Kyoto, pp. 917–926, 1991.
Printed in Great Britain. 

0273–1223/91 $0·00 + ·50


# EFFECT OF ANAEROBIC STABILIZATION OF ACTIVATED SLUDGE ON ITS PRODUCTION UNDER BATCH CONDITIONS AT VARIOUS $S_o/X_o$ RATIOS

P. Chudoba, J. J. Chevalier, J. Chang and B. Capdeville

*Unité de Recherche Traitement Biologique des Eaux, Département de Génie des Procédés Industriels, Institut National des Sciences Appliquées, Avenue de Rangueil, 31077 Toulouse, France*

ABSTRACT

The effect of anaerobic stabilization of returned activated sludge in a continuous system on biomass production under batch conditions at various $S_o/X_o$ ratios was studied. A system without anaerobic sludge stabilization was also operated as a control unit. All experiments were carried out with Toulouse settled wastewater. Biomass growth, substrate removal, oxygen uptake and carbon dioxide production were measured using a quadrupole mass spectrometer (Inficon IQ 200) coupled to a 15-liter fermentor (Biolafitte). The results confirmed that one of the most important parameters in batch cultivation is the initial $S_o/X_o$ ratio. It was found that anaerobic treatment of activated sludge changed its behaviour during batch cultivation. As a consequence, the observed biomass yield ($Y_{obs}$) decreased with increasing $S_o/X_o$ while it remained constant with the sludge from the control unit. In the anaerobic reactor, microorganisms are subject to a physiological shock due to lack of oxygen and food. Under the above conditions, they use ATP as a source of energy. After they are returned to aerobiosis and supplied with exogenous substrate, they rebuild energy reserves at the expense of growth.

KEYWORDS

Activated sludge; anaerobiosis; batch; mass spectrometer; substrate removal; growth; ATP.

## INTRODUCTION

What is the effect of anaerobiosis on the activated sludge process ? One of the first reports on this effect was presented by Wuhrmann (1960) who concluded that the purification capacity of activated sludge remains relatively unaffected by anaerobic intervals. Westgarth *et al.* (1964) demonstrated that anaerobic conditions had a positive effect on suppressing activated sludge filamentous bulking. Another case of anaerobiosis in activated sludge processes which may be mentioned is an anaerobic selector introduced in front of an oxic completely mixed tank (CMT). Thus, Wanner *et al.* (1987a,b ; 1988) used an anaerobic CMT followed by an oxic one to study the behaviour of filamentous microorganisms. They defined anaerobiosis as conditions under which neither molecular oxygen nor nitrite/nitrate nitrogen are present as a final acceptor in the electron transport system. A question arises whether an anaerobic zone is able to affect the biomass production in an activated sludge system. Westgarth *et al.* (1964) found that the insertion of a period of anaerobiosis in the high-rate activated sludge process affected the rate of waste sludge production, which was about half that without anaerobiosis. The aim of this paper is not to describe the mechanisms of suppression of activated sludge filamentous bulking or to study phosphate removal, but to study the behaviour of activated sludge cultivated in oxic-anaerobic conditions during batch experiments under oxic conditions.

## MATERIALS AND METHODS

Laboratory models. A schematic diagram of the models is shown in Figure 1. OSA (Oxic-Settling-Anaerobic) system consisted of an oxic CMT and an anaerobic CMT incorporated into the returned sludge circuit. The conventional activated sludge system involved an oxic CMT which served as a control unit. All technological parameters of both systems are given in Table 1.

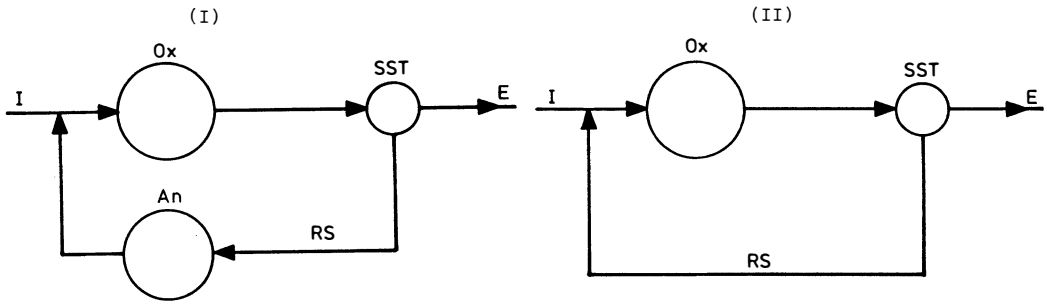


Fig. 1. Laboratory systems used (I-OSA system, II-control unit ; Ox-oxic CMT, An-anaerobic CMT, SST-secondary settling tank, I-influent, E-effluent, RS-returned sludge).

TABLE 1. Technological Parameters of Activated Sludge Systems

| Parameter | Unit | OSA system | control unit |
|---|---|---|---|
| Volume of the oxic CMT | l | 2 - 5 | 2 - 5 |
| Volume of the anaerobic CMT | l | 4.75 | - |
| Volume of the SST | l | 2.5 | 2.5 |
| Recirculation ratio | - | 0.6 - 0.8 | |
| HRT in the oxic CMT | h | 0.5 - 2 | 1 - 3 |
| HRT of sludge in the anaerobic CMT | h | 3 | - |
| SRT in the whole system ($\Theta_x$) | d | 2.5 | 2.5 |
| Sludge loading with TOD ($B_x$)* | $kg.kg^{-1}.d^{-1}$ | 1 - 2 | 1 - 1.6 |
| Volumetric loading with TOD ($B_v$)* | $kg.m^{-3}.d^{-1}$ | 1.6 - 2.3 | 2.6 - 8 |
| Average influent TOD | $mg.l^{-1}$ | 300 | 300 |
| Average influent SS | $mg.l^{-1}$ | 130 | 130 |
| Average effluent TOD | $mg.l^{-1}$ | 140 | 90 |
| Average effluent SS | $mg.l^{-1}$ | 55 | 25 |
| Dissolved oxygen in the oxic CMT | $mg.l^{-1}$ | 5 | 5 |
| Temperature | °C | 20 - 25 | 20 - 25 |
| Ox.-red.potential in the anaerobic CMT | mV | - 250 | - |

*Sludge and volumetric loadings are respectively related to total sludge concentration and to total volume of both systems.

Settled wastewater from the municipality of Toulouse was used as a multi-component substrate solution in both systems. Both systems were operated at sludge age of 2.5 days in order to suppress nitrification.

Determination of optimum HRT in the anaerobic CMT. To determine the optimum time period over which the sludge should be held under anaerobic conditions, a kinetic batch test was performed. A 1-liter glass closed reactor, equipped with a dissolved oxygen probe and pH and oxidation-reduction potential electrodes, was filled with mixed liquor from the oxic CMT of the OSA system. This mixture was magnetically stirred and a liquid sample was taken each hour in order to determine adenosine triphosphate (ATP) content in biomass and volatile fatty acid production. The pH, oxidation-reduction potential and dissolved oxygen values were recorded continuously.

Respirometric studies. The activated sludge from both systems was used for batch respirometric tests. The schematic diagram of the experimental system is shown in Figure 2.

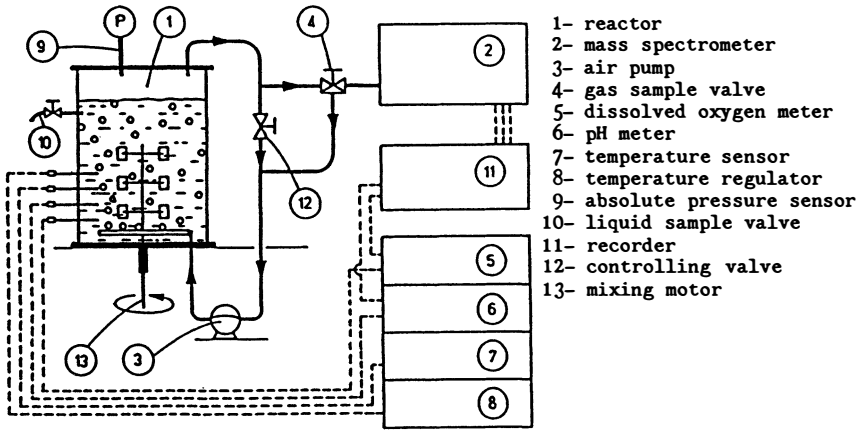


Fig. 2. The respiratory system used.

A 15-liter fermentor (Biolafitte) was equipped with a dissolved oxygen meter, a pH meter and a temperature regulator. The velocity of the agitator was controlled by regulating the speed of the motor. The quadrupole mass spectrometer (Inficon IQ200) is a type of mass spectrometer known as a "residual gas analyzer", an instrument designed for the analysis of the rarefied atmosphere in a high vacuum system. Its mass range of 0-200 AMU (atomic mass unit) makes it suitable for measuring any permanent gas and almost all volatile components that may be important in fermentation technology. It is able to continuously monitor ten different components of gas simultaneously with a very short response time of less than one second. The main advantages of this instrument are its long-term stability and its consumption of less than 14 milliliters of gas sample during continuous measurements over 24 hours.

Using the quadrupole mass spectrometer coupled to fermentor, a respirometric system was created enabling simultaneous measurement of biomass growth, substrate removal, oxygen uptake and carbon dioxide production. The first reports of using the mass spectrometer (MS) for monitoring fermentation processes were presented by Nobbs (1974), Reuss et al. (1975) and Lespinat et al. (1978). Numerous works describe direct use of MS for determination of a variety of volatile compounds and gas composition in biological systems (Bohatka et al., 1983 ; Heinzle et al., 1983 ; Lloyd et al., 1985 ; Heinzle, 1987). Recently, Chang (1988) reported on the results obtained in batch experiments using the quadrupole MS, clearly showing advantages of this device.

All experiments were run aerobically in this system and under carbon-limited conditions. Temperature was maintained at 25 ± 0.1°C. The experimental program was based on different ratios of the initial substrate concentration ($S_o$) to the initial biomass concentration ($X_o$). This ratio is one of the most important parameters in batch experiments (Chudoba, 1969 ; Chang, 1988 ; Pitter and Chudoba, 1989 ; Chudoba, 1989). It determines whether cell multiplication will take place or not during the exogenous substrate removal (Speece et al., 1973 ; Chudoba, 1989).

A preselected volume of excess activated sludge from the oxic CMT's of both systems was taken, injected immediately into the experimental batch reactor and mixed with the chosen volume of substrate (municipal wastewater) from the continuous systems. The total culture volume in the fermentor varied between 7 and 10 liters for different runs with different $S_o/X_o$ ratios. The system was closed by plugging the absolute pressure sensor 9 (Fig. 2) into the fermentor. The liquid sample was taken by means of a syringe through liquid sample valve 10 as rapidly as possible. The analyses in the liquid and gas phases were then conducted.

Analytical methods. In the liquid phase, analyses of COD, TOD and mixed liquor suspended solids (MLSS) were carried out according to Standard Methods (1980) and Normes Afnor (1983). COD was determined by the dichromate semimicromethod described by Jirka and Carter (1975). TOD was determined by means of a TOD analyzer Ionics model 225. The values of pH and dissolved oxygen (DO) were measured by pH and DO electrodes (Biolafitte), respectively. The ATP content was determined by the bioluminiscence method (Martin et al., 1979) using ATP photometer

Nucleotimetre 107 (CLV-Interbio). Volatile fatty acids were determined by gas chromatography.

The air pump 3 (Fig. 2) recycled gas in the fermentor and aerated mixed culture with a sufficient air flow rate. A small part of the gas passed through the gas sample valve 4, from which the mass spectrometer sampled the gas and continuously analyzed $O_2$, $CO_2$ and $N_2$ concentrations. The absolute pressure in the closed batch reactor was measured by the absolute pressure sensor 9 (Membranovac-LH Sogev). All experimental data were processed by means of a Hewlett-Packard 300 computer.

## RESULTS AND DISCUSSION

Batch respirometric experiments. Examples of the shapes of biomass and substrate concentration curves during batch experiments are shown in Figures 3 and 4.

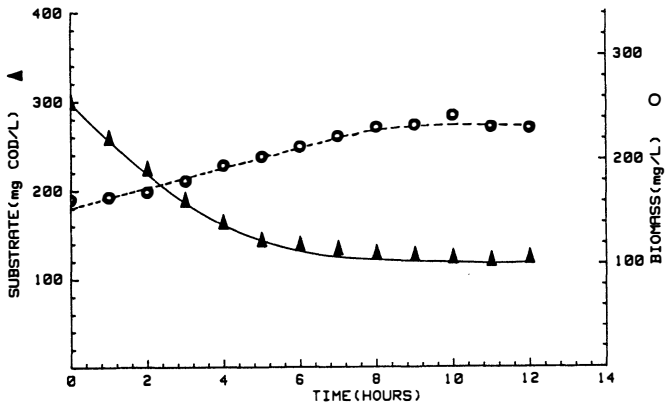


Fig. 3. Substrate removal and biomass growth ($S_o/X_o < 2$, activated sludge from control unit).

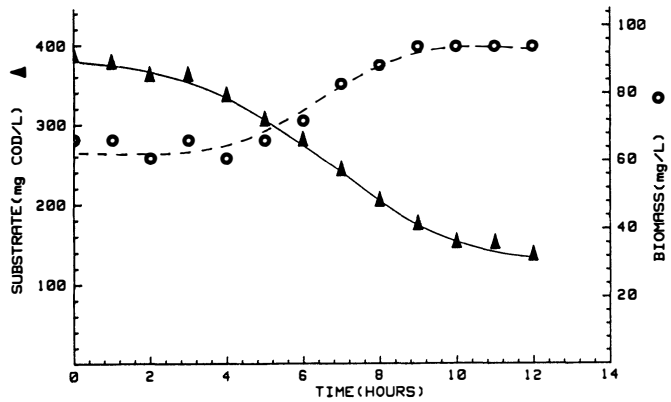


Fig. 4. Substrate removal and biomass growth ($S_o/X_o > 2$, activated sludge from control unit).

If the initial $S_o/X_o$ ratio is low (Fig. 3), a curve of the multi-component substrate removal shows a decreasing rate. In the case of a single-component substrate, the end removal rate is constant (Chudoba, 1969 ; Pitter and Chudoba, 1989). This is a typical example of a system without cell multiplication during substrate removal (Pitter and Chudoba, 1989). At higher initial $S_o/X_o$ ratio (usually higher than 2), the cell division occurs, which is demonstrated by an acceleration of the substrate removal (Fig. 4). Similar relationships for oxygen consumption and carbon dioxide production are shown in Figures 5 and 6. In figures 4 and 6, a three-hour lag period can be observed on the curves of biomass growth and $CO_2$ production, respectively. This type of lag phase is called "apparent lag phase" and determines the time

necessary for multiplication of an originally small population to concentrations which bring about a noticeable decrease in substrate concentration (Wiggins et al., 1987 ; Pitter and Chudoba, 1989).

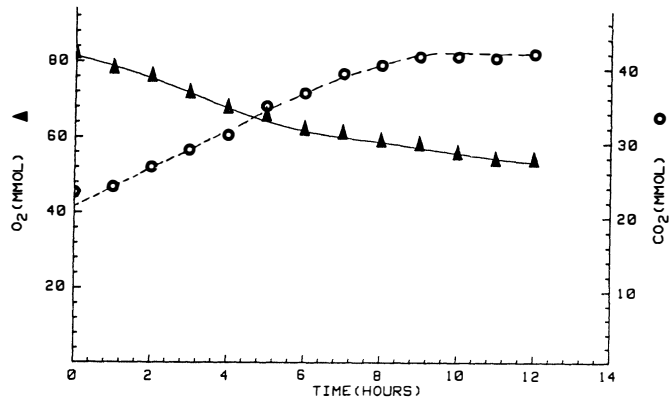


Fig. 5. Oxygen consumption and carbon dioxide production ($S_o/X_o$ < 2, activated sludge from control unit).

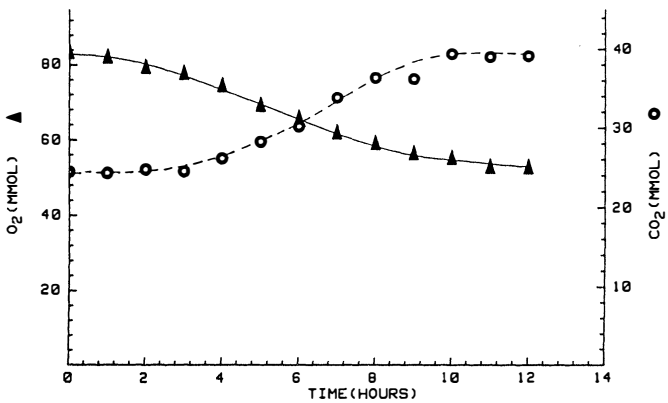


Fig. 6. Oxygen consumption and carbon dioxide production ($S_o/X_o$ > 2, activated sludge from control unit).

For each run, the observed biomass yield coefficient was calculated as follows :

$$Y_{obs} = \frac{X - X_o}{S_o - S} \quad (1)$$

where

$Y_{obs}$ : observed biomass yield coefficient, $mg.mg^{-1}$ (MLSS per COD);
$X$ : biomass maximum concentration, $mg.l^{-1}$;
$X_o$ : initial biomass concentration, $mg.l^{-1}$;
$S$ : substrate minimum concentration, $mg.l^{-1}$ (as COD);
$S_o$ : initial substrate concentration, $mg.l^{-1}$ (as COD).

It is necessary to note that $Y_{obs}$ stayed constant throughout a run. Figures 7 and 8 show the relationships between the $Y_{obs}$ values and the $S_o/X_o$ ratios obtained in the above systems.

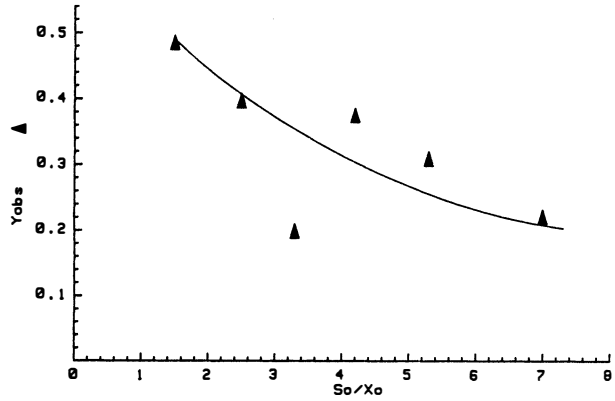


Fig. 7. Relationship between $Y_{obs}$ and $S_o/X_o$ (OSA system).

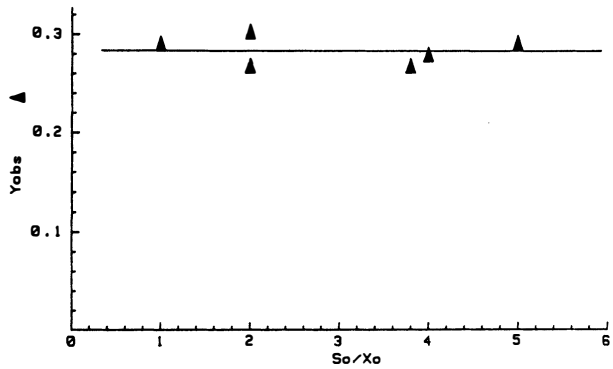


Fig. 8. Relationship between $Y_{obs}$ and $S_o/X_o$ (control unit).

It was observed in batch experiments with activated sludge from OSA system that the observed biomass yield coefficient decreased as the $S_o/X_o$ ratio increased. A similar relationship as shown in Figure 7 was also obtained by Chang (1988) (Fig. 9). He used the activated sludge grown in a laboratory system with an anaerobic CMT in the returned sludge circuit and fed on synthetic wastewater. He found that the higher the $S_o/X_o$ ratio, the lower the biomass production and the greater the quantity of substrate oxidized per unit of substrate removed.

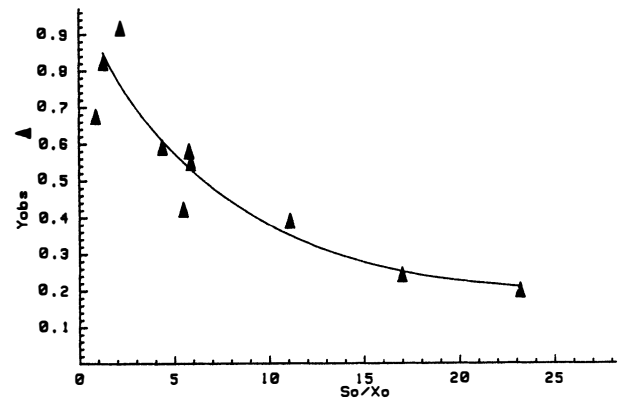


Fig. 9. Relationship between $Y_{obs}$ and $S_o/X_o$ (Chang, 1988).

An explanation given for this phenomenon was a higher contribution of substrate removal for maintenance energy requirement (Chang, 1988). However, it may be remarked that the phenomenon of $Y_{obs}$ diminution with increasing $S_o/X_o$ ratio in batch experiments was observed only with activated sludge grown in a system where it was periodically exposed to anaerobic conditions. But the value of $Y_{obs}$ of the activated sludge from the control unit used in batch experiments remained constant with varying $S_o/X_o$ ratio (Fig. 8). Supposing that microorganisms can considerably multiply only at higher $S_o/X_o$ ratios (Speece et al., 1973 ; Pitter and Chudoba, 1989) the above phenomenon can be explained as follows. For their multiplication, the cells need more energy for enzyme, protein, RNA and DNA synthesis. At low initial $S_o/X_o$ ratios the increase of biomass reflects practically only the accumulation of reserve substances, which is energetically less demanding than the synthesis of all the substances required for reproduction. Thus, at low $S_o/X_o$ values the ratio between Anabolism (Synt) and Catabolism (Ox) is usually higher than at high $S_o/X_o$ values. Similar conclusions were drawn by Rao and Gaudy (1966).

However, the behaviour of the two activated sludges at low initial $S_o/X_o$ ratios is different. Sludge grown in the system with an anaerobic returned sludge zone was kept for some time under starvation and anaerobic conditions. The carbon stores were thus depleted and many enzymes needed for balanced replication processes may have been broken down in order to help provide for the endogenous energy requirement of the cells (Clifton, 1957). When these cells are contacted with a fresh supply of the same substrate on which they were originally grown, there is a period during which the cells grow in the sense that they increase in mass but do not yet begin to replicate (Rao and Gaudy, 1966). The mode of substrate utilization during this period then depends on the initial inoculum of cells present in the system and thus on the $S_o/X_o$ ratio.

Continuous activated sludge systems. In a formal discussion of the paper reported by Westgarth et al. (1964), a question arose whether the definition of anaerobic conditions in the returned sludge CMT was correct. Rohlich and Boyle (1964) thought that the time period over which the sludge had been held under anaerobic conditions required closer study. According to Sawyer (1964), no data were present to show that anaerobic conditions had existed in the sludge storage units. It was suggested that true anaerobic conditions had not prevailed. To resolve this question, a kinetic batch experiment was made in order to determine the operating conditions of the anaerobic CMT. The results of this experiment are depicted in Figure 10. The ATP content decreased quickly during the first hour and stabilized at a minimum value after 4 hours. The oxygen concentration and oxidation-reduction potential decreased similarly to reach minimum values of 0 $mg.l^{-1}$ and -380 mV, respectively. The volatile fatty acid production was not detected and thus, the decrease of pH values was negligible. According to these results, an HRT of 3 hours was chosen in the anaerobic CMT.

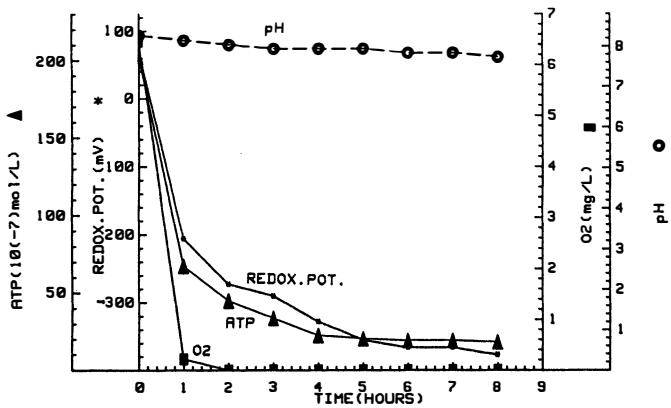


Fig. 10. Anaerobic batch test.

As ATP is an essential component in the biochemical substrate utilization and in new cell synthesis, it has been chosen by numerous investigators to describe the microbial activity (Weddle and Jenkins, 1971 ; Patterson et al., 1970). It is specific for living organisms only and thus may relate directly to the viable biomass (Weddle and Jenkins, 1971). When the microorganisms are subjected to a physiological shock created by a lack of oxygen, they are not able to produce the essential energy and they have to use their reserves of ATP as a

source of energy. After they are returned to aerobic conditions, they rebuild energy reserves at the expense of growth. According to Westgarth et al. (1964) and Ecker and Lockhart (1961), the aerobically grown cells exposed to anaerobic conditions are able to utilize oxygen readily when returned to aerobic conditions. In addition, it was found that the amount of excess sludge produced by the unit with anaerobic recirculation zone under high-rate loading was only about half the amount produced by the completely mixed aerobic unit. Westgarth et al. (1964) concluded that their observations of much less excess sludge production and more substrate oxidation in the aerobic-anaerobic high-rate activated sludge process were microbiologically not clear. These findings support the validity of the concept of ATP depletion under anaerobic conditions and its consequent resynthesis under aerobic conditions at the expense of cell growth.

The insertion of anaerobic regeneration into the returned sludge circuit resulted in suppression of filamentous bulking (Fig. 11), though small amounts of Beggiatoa were observed. The capability of anaerobic conditions to suppress some filamentous microorganisms' growth is well known (Westgarth et al., 1964 ; Wanner et al., 1987 a,b, 1988). On the other hand, the oxic CMT produced a mixed culture with high SVI as shown in Fig. 11. Microthrix parvicella was identified as a causative microorganism.

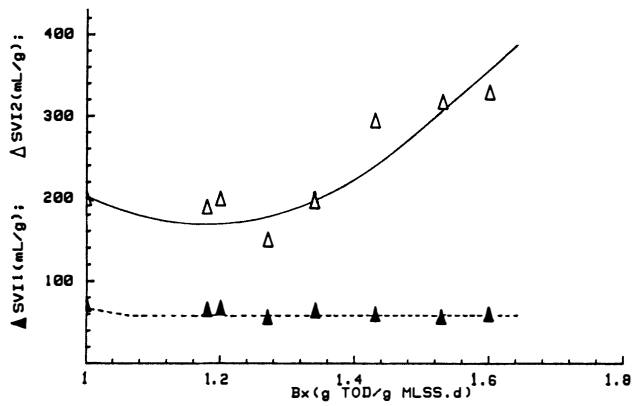


Fig. 11. Relationship between SVI and $B_x$ (▲ OSA system, Δ control unit).

The treatment efficiency and effluent quality in the OSA system can be affected by a disintegration of the activated sludge floc. This drawback might be suppressed by optimizing the HRT of activated sludge in the anaerobic zone, which is a subject of our current research.

## CONCLUSIONS

Two laboratory activated sludge units were run. An OSA (Oxic-Settling-Anaerobic) system with an anaerobic stabilization zone in sludge recirculation and conventional activated sludge system, which served as a control unit. Excess sludge was used for batch respirometric experiments in a unit formed by a reactor coupled with a mass spectrometer. The batch experiments were carried out at various initial $S_o/X_o$ ratios. The observed biomass yield coefficients ($Y_{obs}$) were calculated for each experiment. The results can be summarized as follows :

1. At low initial $S_o/X_o$ ratios, the curves of COD removal showed decreasing rates. At high initial $S_o/X_o$ ratios, the curves of COD removal showed increasing rates, indicating a considerable cell multiplication.
2. The insertion of the anaerobic stabilization zone into the returned sludge circuit changed the composition and behaviour of activated sludge microorganisms.
3. Under batch conditions, the observed biomass yield ($Y_{obs}$) decreased with increasing $S_o/X_o$ ratio. No increase was observed with activated sludge microorganisms grown in the control unit.
4. Anaerobically treated activated sludge had SVI values around 60 $ml.g^{-1}$ while sludge from the oxic CMT had SVI values around 200 $ml.g^{-1}$.
5. The anaerobically treated microorganisms are subject to a physiological shock created by a lack of oxygen and food. Under the above conditions, they use ATP as a source of energy. After they are returned to aerobiosis and supplied with exogenous substrate, they rebuild energy

reserves at the expense of growth.
6. The good settleability and production of activated sludge could be controlled by means of an anaerobic CMT in the returned sludge circuit. These findings may be important for sludge management in the activated sludge process. A study is currently being conducted in order to apply the obtained knowledge for continuously grown culture conditions.

REFERENCES

AFNOR (1983). Eaux - méthodes d'essai. 2ème edition. Association Française de Normalisation, Paris.

APHA (1980). Standard Methods for the Examination of Water and Wastewater. 15th edition. American Public Health Association, Washington, D.C.

Bohatka, S., Polya, K., Langer, G. and Szilagyi, J. (1983). Quadrupole mass spectrometer analyzer system for monitoring fermentation processes. Conf. Advances in Fermentation, 131-137.

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

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

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

Clifton, C.E. (1957). Introduction to Bacterial Physiology, McGraw-Hill Book Co. (Ed.), New York.

Ecker, R.E. and Lockart, W.R. (1961). Specific effect of limiting nutrient on physiological events during culture growth. J. Bacteriol., 82, 511-516.

Heinzle, E., Furukawa, K., Dunn, I.J. and Bourne, J.R. (1983). Experimental methods for on-line mass spectrometry in fermentation technology. Bio/technology, April, 181-188.

Heinzle, E. (1987). Mass spectrometry for on-line monitoring of biotechnological processes. In : Advances in Biochemical Engineering/Biotech. Biotechnol. Methods, A. Fiechter (Ed.) Pergamon Press, 35, 1-47.

Jirka, A.M. and Carter, M.J. (1975). Micro semi-automated analysis of surface and wastewaters for chemical oxygen demand. Analyt. Chem., 47, 1397-1402.

Lespinat, P.A., Gerster, R. and Berlier, Y. (1978). Direct mass-spectrometric determination of the relationship between respiration, hydrogenase and nitrogenase activities in Azobacter Chroococcum, Biochimie, 60, 339-341.

Lloyd, D., Bohatka, S. and Szilagyi, J. (1985). Quadrupole mass spectrometry in the monitoring and control of fermentations. Biosensors, 1, 179-212.

Martin, G., Nam, S.H., Vitrac, O. and Reinbult, M. (1979). Utilisation de l'ATP dans les systèmes d'épuration biologique. Mise au point d'une technique d'extraction et de dosage de l'ATP dans les boues activées. Eau Ind., 32, 46-53.

Nobbs, J.M. (1974). Mass spectrometric determination of oxygen. Proc. of an interdisciplinary symposium. Odense, 209-225.

Patterson, J.W., Brezonik, P.L. and Putnam, H.D. (1970). Measurement and significance of adenosine triphosphate in activated sludge. Environ. Sci. Technol., 4 (7), 569-575.

Pitter, P. and Chudoba, J. (1989). Biological Degradation of Organic Substances in the Aquatic Environment. Chapter 2, CRC Press, U.S.A., (In press).

Rao, B.S. and Gaudy, A.F. Jr. (1966). Effect of sludge concentration on various aspects of biological activity in activated sludge. J. Water Pollut. Control. Fed., 38 (5), 794-812.

Reuss, M., Piehl, H. and Wagner, F. (1975). Application of mass spectrometry to the measurement of dissolved gases and volatile substances in fermentation. European J. Appl. Microbiol., 1, 323-325.

Rohlich, G.A. and Boyle, W.C. (1964). Formal discussion of : Anaerobiosis in the activated-sludge process. Presented at the 2nd IAWPRC Conference, Tokyo, 55-57.

Sawyer, C.N. (1964). Floor discussion of : Anaerobiosis in the activated-sludge process. Presented at the 2nd IAWPRC Conference, Tokyo, 60.

Speece, R.E., Engelbrecht, R.S. and Aukamp, D.R. (1973). Cell replication and biomass in the activated sludge process. Water Research, 7, 361-374.

Wanner, J., Kucman, K., Ottova, V. and Grau, P. (1987a). Effect of anaerobic conditions on activated sludge filamentous bulking in laboratory systems. Water Research, 21 (12), 1541-1546.

Wanner, J., Ottova, V. and Grau, P. (1987b). Effect of an anaerobic zone on settleability of activated sludge. In : Biological phosphate removal from wastewaters, R. Ramadori (Ed.). Pergamon Press, 155-164.

Wanner, J. and Grau, P. (1988). Filamentous bulking in nutrient removal activated sludge systems. Wat.Sci. Tech., 20 (4/5), 1-8.

Weddle, C.L. and Jenkins, D. (1971). The viability and activity of activated sludge. Water Research, 5 (8), 621-640.

Westgarth, W.C., Sulzer, F.T. and Okun, D.A. (1964). Anaerobiosis in the activated-sludge process. Presented at the 2nd IAWPRC Conference, Tokyo, 43-55.

Wiggins, B.A., Jones, S.A. and Alexander, M. (1987). Explanations for the Acclimation Period Preceding the Mineralization of Organic Chemicals in Aquatic Environments. Appl. Environ. Microbiol., 53 (4), 791-796.

Wuhrmann, K. (1960). Effect of oxygen tension on biochemical reactions in sewage purification plants. Manhattan College Conf. on Biological Waste Treatment. Paper n° 3, pp 10.