SIMULTANEOUS SULFIDE AND ACETATE OXIDATION IN A DENITRIFYING FLUIDIZED BED REACTOR—I START-UP AND REACTOR PERFORMANCE

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Abstract—Part of the post-treatment of an anaerobic wastewater treatment plant described in a recent patent (EPA 0051.888) is a denitrifying fluidized bed reactor in which sulfide and lower fatty acids are removed. In this reactor the sand particles covered with biomass were kept in a fluidized state by the upflowing wastewater. A more detailed insight into the performance and resilience of the reactor under different operating conditions was required to assess the applicability of this type of reactor for a variety of wastewaters. To this goal a laboratory model of this denitrifying, fluidized bed reactor was built. The main objective was to show that sulfide and acetate could be removed from a synthetic wastewater using nitrate as the electron acceptor, and that the laboratory reactor could be used for further study of the mechanisms and performance of the pilot plant reactor. As an initial step, the start-up and overall performance were studied. Sulfide ($2-3 \text{ kg S m}^{-3} \text{ d}^{-1}$), acetate ($4-6 \text{ kg C m}^{-3} \text{ d}^{-1}$) and nitrate ($5 \text{ kg N m}^{-3} \text{ d}^{-1}$) were effectively removed. Only after the removal of oxygen from the influent with a pre-treatment column did the occurrence of elemental sulfur as an intermediate of the sulfide oxidation no longer lead to the build up of high sulfur concentrations in the reactor (only 0.3% of the sulfide added was converted to sulfur as long as nitrate was supplied in excess). Bacterial colonization of the sand particles and biomass growth was rapid in the first 4 days despite the high flow (dilution) rates applied. Biomass production was calculated from redox/electron balances in addition to being measured. From the redox balances, it was concluded that biomass production increased with increasing loading rates. Plotting data from the individual days suggested that an average loading rate of $20-40 \text{ mequiv electrons l}^{-1} \text{ reactor h}^{-1}$ would result in roughly no net growth. The redox balances show that net CO_2 fixation may take place when sulfide plus sulfur turnover rates are high relative to the acetate turnover rate. The ratios of sulfide plus sulfur to acetate turnover rates above which CO_2 fixation started agreed well with data from chemostat studies of the facultative autotroph, *Thiobacillus versutus*. Substrate removal rates and intermediates found in the laboratory system were representative for the behaviour of the pilot plant reactor as described in the above mentioned patent. The results indicate that further studies on the resilience of the reactor to changes in the influent composition and in the operating conditions should be performed. The system is also sufficiently well defined and controllable to allow a study on the physiological behaviour and the microbial selection processes that occur in this system.

Key words—denitrification, sulfide-oxidation, fluidized bed, wastewater treatment, mixotrophic growth, elemental sulfur, acetate oxidation, mass balances, redox balances, conversion rates

INTRODUCTION

The biological treatment of large volumes of strongly polluted wastewaters calls for high performance systems such as fluidized bed reactors. For several types of conversion (aerobic removal of organic compounds, methane production, denitrification and nitrification) the merits of this type of reactor have been demonstrated in laboratory, pilot plant and full scale reactors (Gauntlett, 1981; Hawkins *et al.*, 1981). The increasing use of anaerobic, methanogenic treatment during the past decade has resulted in an increasing need for appropriate post-treatment of the effluent. Typical compounds in the effluent of such reactors are lower fatty acids (mostly acetate), ammonium and hydrogen sulfide. The concentrations of the latter two depend on the nitrogen and sulfur content of the wastewater. Both sulfide and ammonium must be removed from the effluent, and the use of fluidized

bed reactors for this post-treatment might be an interesting option. In a recent patent (Mulder, 1982; EPA 0051.888) an integral wastewater treatment system based on a series of fluidized bed reactors has been described. After the anaerobic conversion of most of the carbon content to CO_2 and methane, the effluent passes through denitrification and nitrification steps. In the denitrification reactor, both sulfide and the lower fatty acids act as electron donors and the nitrate from the nitrification reactor serves as the electron acceptor. This makes this system rather different from other reactor configurations which have, until now, employed either heterotrophic (methanol or wastewater BOD) or completely autotrophic (reduced sulphur compounds) removal of nitrate.

Although a pilot plant reactor had been operating successfully for over a year, the microbial processes in the denitrification reactor had not been studied in

Fig. 1. Laboratory set-up for a fluidized bed denitrification reactor; (a) flow meter; (b) heat exchanger; (c) medium reservoir for the pre-treatment column; (d) medium pump; (e) pre-treatment column; (f) settler region; (g) recycling pump; (h) main pump; (i) pulse reduction vessel; (j) medium pump; (k) medium reservoirs for denitrification reactor; (l) measurement station; (m) pH-meter and recorder; (n) denitrification reactor; (o) recycling pump; and (p) nitrogen gas cylinder.

detail. A more detailed insight into the performance and resilience of the reactor under different operating conditions was required in order to assess the applicability of this type of reactor to a variety of wastewaters. To this goal, not only a black box study of the overall behaviour of the reactor is required, but also an understanding of the most important processes that govern the microbial selection and physiology in fluidized bed reactors. Therefore, it was decided to construct a laboratory-scale fluidized bed reactor in order to be able to work with a system well defined in terms of operating conditions and wastewater composition. A mathematical and technical description of this work has been published elsewhere (Gommers *et al.*, 1986). This paper describes the overall performance of the laboratory denitrification reactor with special emphasis on data concerning surplus sludge production, conversion rates, redox balances and production of intermediates. Future work will concentrate on the influence of these parameters on the microbial composition and physiological behaviour of the biomass.

MATERIALS AND METHODS

Experimental set up of the fluidized bed reactor

Figure 1 shows a schematic representation of the laboratory fluidized bed reactor used in this study. The plexiglass reactor (dia 0.04 m, height 3 m) was fed with tap water heated to 35°C. A concentrated (1000–2000 times) medium was added to the tap water and passed through a pre-treatment column (dia 0.04 m, height 1 m). This pre-treatment column served for the removal of oxygen from the tap water (usually 200 µM), to provide nearly anaerobic

conditions. Oxygen concentrations entering the denitrification reactor were typically 5–10 µM with an occasional maximum of 40 µM. Just before entering the reactor, sulfide was mixed with the complete medium. The final concentration of the medium entering the denitrification reactor was as follows (mmol l⁻¹): Na-acetate, 0.3; Na₂S, 0.3; NaNO₃, 1.5; NH₄ Cl, 0.9; KH₂PO₄, 0.1; MgSO₄, 0.08.

Yeast extract was added to a concentration of 1.5 mg l⁻¹ and a trace element solution (Vishniac and Santer, 1957) was diluted in a 1:30,000 dilution ratio. Both the pre-treatment column and the denitrification reactor were equipped with a recycling pump (recycle ratio 1:1.5). An inlet for N₂-gas sparging was built in the denitrification reactor, but was not used in this study. The column was provided with sample ports (dia 1.5 cm) at 10 cm intervals. Liquid samples were taken through a syringe and needle, while the biomass samples were taken using a wide ended syringe. Influent and effluent samples were taken through septa installed at the entrance and exit of the reactor.

Start-up

The reactor was filled with 860 g sand (dia 0.5 mm) and inoculated with 1 l. of sludge from a small canal. Directly after inoculation the liquid flow was adjusted to approx. 40 l h⁻¹, corresponding with a residence time of 6 min within the reactor. After 2 days, biomass started accumulating on the sand and substrate removal commenced.

Element balance and profile measurements

Samples from several heights in the reactor were taken to construct profiles. Part of every sample was used immediately for the determination of the sulfide concentration. The rest of the sample was used for the HPLC determination of the other compounds (see below). To avoid interference by the sulfide the sample was first mixed with 2.0 mg Zn-salicylate in a centrifuge tube. The precipitated ZnS was centrifuged off and the supernatant was stored at -18°C for later determination. The salicylate salt was used to avoid the appearance of an extra peak in the HPLC-chromatogram.

Chemical analyses

Sulfite and sulfide were measured as described by Trüper and Schlegel (1964). Elemental sulfur was measured after extraction with acetone as described by Bartlett and Skoog (1954). Thiosulfate was determined as described by Sörbo (1957) and with HPLC ion chromatography. The cyanolytic method used for the determination of thiosulfate also detects polysulfides and negative thiosulfate results were therefore interpreted as also negative for polysulfides. The HPLC-system consisted of an IONOSPHER-A column (250×4.6 mm, Chrompack) and refraction index detection (Waters 410). The eluting liquid was a 4 g l^{-1} (pH 6.0) sodium salicylate solution. Acetate, nitrate, nitrite, sulfate and the thiosulfate could be detected as separate peaks. Oxygen was measured with the Winkler method (Rand *et al.*, 1975).

Biomass determination

The biomass volume in the reactor was determined by measuring the settled bed height after stopping the liquid flow. The settled bed volume of the biomass samples taken from the reactor was also measured prior to the dry weight measurement. Dry weight was determined by drying of the sample at 65°C for 24 h. The quantity of sand in the material was measured after ashing at 700°C for 1 h. The dry weight minus the ashed weight is hereafter termed volatile solids (VS). This was not corrected for elemental sulfur as its content was usually below 5% of the VS. The VS concentration in the reactor was calculated by multiplying the ratio of the settled bed volumes of the reactor and the sample by the VS in the sample.

RESULTS AND DISCUSSION

Start-up/general operation

The laboratory fluidized bed reactor was fed with artificial wastewater containing 1.5 mM nitrate as the electron acceptor. This was in excess of the requirement for the oxidation of the two electron donors sulfide (0.3 mM) and acetate (0.3 mM). The hydraulic residence time in the reactor was approx. 10 min. This is far below the time required for suspended growth of any known acetate or sulfide oxidizing bacterium, which have doubling times of at least 50 min. In this way a strong selective force is exerted in favour of attached growth on the fluidized sand particles (Heijnen, 1984). Indeed, within 4 days after inoculation, the sand particles in the reactor were covered with enough biomass to completely remove acetate and sulfide from the wastewater. This shows that in spite of a very high flow rate, an efficient colonization of the particles had taken place.

During the initial runs, before the installment of the pre-treatment column, substantial formation of elemental sulfur took place. The sulfur accumulated on the surface of the particles and thus reduced the activity of the biofilm. A second problem caused by the presence of sulfur was its spontaneous reaction with sulfide to produce polysulfides, as judged by the yellow-green colour of the effluent. This also reduced the activity of the biofilm. In subsequent experiments, sulfur formation was avoided by the biological removal of oxygen from the influent prior to the addition of the sulfide (see Materials and Methods).

Fig. 2. Settled bed height of the sand particles with attached biomass in the denitrification reactor during the first 25 days.

Biomass growth

Although biomass was visible on the sand particles after 2 days the settled bed height did not increase until after the fifth day (Fig. 2). After that, the bed expanded steadily. The fluidized bed height was automatically kept constant at 3.0 m by the overflow device (Fig. 1). Biomass was removed from the settler compartment twice a week. The removed biomass, which represents the net growth of the system, corresponded with a biomass production of 2 g VS day^{-1} (about $1 \text{ g carbon day}^{-1}$ being equivalent to an average specific growth rate of 0.03 day^{-1} , given a content of 60 g VS in the reactor). The acetate load was $7 \text{ g carbon day}^{-1}$ so that 15% of the acetate was assimilated. The percentage of acetate converted to biomass reported for pure culture studies (and enrichments) was significantly higher: 30–40% (Stouthamer *et al.*, 1980). However, the growth yield of *Thiosphaera pantotropha*, an organism isolated from the pilot plant version of this reactor (Robertson and Kuenen, 1983a), in the chemostat also assimilates approx. 15% of the acetate during denitrification (L. A. Robertson, personal communication).

The particle size and the gram volatile solids (VS) per g sand increased continuously during the first 30 days. To avoid blockage problems caused by oversized or flocculent particles, an equivalent amount of sand was added to the reactor each time the excess biomass was removed. This procedure stabilized the particle size at $2\text{--}3 \text{ mm}$ with a terminal settling velocity of $3.7 \pm 0.6 \text{ cm s}^{-1}$. The biomass to sand ratio stabilized at about $100 \text{ mg VS g}^{-1} \text{ sand}$ (Fig. 3) suggesting a loosely packed biolayer ($\approx 1\%$ VS). Observed fluctuations were due to the batch-wise addition of the cleaned sand. In this system the presence of clean sand at the bottom of the reactor proved to be a preventive and curative method of controlling both particle size and flocculent growth.

Sulfur balance

The end product of the oxidation of sulfide was sulfate. However, sulfide and sulfate measurements showed that an intermediate was formed (Fig. 4). No elemental sulfur, sulfite, polysulfides, thiosulfate or

Fig. 3. Biomass development in the denitrification reactor as mg volatile solids (VS) g^{-1} sand.

any higher thionates were detected in the effluent and it was concluded that elemental sulfur accumulated within the reactor in association with the biomass particles. Chemical analysis and microscopic examination revealed elemental sulfur concentrations up to $0.026 \text{ mg S mg VS}^{-1}$ within the particles. Sulfur balances made over a period time (Fig. 4), however, revealed that both periods of elemental sulfur accumulation and consumption occurred. Although no explanation as yet exists for this phenomenon, the net effect of this unstable behaviour was that only a very small percentage of the sulfide added to the reactor was converted to elemental sulfur (0.3%). The sulfur accumulated within the reactor did not hamper its performance.

Because of the periods of sulfur consumption and production, it was concluded that sulfur is an inter-

mediate of the sulfide oxidation. This was confirmed by subsequent activity measurements on the biomass taken from the reactor (published separately, see following paper). It may well be that the organisms responsible for sulfide oxidation are also responsible for the further oxidation of the elemental sulfur to sulfate, or two different groups may be responsible for these two steps. Elemental sulfur also occurred as an intermediate in experiments with pure cultures of *Thiobacilli* grown on sulfide (Beudeker *et al.*, 1982). In studies using autotrophic sulfide oxidation for nitrate removal, sulfur has also been reported to be an intermediate (Kobayashi *et al.*, 1983; Bisogni and Driscoll, 1977; Driscoll and Bisogni, 1978). Sulfur formation was only negligible under strict sulfide limitation (Beudeker *et al.*, 1982). The fluidized bed reactor, although being fed with a sulfide limited medium, is best described as a plug flow reactor with a limited amount of back-mixing (Gommers *et al.*, 1986). At the bottom of the reactor, therefore, the biomass will not be sulfide limited. The sulfide loading of the reactor was $2\text{--}3 \text{ kg S m}^{-3} \text{ d}^{-1}$ and experiments involving higher loadings are now being performed. This loading is very close to that claimed for the pilot plant reactor ($3.15 \text{ kg S m}^{-3} \text{ d}^{-1}$) as reported in the patent EPA 0051.888 and in Mulder *et al.* (1986). Other denitrifying reactors using reduced sulfur compounds were based upon strictly autotrophic conversions. Reported loadings ranged from 0.1 to $4.0 \text{ kg S m}^{-3} \text{ d}^{-1}$ (Gaid *et al.*, 1980; Cloirec *et al.*, 1985; Driscoll and Bisogni, 1978; Batchelor and Lawrence, 1978). These reactors were usually operated under electron acceptor-limited conditions to

Fig. 4. Mass balance of sulfur compounds (subscript I = influent, E = effluent concentration). Sulfite, elemental sulfur, thiosulfates and other thionates were not present in either the influent or the effluent in detectable concentrations. Only changes in concentrations are shown. For sulfide the concentration decrease was equal to the influent concentration (except for the first 2 days) as no sulfide was present in the effluent. The sulfate concentration in the influent was typically $0.8\text{--}1.0 \text{ mM}$. The areas between the two lines indicate elemental sulfur accumulation (sulfide decrease $>$ sulfate increase) or elemental sulfur consumption (sulfide decrease $<$ sulfate increase) in the denitrification reactor.

Fig. 5. Mass balance of nitrogen compounds (subscripts as in Fig. 4). Only changes in concentrations are shown. Nitrate was always present in the effluent, typically 0.2–0.5 mM. Nitrite was not present in the influent. The area between the lines indicates the amount of nitrate converted to nitrogen gas.

ensure complete removal of the nitrogen compounds and were not focused on the complete conversion of the reduced sulfur compounds to sulfate. Due to the accumulation of elemental sulfur in these reactors, comparison of their values with the data reported here is not possible.

Nitrogen balances

Denitrification in the reactor followed the classic pattern via nitrite to nitrogen gas (Fig. 5). The reactor was operated under electron donor limited conditions and therefore nitrate and nitrite were always present in the effluent.

The amount of nitrogen gas produced from the low concentration of nitrate (1–2 mM) used in this study was low enough to remain in solution, and therefore little or no gas production was visible within the reactor. This may be of importance in scaling up to larger systems using more concentrated influents. The effect of gas produced within a fluidized bed reactor appeared to have a limited effect on the mixing in the reactor (Schoutens *et al.*, 1986). However, externally supplied gas was shown to have a more profound effect on both the mixing of the liquid and the solid phases (Gommers *et al.*, 1986).

Nitrogen conversion in the reactor was $5 \text{ kg N m}^{-3} \text{ d}^{-1}$. This is substantially higher than the $2.5 \text{ kg N m}^{-3} \text{ d}^{-1}$ mentioned in the patent (EPA 0051.888) for the comparable pilot plant reactor. Heterotrophic denitrification rates in fluidized bed reactors range from 1 to $60 \text{ kg N m}^{-3} \text{ d}^{-1}$ (Vossoughi *et al.*, 1982; Gauntlett, 1981). The higher values were found only in very heavily (highly concentrated) loaded systems with nitrate concentrations in the effluent as high as 30 mmol l^{-1} (Walker *et al.*, 1981). Autotrophic systems can convert 0.1 to

$3.0 \text{ kg N m}^{-3} \text{ d}^{-1}$ (Driscoll and Bisogni, 1978; Gaid *et al.*, 1980; Bisogni and Driscoll, 1977; Batchelor and Lawrence, 1978) with low nitrate influent concentrations ($< 100 \text{ mg N l}^{-1}$) and 0.1 to $5.6 \text{ kg N m}^{-3} \text{ d}^{-1}$ (Steinmuller and Kutzner, 1981; Claus and Kutzner, 1985) with higher nitrate concentrations ($> 400 \text{ mg N l}^{-1}$).

Redox/electron balances

In the reactor described in this paper, several potential electron donors and electron acceptors were present. Table 1 shows their value expressed as moles of electrons potentially accepted or donated per mole of compound. If the electron donor, sulfide, is only partially oxidized and sulfur accumulates, the amount of sulfur could be regarded as a non-utilized electron donor in the effluent, even though it is not actually present in the effluent of the reactor. When sulfur is consumed, however, this amount should be regarded as an electron donor in the influent. The complete donor and acceptor concentrations in both influent and effluent are shown in Fig. 6. The sulfur accumulation and consumption, together with the nitrite production can also be seen in this figure. The complete removal of acetate is also clear. Inter-

Table 1. Stoichiometric values for the number of electrons potentially accepted or donated per mole by the compounds present in the denitrification reactor

Compound	Acceptor/donor	No. electrons molecule ⁻¹
NO_3^-	Acceptor	5
NO_2^-	Acceptor	3
S^0	Acceptor	2
CO_2	Acceptor	4
S^{2-}	Donor	8
Ac^-	Donor	8
S^0	Donor	6

Fig. 6. Redox (or electron) balance of the denitrification reactor showing the potential electron donor and electron acceptor concentration in the influent and effluent. The number of electrons per mole of compound are shown in Table 1. Sulfate and nitrogen gas were considered as end products with no potential electron donor or acceptor value. The value of elemental sulfur in the reactor is explained in the results section.

pretation of these electron balances is based upon the fact that any electrons donated by one of the electron donors should correspond with the disappearance of the equivalent amount of electron acceptor. If not, these electrons must have accumulated in the reactor (organic carbon levels in the effluent were below 1 ppm). Exploratory experiments on the effluent of the pilot plant reactor showed that under normal operating conditions N_2O -levels were not detectable. Therefore we assumed that N_2O -levels in the laboratory systems were negligible and thus N_2O was not measured routinely. However, it should be noted that sulfide can inhibit N_2O -reductase (Robertson and Kuenen, 1983b). If sulfide concentrations in the reactor increase substantially N_2O may be present in the effluent. As we have already taken the accumulated sulfur into account in the balance, these accumulated electrons are a direct measure of the incorporation of carbon compounds. Acetate and possibly CO_2 may be incorporated into products as biomass and other organic compounds (for example, polymers needed for adhesion or storage products). As yet, no distinction between these two types of carbon incorporation can be made. Considering them both as electrons fixed in VS does give an independent check of the measured biomass production. The amount of electron equivalents accumulated per day, recalculated as VS (assumed to be CH_2O ; 8 electrons $\rightarrow$ 48 mg VS) represent a biomass production rate of approx. 4 g VS d^{-1} for the first 25 days and 2 g VS d^{-1} if the first 40 days are considered. The fact that the values for the carbon incorporation and the actual biomass produced (2 g VS d^{-1}) are in agreement, shows that redox/electron balances can be a useful tool for the description of this type of system.

The amount of electrons fixed in the reactor increased with the electron donor load of the reactor (Fig. 7). At low loadings, the electron-content of the reactor decreased. This can be explained if the rates of electron releasing processes (e.g. biomass decay, conversion of storage products) within the reactor were higher than the rates of the electron storing processes (e.g. growth, storage product formation). At high reactor loadings, the difference in the respective rates of the two types of processes resulted

Fig. 7. Net electron accumulation within the denitrification reactor (calculated from the redox balances) as a function of the electron donor load of the reactor (sum of electrons from sulfide, acetate and elemental sulfur). During net sulfur consumption periods, sulfur was considered as an additional electron donor. Positive values indicate a net accumulation of electrons within the reactor (not accountable as sulfur) while negative values correspond with a net decrease of the amount of electrons present in the reactor.

in the net increase of the electron-content of the reactor. At a loading of 20–40 mequiv $l^{-1} h^{-1}$, the electron content of the reactor would be more or less stable which is in good agreement with the no-growth loading found by Heijnen (1984) for an aerobic fluidized bed reactor.

Although this implies that the average growth is zero this does not correspond with a no-growth state of biomass at all positions in the column and or at all positions in the biolayers. An unknown amount of CO_2 might be fixed within the biomass and act as an electron sink. That this is indeed microbiologically feasible can be seen if one considers the type of organisms potentially present in this reactor. These organisms will either be obligate or facultative autotrophs. In a study of the microbial composition of the pilot plant reactor, which was fed with an influent comparable to the reactor under study, no obligate autotrophs were found and only facultative autotrophs and heterotrophs were isolated (Robertson and Kuenen, 1983a). Aerobic, pure culture studies with *Thiobacillus versutus*, a facultative autotroph, showed that under mixotrophic growth conditions (simultaneous consumption of an inorganic and an organic electron donor) CO_2 fixation only occurred if the ratio between the inorganic and the organic compound exceeded 1.5 (electron/electron) (Gottschal and Kuenen, 1980). To check our data with this finding, the amount of electrons fixed for the individual days were calculated. When sulfur was consumed in the reactor this was added to the amount of sulfide used, to give the total amount of inorganic substrate used. The days with no net electron storage (see also Fig. 7) had to be omitted because the use of (probably organic) electron donors within the reactor on these days could not be correctly interpreted. The remaining days were sorted into two groups, one with days when the inorganic to organic electron consumption was < 1.5 and the other when the ratio was higher. The differences in this ratio were not caused by fluctuations in the influent composition, but by the dynamic behaviour in respect to the sulfur accumulation or consumption in the reactor. Each day was then examined for the amount of electrons fixed as calculated from the redox balances (Fig. 6). The amount of electrons assimilated in the reactor was converted to acetate fixed and compared with the amount of acetate actually added on that day. Three metabolic categories were distinguished:

- (1) no net fixation of acetate occurred;
- (2) between 0–79% of the acetate supplied was incorporated; and
- (3) $> 79\%$ of the acetate supplied was assimilated in the reactor. The last category was considered to be the one in which CO_2 fixation must occur. The division at 79% was taken from Gommers *et al.* (1988a) who showed that even if all of the acetate supplied to a culture is converted to biomass, some CO_2 must still be produced because of the limitations

Fig. 8. Effect of the ratio of inorganic (sulfide plus sulfur) to organic (acetate) compounds consumed on the amount of electrons fixed in the reactor. A detailed explanation is presented in the results section.

(I) No net fixation of electrons (and thus acetate) occurred.

(II) The amount of electrons fixed corresponds with 0–79% of the acetate consumed (=supplied).

(III) The amount of electrons fixed corresponds with $> 79\%$ of the acetate supplied and implies the occurrence of CO_2 fixation.

The individual redox balances of the days with a loading rate higher than 35 mequiv electrons $l^{-1} h^{-1}$ were selected. First for their "fixation category" [(I)–(III) as mentioned above] and second for their inorganic to organic substrate ratio.

of the biochemical pathways involved. As can be seen in Fig. 8, the behaviour of the microbial population differed radically if the ratio of inorganic to organic electrons was changed from $<$ to > 1.5 . Only the days with higher ratios showed electron fixation values that would imply CO_2 fixation. The fact that no net CO_2 fixation occurred on some days does not mean that CO_2 fixation did not occur at any place in the reactor, but that the CO_2 production (from acetate) was larger than the CO_2 fixation on that particular day.

FINAL COMMENTS

The denitrifying, fluidized bed reactor in which both sulfide and acetate are oxidized simultaneously was built as a laboratory model for a pilot plant reactor (EPA 0051.888). The main objective was to show that sulfide, acetate and nitrate could be removed from a synthetic wastewater and that the laboratory reactor could be used for further studies of the mechanisms and performance of the pilot plant reactor.

The present experiments have shown an effective removal of sulfide, acetate and nitrate at rates at least similar to those of the pilot plant. The intermittent accumulation of small amounts of sulfur in the reactor did not interfere with the proper functioning of the system. The biomass production was relatively low and closely related to the reactor loading. As the reactor was operated under electron donor-limited conditions, nitrate and nitrite were always present in the effluent. In future experiments, a smaller excess of nitrate in the influent should reduce or avoid this problem.

The microbiological behaviour of the biomass bears a clear resemblance to that of the facultatively chemolithotrophic *Thiobacillus versutus* grown in aerobic chemostats (Gottschal and Kuenen, 1980). This implies that the known physiological adaptation of this type of organisms to varying substrate regimes may dramatically alter the performance of the reactor. Therefore, it will be necessary to carry out further studies on the resilience of the reactor to changes in the effluent composition and in the operation conditions. Indeed the laboratory reactor system appears to be sufficiently well defined and controllable to allow a study on the physiological behaviour and the microbial selection processes that occur in this system. A further description of the activity of the biomass in the reactor is published in Part II (Gommers *et al.*, 1988b).

CONCLUSIONS

(1) The constructed laboratory scale fluidized bed reactor performed similar to the pilot plant reactor and showed an efficient conversion of sulfide, acetate and nitrate.

(2) During electron donor limitation nitrite accumulated. In spite of the donor limitation an intermittent formation of small quantities of sulfur (0.3% of total sulfide converted) took place, however without effecting the good performance of the reactor.

(3) Estimates of biomass production based on redox and electron balances agreed well with direct measurements of biomass yields.

(4) In line with previous predictions, the microbiological behaviour of the reactor was similar to that of facultatively chemolithotrophic sulfide oxidizing bacteria.

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