The performance and mixing characteristics of an anaerobic hybrid reactor treating a synthetic fatty acid containing substrate*

WA Joubert and TJ Britz**
Department of Microbiology, University of the Orange Free State, P.O. Box 339, Bloemfontein 9300, South Africa.

Abstract

An anaerobic hybrid reactor combining an upflow sludge blanket and fixed film reactor was designed and characterised. A strict anaerobic non-methanogenic bacterial count of 10^9 viable bacteria/mℓ was obtained in the digester fluid. The aerobic and facultative anaerobic bacteria in the fluid gave consistent counts, of 10^5 and 10^6 viable bacteria/mℓ, over a 45 d period. Flow within the reactor was mainly of the plug flow type with a large degree of longitudinal dispersion. Gas production did not alter the mode of flow, but decreased the dead reactor space significantly. Effluent recirculation markedly affected the COD removal efficiency and mixing characteristics of the reactor. The highest efficiency was obtained with a recirculation ratio of 6,4 : 1. The reactor attained a 90,0% COD reduction of the synthetic fatty acid substrate (pH 5,0) loaded at a rate of 15,0 kg COD/m^3.d and at a temperature of 37°C. Recirculation improved the mixing characteristics of the reactor and changed the flow to that of the completely mixed type.

Introduction

Anaerobic micro-organisms can only degrade organic compounds in waste waters to biogas, if the substrate is brought into close contact with the organisms and sufficient time is allowed for the reaction to occur. The rate at which the bioconversion takes place is primarily dependent upon the bacterial concentrations in the reactor. Although many reactor configurations are presently known to fulfill the requirements for anaerobic catabolisms, most of them exhibit some sort of shortcoming (Van den Berg, 1984).

The anaerobic fixed film reactor has been shown to be superior, both with respect to biomass retention and the overall process stability (Murray and Van den Berg, 1981; Van den Berg and Kennedy, 1981). One shortcoming of the fixed film reactor, however, is the long periods necessary for the formation of high bacterial concentrations on the inert fixed supports. The upflow sludge blanket reactor (USBR) has, however, been shown to exhibit less dead reactor space (Buijs *et al.*, 1982) and is able to operate effectively at low hydraulic residence times (Christensen *et al.*, 1984). The disadvantage of this reactor type is the long periods necessary for start-up and the formation of granular settleable sludge. These reactors invariably exhibit some degree of backmixing and dead reactor space which affect reactor performance and available working volume. Excessive backmixing in an experimental reactor makes the interpretation of results on product and by-product yields uncertain. Thus, in order to increase the basic knowledge of the performance characteristics of this reactor type, a measure of the extent of backmixing, characterised by the longitudinal dispersion number ($D/\mu L$), is necessary (Levenspiel and Bischoff, 1959).

The objective of this study was thus to design and characterise the performance of an anaerobic hybrid reactor combining an USBR and a fixed film reactor. Tracer studies were conducted to determine the effects of gas production and effluent recirculation on dead reactor space and flow patterns within the reactor.

Materials and methods

Reactor

A laboratory-scale anaerobic hybrid reactor (working volume = 1,9 ℓ; inside diameter = 80 mm; height = 440 mm), combining an upflow sludge blanket and fixed film, was used in this study (Figure 1). A porous polyethylene foam (density = 77,0 kg/m^3), fitted to the inside reactor wall, was used as fixed film support. The reactor was originally inoculated using a mixture of active laboratory digester sludge and municipal sewage sludge and allowed to acclimatise for 260 d on a synthetic substrate. An operating temperature of 37°C was maintained using a heating tape (Meyer *et al.*, 1983). Gas production was measured by displacement of a brine solution and volumes corrected for standard temperature and pressure (STP). Gas composition and volatile fatty acids (VFA) were determined gas chromatographically (Britz *et al.*, 1983) and chemical oxygen demand (COD) was analysed in accordance with Standard Methods (American Public Health Association, 1976). Calculations of the total COD removal efficiency of the reactor were based on the soluble and insoluble components in the effluent.

Substrate

The synthetic substrate (COD = 9 850 mg/ℓ) consisted of (mg/ℓ): acetate 4 500; propionate 2 900; butyrate 1 400; NH_4Cl 500; KH_2PO_4 500; water 1,0 ℓ and 0,1 mℓ/ℓ trace metal solution (Nel *et al.*, 1985). The substrate pH was adjusted to pH 5,0 using a solution consisting of 3N $Ca(OH)_2$, 6N KOH and 6N NaOH. The substrate was boiled for 5 min and allowed to cool to room temperature before use. Gas production was precluded for certain tracer experiments, by using the synthetic substrate devoid of all carbon sources.

Bacterial counts

Aerobic, facultative anaerobic and strict anaerobic non-methanogenic bacteria were counted in the digester fluid and effluent using glucose or fatty acid (FA) containing media. The digester was sampled using sterile disposable syringes. The glucose medium (g/ℓ) consisted of: yeast extract 4; glucose 10;

---

*Part of this article was presented as a poster at the Anaerobic Digestion Symposium, Bloemfontein, 1986.

**To whom all correspondence should be addressed.

Received 12 August 1986.

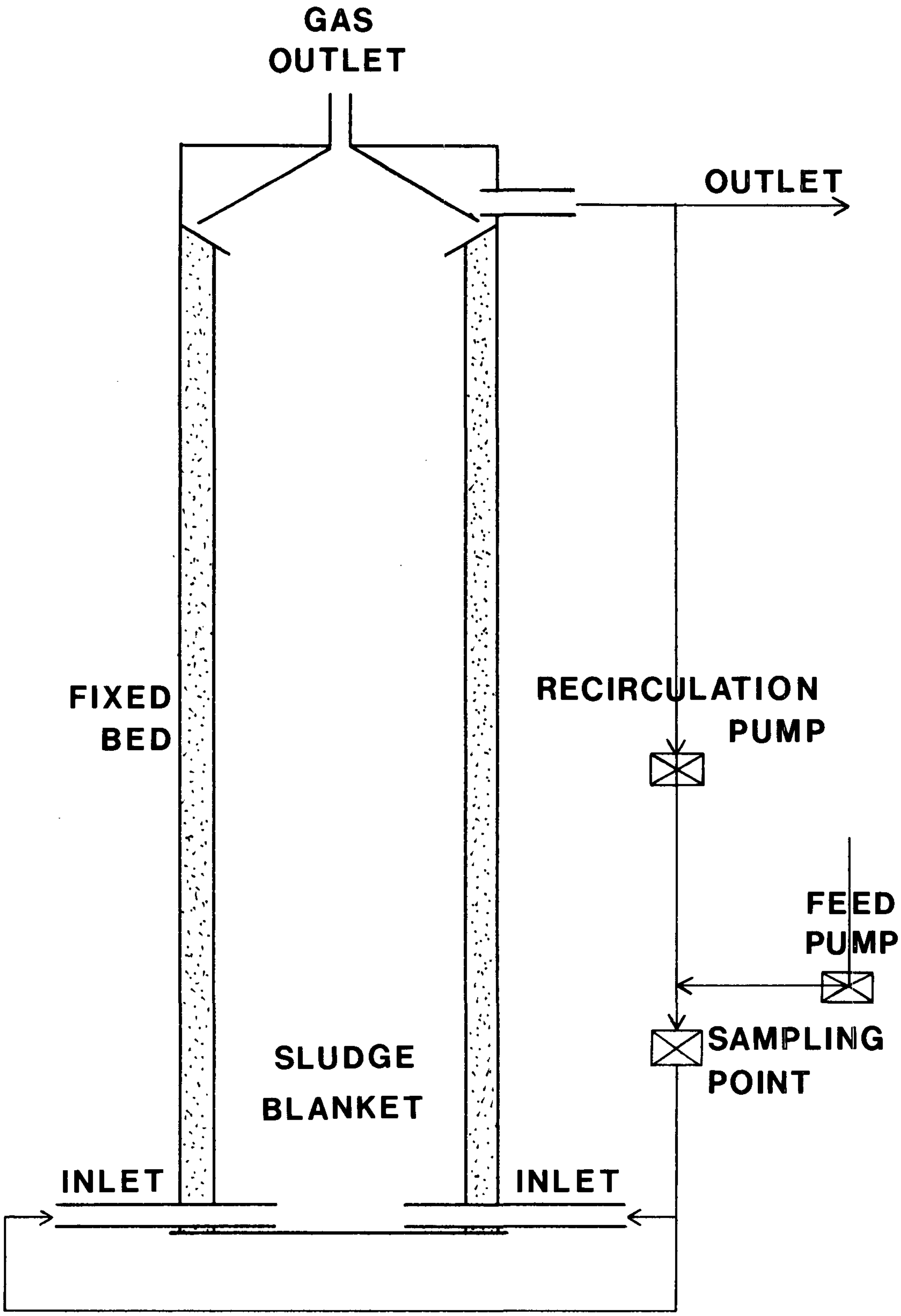

Figure 1
Anaerobic hybrid reactor.

KH_2PO_4 1,6; K_2HPO_4 3,2; NH_4Cl 0,5; $CaCl_2.2H_2O$ 0,2; $MgCl_2.2H_2O$ 0,2 and water 1,0 ℓ. The fatty acid medium consisted of (g/ℓ): yeast extract 4; acetate 4,5; propionate 2,9; butyrate 1,4; NH_4Cl 0,5; KH_2PO_4 0,5; water 1,0 ℓ and 0,1 mℓ/ℓ trace metal solution (Net *et al.*, 1985). The pH of the media, after sterilisation, was 7,0.

Aerobic bacteria were counted after incubating the pour plates at 37°C for 48 h. Facultative anaerobic bacteria were counted after incubating the plates in a Gaspak jar for 48 h at 37°C. Anaerobic counts were performed on both media to which cysteine hydrochloride (0,5 g/ℓ) had been added. Anaerobic media were prepared under a stream of oxygen-free nitrogen using a modified (Miller and Wolin, 1974) Hungate technique (Hungate, 1969). Agar (2,0%) was dispensed into the vials prior to the addition of the media. Vials were sealed with butyl rubber stoppers and aluminium crimps before incubation in an anaerobic cabinet for 48 h at 37°C.

Mixing studies

Tracer studies, with lithium chloride (15 mg/ℓ) as tracing medium, were commenced after the continuous operation of the reactor for a period of 280 d. The tracer was imposed as a step input, by continuously introducing the tracing solution to the incoming substrate, at time t = 0 (Danckwerts, 1953). Samples (10 mℓ) were taken over a period of 2,0 arithmetic mean (theoretical) residence times (2,0 τ) at intervals of 0,1 τ. All samples were filtered through a 0,22 μm pore filter prior to analysis in a Perkin Elmer 603 atomic absorption spectrophotometer.

The output signal of the step function input, the F-curve, was presented in dimensionless form by relating C/Co to the corresponding time (v.t/V). The F-curve was reduced to the corresponding C-curve by taking slopes of the F-curve (Levenspiel and Smith, 1957; Levenspiel, 1958). The vessel dispersion number, D/μL, was calculated using the dispersion model (Levenspiel, 1972) for reactors with plug flow characteristics with large degrees of dispersion. Mixed flow patterns with dead reactor space were analysed using the multiparameter model (Levenspiel, 1972). The dispersion number was calculated using the following equations:

$$\sigma_\Theta^2 = \sigma^2/t^2 \quad \ldots\ldots \quad (1)$$
$$= 2(D/\mu L) - 2(D/\mu L)^2(1 - e^{-\mu L/D}) \quad \ldots\ldots \quad (2)$$

where

$$\sigma^2 = (\Sigma t_i^2.C_i.\Delta t_i)(\Sigma C_i.\Delta t_i)^{-1} - \bar{t}^2 \quad \ldots\ldots \quad (3)$$

and

$$\bar{t} = (\Sigma t_i.C_i.\Delta t_i)(\Sigma C_i.\Delta t_i)^{-1} \quad \ldots\ldots \quad (4)$$

Dead space in mixed flow regions was calculated using the equation:

$$C/C_o = X.e^{-X.\Theta} \quad \ldots\ldots \quad (5)$$
$$\text{where } X = V/Vm \quad \ldots\ldots \quad (6)$$

Results and discussion

A startup period of 300 d was allowed before the first mixing experiments were started. The results of a 2^2 factorial experiment conducted during this time, indicated that a temperature of 37°C must preferably be used in the reactor to obtain the best performance characteristics (Joubert *et al.*, 1985). A space loading rate of 10,8 kg COD/m^3.d and a temperature of 37°C were thus used to obtain a total COD removal efficiency of 68,1% and a corresponding methane yield of 0,249 m^3/kg COD removed per day. The effect of effluent recirculation on the total COD removal efficiency was determined using a constant loading rate of 10,8 kg COD/m^3.d and different recirculation ratios varying from 2,0 to 10,0. A recirculation ratio of 6,4 yielded the best removal efficiency of 90,2% (Figure 2). A recirculation ratio of 6,4 : 1 was therefore used to determine the best performance characteristics of this reactor type at a COD removal efficiency greater or equal to 90%. The performance characteristics of the reactor are given in Table 1 and the effect of residence times on the total COD removal efficiency is given in Figure 3.

The reactor was in continuous operation for a period of 370 d before the bacterial numbers were assessed. A four-cycled count was performed with two weeks between successive counts. The viable bacterial count was used as a measure of the bacterial concentration in the digester fluid. The mean counts acquired on the different media are given in Table 2 and indicate that the anaerobic bacteria, in the digester fluid, were about 1,6 × 10^3 and 1,1 × 10^3 times higher than the respective aerobic and facultative anaerobic bacteria. The high viable, non-methanogenic, cell numbers obtained in this system are in accordance with those found in other anaerobic digesting environments (Mah and Sussman, 1967).

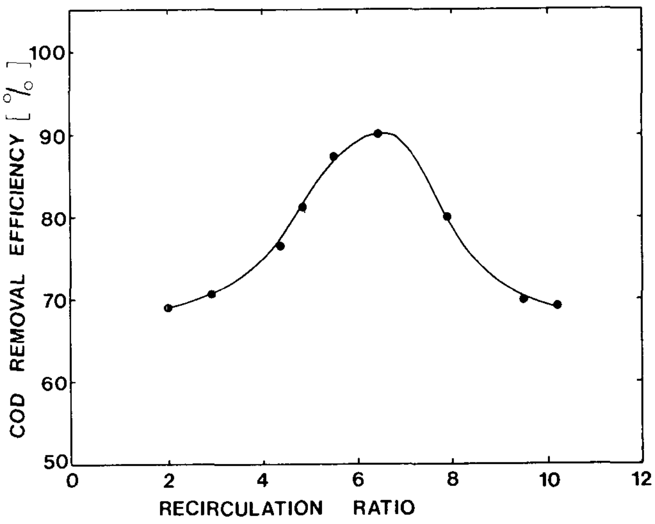

Figure 2
The effect of different recirculation ratios (R) on the total COD removal efficiency.

The data of the mixing studies, given in Table 3, indicate that, if no recirculation is used, the reactor operates in a plug flow mode with a large degree of longitudinal dispersion. Gas production was found to lower the dispersion and dead reactor space, but did not seem to alter the mode of flow inside the reactor. Effluent recirculation in comparison to gas production, was found to dramatically improve the mixing characteristics of the reactor. Dead reactor space was markedly reduced and fluid flow changed to that of the completely mixed type (Table 4). The design of the reactor inlets used in this study is such that it tends to force the incoming substrate away from the reactor walls. If gas production is prevented in the reactor, the incoming substrate chooses the path of least resistance and channels along the axis of the reactor leaving a stagnant fluid layer in the vicinity of the fixed film. At increased loading rates the width of the stagnant fluid annulus was progressively broadening, causing the increase in dead reactor space. The high bacterial concentration in the sludge may also promote channelling of the fluid, resulting in the occurrence of other dead space regions. The dispersion numbers calculated for the reactor without gas production and recirculation (Table 3) decreased at lower residence times. These findings are in contrast with those obtained by Samson *et al.* (1985), but agree with those obtained by Forster *et al.* (1982) using an upflow sludge blanket reactor. This apparent discrepancy can be explained in terms of the different reactor configurations used. In the reactor used in this study, the gas bubbles and substrate are in parallel upward flow, whereas in the downflow fixed film reactor used by Samson *et al.* (1985) the gas and substrate moved countercurrently. This decrease in dispersion number indicates a decline in the spread of the stimulus response (σ^2) at the lower residence times. It might be that, at increased loading rates, conditions were created to promote a flatter liquid velocity profile through the active reactor volume, resulting in the decreased response variance.

Gas production was found to significantly reduce dead space within the reactor. The small rising gas bubbles in the reactor fluid have been found to carry a wake of solid particles behind them (Kunii and Levenspiel, 1968). To counter this upward flow of minute particles, other suspended particles must move downwards in the reactor fluid. This forced circulation caused by the rising gas bubbles will disturb the stagnant regions near the carrier, thereby reducing the dead reactor space. The increased gas production encountered at lower residence times reduced this stagnant annulus region even further. This can be observed by the decrease in dead reactor space given in Table 3. The results given in Tables 3 and 4 indicate that effluent recirculation has a more pronounced effect on dead reactor space than gas production. Over the span of residence times investigated, the average reduction in dead water as a result of gas production was about 15% and, as a result of effluent recirculation (ratio = 6,4 : 1), about 22%. A significant part of the dead space still encountered in the reactor may be attributed to bacterial growth in the sludge bed and fixed film. An increase in dead reactor space, caused by the accumulation of biomass in the lower reactor parts and the formation of thick bacterial layers on fixed films, has also been found by other workers (Samson *et al.*, 1985).

TABLE 1
CHARACTERISTICS OF THE HYBRID REACTOR OPERATED AT A RECIRCULATION RATIO OF 6,4

Mean residence time (h)	15,8
Loading rate (kg COD/m^3.d)	15,0
Total COD removal (%)	90,0
Soluble COD removal (%)*	90,4
Methane content of biogas (%)	67,1
Total gas production (m^3/m^3)	5,1
Methane yield (m^3 CH$_4$/kg COD removed per day	0,254

*COD of supernatant after centrifugation of the sample at 10 000 x g for 15 min.

TABLE 2
VIABLE NON-METHANOGENIC BACTERIAL COUNTS (BACTERIA/mℓ) IN A SINGLE PHASED ANAEROBIC HYBRID REACTOR

		FA medium	Glucose medium
Aerobic	Digester fluid	$6{,}80 \times 10^5$	$15{,}40 \times 10^5$
	Effluent	$3{,}80 \times 10^3$	$6{,}30 \times 10^3$
Facultative anaerobic	Digester fluid	$1{,}31 \times 10^6$	$2{,}27 \times 10^6$
	Effluent	$1{,}18 \times 10^3$	$1{,}19 \times 10^3$
Strict anaerobic	Digester fluid	$1{,}58 \times 10^9$	$2{,}49 \times 10^9$
	Effluent	$3{,}30 \times 10^5$	$3{,}14 \times 10^5$

The results obtained in this study indicate that less dead reactor space will probably be encountered if the substrate is dispersed homogeneously at the inlet of an USBR and if recirculation is used. A degree of dead space will, however, always be found as a result of bacterial growth regardless of the efficiency of substrate dispersion.

TABLE 3
THE EFFECTS OF GAS PRODUCTION ON THE MIXING CHARACTERISTICS OF THE HYBRID ANAEROBIC REACTOR OPERATED WITHOUT RECIRCULATION

	Without gas production				With gas production			
Arithmetic mean residence time (h)^a	**10,0**	**13,0**	**16,0**	**25,0**	**10,0**	**13,0**	**16,0**	**25,0**
Calculated residence time (h)^b	4,1	5,5	7,0	12,2	10,2	9,5	11,1	14,9
Gas production (m^3/m^3)	—	—	—	—	7,9	5,5	4,9	3,6
Dispersion number^c	0,15	0,16	0,17	0,19	0,12	0,13	0,14	0,14
Per cent of volume as dead space^d	59	58	56	51	—	27	31	40

^{a} Calculation based on original working volume
^{b} Calculated using the dispersion model
^{c} Calculated using the stimules response data
^{d} Calculated using the multiparameter model

TABLE 4
THE EFFECTS OF GAS PRODUCTION ON THE MIXING CHARACTERISTICS OF THE HYBRID ANAEROBIC REACTOR OPERATED WITH AN EFFLUENT RECIRCULATION OF 6,4 : 1

	Without gas production				With gas production			
Arithmetic mean residence time (h)[a]	**10,0**	**13,0**	**16,0**	**25,0**	**10,0**	**13,0**	**16,0**	**25,0**
Calculated residence time (h)[b]	7,6	9,6	12,0	18,1	8,1	10,5	12,8	19,8
Gas production (m^3/m^3)	—	—	—	—	8,3	5,8	5,1	3,8
Per cent of volume as dead space	24	26	25	28	19	19	20	21

[a] Calculation based on original working volume
[b] Calculated using the dispersion model

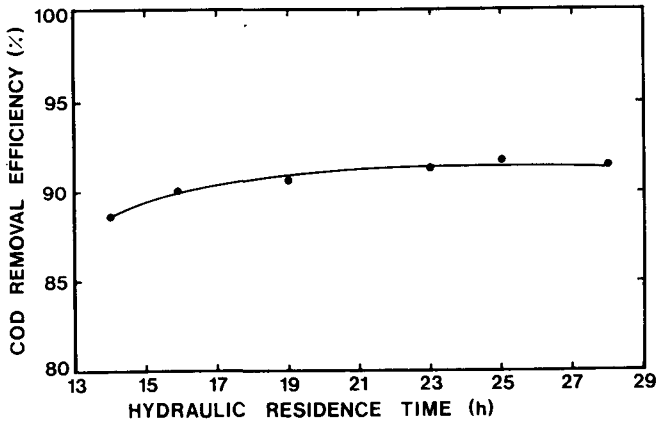

Figure 3
The effect of hydraulic residence times on the COD removal efficiency of the hybrid reactor operated at a recirculation ratio of 6,4 : 1.

Conclusions

- The upflow anaerobic hybrid reactor used in this study effectively treated a synthetic volatile fatty acid (VFA) substrate (COD = 9 850 mg/ℓ), pH 5,0, at 37°C. A recirculation ratio of 6,4 : 1 increased the total COD removal efficiency from 68,1% to 90,2% at a loading rate of 10,8 kg COD/m^3.d.
- Using a recirculation ratio of 6,4 : 1, the reactor was capable of a 90,0% substrate COD reduction at a loading rate of 15,0 kg COD/m^3.d.
- Conditions in the reactor were primarily anaerobic with a viable non-methanogenic cell count in the sludge of about 10^9 bacteria/ml. The aerobic and facultative anaerobic bacteria gave consistent cell counts of about 10^5 and 10^6 bacteria/mℓ respectively, indicating a possible functional role in the reactor.
- From the stimulus response experiments it was found that the reactor operated in the plug flow mode with a degree of longitudinal dispersion if no recirculation was used. Flow changed to that of the completely mixed type if recirculation was used. Recirculation had a more pronounced effect on dead reactor space than gas production. Bacterial growth contributed to the dead space which adversely affected reactor performance.

Nomenclature

C	tracer concentration (mg/ℓ)
C_i	tracer concentration at time i (mg/ℓ)
C_o	up-step tracer concentration (mg/ℓ)
$(D/\mu L)$	vessel dispersion number (dimensionless)
F	response on an up-step stimulus (dimensionless)
t	time (h)
$\bar{t}$	mean residence time (h)
Δt	time-interval (h)
τ	arithmetic mean residence time (h)
v	volumetric flow rate (ℓ/h)
V	volume of vessel (ℓ)
V_m	volume of mixed zone (ℓ)
σ^2	variance

Acknowledgements

This study was supported by a research grant from the Central Research Fund of the University of the OFS and the financial support of Sasol.

References

AMERICAN PUBLIC HEALTH ASSOCIATION. (1976) *Standard methods for the examination of water and waste water.* 14th edit. American Public Health Association, American Water Works Association, Water Pollution Control Federation, Washington, DC.

BRITZ, T.J., MEYER, L.C. and BOTES, P.J. (1983) Anaerobic digestion of a petrochemical effluent. *Biotechnol. Lett.* 5 113-118.

BUIJS, C., HEERTJES, P.M. and VAN DER MEER, R.R. (1982) Distribution and behaviour of sludge in upflow reactors for anaerobic treatment of wastewater. *Biotechnol. Bioeng.* 24 1975-1989.

CHRISTENSEN, D.R., GERICK, J.A. and EBLEN, J.A. (1984) Design and operation of an upflow anaerobic sludge blanket reactor. *J. Water Pollut. Contr. Fed.* 56 1059-1062.

DANCKWERTS, P.V. (1953) Continuous flow systems. Distribution of residence times. *Chem. Eng. Sci.* 2 1-13.

FORSTER, C.F., ROCKEY, J.S., WASE, D.A.J. and GODWIN, S.J. (1982) Mixing characteristics of fixed film anaerobic reactors. *Biotechnol. Lett.* 4 799-804.

HUNGATE, R.E. (1969) A role tube method for cultivation of strict anaerobes. J.R. Norris and D.W. Ribbons (Eds). *Methods in Microbiology*, Vol. 3B.

JOUBERT, W.A., BRITZ, T.J. and KOCK, J.L.F. (1985) Improved anaerobic digester performance using a simple statistical technique. *J. Ferment. Technol.* **63** 575-578.

KUNII, D. and LEVENSPIEL, O. (1968) Bubbling bed model. Model for the flow of gas through a fluidized bed. *Ind. Eng. Chem. Fundamentals* 7 46-452.

LEVENSPIEL, O. (1958) Longitudinal mixing of fluids in flowing in circular pipes. *Ind. Eng. Chem.* **50** 343-346.

LEVENSPIEL, O. (1972) *Chemical reaction engineering*. 2nd edit. Wiley, New York.

LEVENSPIEL, O. and BISCHOFF, K.B. (1959) Backmixing in the design of chemical reactors. *Ind. Eng. Chem.* **51** 1431-1434.

LEVENSPIEL, O. and SMITH, W.K. (1957) Notes on the diffusion-type model for the longitudinal mixing of fluids in flow. *Chem. Eng. Sci.* **6** 227-233.

MAH, R.A. and SUSSMAN, C. (1967) Microbiology of anaerobic sludge fermentations. *Appl. Microbiol.* **16** 358-361.

MEYER, L.C., HUGO, A.B., BRITZ, T.J., DE WITT, B. and LATEGAN, P.M. (1983) Temperature control for laboratory-scale anaerobic digesters. *Water SA* **9** 79-80.

MURRAY, W.D. and VAN DEN BERG, L. (1981) Effect of support material on the development of microbial fixed films converting acetic acid to methane. *J. Appl. Bacteriol.* **51** 257-265.

MILLER, T.L. and WOLIN, M.J. (1974) A serum bottle modification of the Hungate technique for cultivating obligate anaerobes. *Appl. Microbiol.* **27** 985-987.

NEL, L.H., BRITZ, T.J. and LATEGAN, P.M. (1985) The effect of trace elements on the performance efficiency of an anaerobic fixed-bed reactor. *Water SA* **11** 107-109.

SAMSON, R., VAN DEN BERG, L. and KENNEDY, K.J. (1985) Mixing characteristics and startup of anaerobic downflow stationary fixed film (DSFF) reactors. *Biotechnol. Bioeng.* **27** 10-19.

VAN DEN BERG, L. (1984) Developments in methanogenesis from industrial matter. *Can. J. Microbiol.* **30** 975-990.

VAN DEN BERG, L. and KENNEDY, K.J. (1981) Support materials for stationary fixed film reactors for high-rate methanogenic fermentations. *Biotechnol. Lett.* **3** 165-170.