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CHARACTERIZATION AND COMPARISON OF BIOMASS FROM MESOPHILIC AND THERMOPHILIC FIXED BED ANAEROBIC DIGESTERS

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ABSTRACT

Two lab-scale mesophilic (MAF) and thermophilic (TAF) anaerobic filters treating effluents from a mussel cooking factory were operated at their maximum organic load rate (OLR) for a long period of time. The biomass profiles and the distribution of occluded and attached biomass were determined. Biomass distribution in the MAF was more homogeneous along the filter. Most of the biomass in the TAF was attached while both attached and occluded biomass was observed in the MAF. The hydrolytic, acidogenic, acetogenic and methanogenic activities of sludges at different levels were determined. An attempt is made to explain the behaviour of both reactors as a function of these activities and other kinetic parameters.

KEY WORDS

Anaerobic Filter; Fixed Film; Mesophilic; Thermophilic; Biomass Distribution; Kinetic Characterization of Biomass.

INTRODUCTION

One of the most useful systems to treat high strength wastewater is the Upflow Anaerobic Filter (UAF) (Young and Yang, 1989). One of the more critical points of this system is the development of sludge which can cause an excessive accumulation of solids so that the digester becomes completely clogged. Inspite of that, there are only a few articles studying extensively this problem. Most of them study the volatile suspended solids (VSS) distribution along the filter (De Walle and Chian, 1976; Weiland, 1987) while others are focussed on the characterization of the biomass (Wilkie *et al.*, 1984),the relative distribution of different methanogens(Ehlinger *et al.*, 1987) or acidogenic/methanogenic bacteria (Kennedy and Guiot, 1987).

The main objectives of this article are to: a) determine the distribution and the methanogenic and non-methanogenic activities of the sludge in two identical mesophilic (MAF) and thermophilic (TAF) anaerobic filters and b) correlate their performance with the activities of their occluded and attached biomass.

EXPERIMENTAL

Analytical Methods

Determinations of total solids (TS), suspended solids (SS), volatile solids (VS) and volatile suspended solids (VSS) were carried out as proposed by Standard Methods (APHA, 1985). A semi-micro method, previously developed (Soto *et al.*, 1989), was used to determine accurately the chemical oxygen demand, both total (CODt) and soluble (CODs), in samples with high chloride concentrations. Glucose concentration was evaluate by determining the amount of reducing sugars in the sample using the di-nitro salicylic acid (DNS) reaction, while glucogen was estimated as the difference between the total sugar (by using the phenol-sulphuric reagent) and the reducing sugar amounts in the sample. Volatile fatty acids (VFA) and gas composition (CH_4, CO_2 and H_2S) were analysed by means of a Gas Chromatograph equipped with a FID and TCD, respectively (Méndez *et al.*, 1989).

The Upflow Anaerobic Filters: Characteristics and Operation

Two upflow fixed bed anaerobic filters (Figure 1) with a working volume of 0.92 L each, packed with raschig rings of corrugated polyvinylchloride plastic (Porosity 94%), were started and operated at thermophilic (55°C) and mesophilic (37°C) ranges of temperature for 2 and 3 years respectively.

Both reactors were seeded with sludges from an Upflow Anaerobic Sludge Blanket (UASB) reactor treating wastewaters from a sugar factory, with a high content in non-volatile solids (Table 1). A high recycling rate was applied during filter start-up (3-4 L/h), decreasing it for operational periods (0.4-1 L/h). Both digesters were operated treating a very saline wastewater coming from the industrial processing of mussels. Its main characteristics are presented in Table 2.

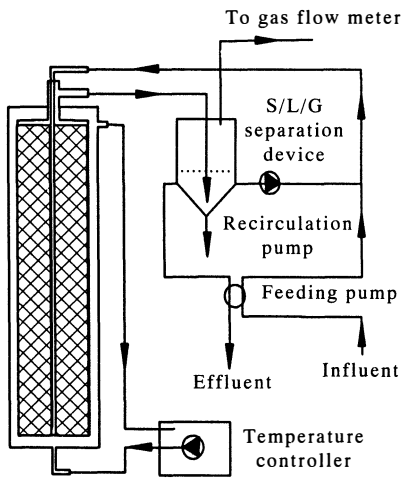

Fig. 1. Diagram of anaerobic filter.

Table 1. Inoculum Characteristics.

TS = 183.3	VS = 43.7	CODs = 0.41
SS = 113.8	VSS = 28.5	pH = 7.2

All, except pH, in g/L.

Table 2. Wastewater Characteristics (g/L).

COD =	11.5-26.6	Protein =	1.5-3.1
SS =	1.1-1.5	Glucogen =	8.0-20.0
VSS =	1.0-1.4	Cl^- =	9.0-15.0

The performance of the operation was reported in previous papers (Lema *et al.*, 1987a, 1988). Stability studies (Lema *et al.*, 1987b) indicated a good resistance to organic and hydraulic overloads, temperature shocks, non-feeding periods and toxic (salinity) inputs.

Biomass Separation and Classification

At the end of the operation, the VSS content of interstitial drained liquid was determined. This value is considered representative of suspended biomass in digesters. The volume of reactor occupied by the biomass (occluded and attached biomass) was determined by a controlled drainage of the liquid.

Once both filters were opened, the occluded biomass (O) and the attached biomass (A) from three reactor zones [lower (L), (0-10 cm high); middle (M), (10-30 cm); and upper (U), (30-50 cm)] were collected separately. SS and VSS were determined on samples of occluded (OL, OM, OU) and attached (AL, AM, AU) biomass obtained from the three different levels.

Anaerobic Assays

A methodology to perform methanogenic and non-methanogenic anaerobic tests requiring the use of small sludge amounts was established previously (Soto, 1990). Two different test methodologies were considered in order to determine : *a)* the specific microbial activity, employing the same substrate concentration and *b)* the substrate removal kinetics, by using different substrate concentrations.

Materials. Reactors of 100 mL were used for specific methanogenic activity tests. The methane production was measured by means of an inverted Mariotte flask filled with 2.5% NaOH solution, and connected to the culture flask. For non-methanogenic tests, the reactors of 100 mL were closed with a septum cap. Samples of 0.5-1.5 mL were taken periodically and centrifuged at 5000 rpm (4000 g) for 15 minutes, the supernatant being properly analysed.

A different system was used to determine the methanization kinetics of several substrates: acetic (HAc), propionic (HPr) and n-butyric (HnBu) acids. These assays were carried out in closed vessels of total and useful volumes of 126 and 50 mL respectively. Methane production was monitored by determining the gas composition in the vessel headspaces. Assuming an ideal behaviour in the gas phase, atmospheric conditions at the starting point, and the initial composition of gas (X^o_{N2}) that of the gas mixture (N_2/CO_2) bubbled up into the liquid, once the vessels were tightly closed, the methane production can be calculated as follows:

$$V_{CH4} = V * X^o_{N2} * X_{CH4} * (1-X_{CH4}-X_{CO2})^{-1}$$

where X is the molar fraction and V is the volume of the gas phase (76 mL). Net production of nitrogen was considered negligible in relation to methane production. Samples of gas (0.25 mL) were periodically analysed.

Methodology. The conditions for each experiment (substrate and sludge concentration) are presented in Tables 3 and 4. The Volatile Fatty Acids (VFA) solutions used for methanogenic activity testing and kinetic assays were previously neutralized with NaOH. Mussel processing wastewater, with a high content of glucogen, and glucose solutions were used as substrates for the hydrolytic and acidogenic activity determinations respectively.

Table 3. Assay Conditions for Activity Tests.

Run	Sludge Sample	Sludge gVSS/L	Substrate (g/L)
1	OL	1.82	Glucogen (1.5)
2	OM	3.01	"
3	OU	2.19	"
4	OL	1.42	Glucose (1.5)
5	OM	3.01	"
6	OU	2.19	"
7	OL	4.07	VFA mixture*
8	OM	2.34	"
9	OU	2.71	"
10	AL	5.92	"
11	AM	4.99	"
12	AU	6.62	"

* The VFA mixture is composed of HAc (2.0 g/L), HPr (0.5) and HnBu (0.5).

Redox potential was adjusted by the addition of 100 mg/L of $Na_2S \cdot 9H_2O$, while 1 g/L of Na_2CO_3 was added to increase the alkalinity. pH was adjusted with 1N or 5N solutions of HCl and NaOH. No addition of nutrients was made.

The step by step procedure was: *a)* introduction into reactors of a previously calculated amount of dilution water, *b)* addition of reducer solution and Na_2CO_3, *c)* addition of the sludge, *d)* pH adjustment between 7.0-7.1, *e)* bubbling of N_2/CO_2 85/15% gas mixture, *f)* sealing of flasks and connection to the biogas measure device when it was pertinent, *g)* addition of the substrates into the flasks and homogenization by a gently shaking.

The specific activity was calculated as the quotient of the slope of substrate removal or methane production curves and the VSS sludge concentration used.

Table 4. Assay Conditions for Kinetic Studies.

Run	Sludge Sample	Sludge g VSS/L	Substrate (g/L)
1	OL+OM+OU	2.34	Glucogen (0.1)
2	"	"	" (0.25)
3	"	"	" (0.50)
4	"	"	" (1.0)
5	"	"	" (2.0)
6	"	"	" (4.0)
7	OL+OM+OU	1.45	Glucose (0.25)
8	"	"	" (0.5)
9	"	"	" (1.0)
10	"	"	" (2.0)
11	"	"	" (4.0)
12	"	"	" (8.0)
13	OL	4.05	HAc (0.25)
14	"	"	" (0.5)
15	"	"	" (1.0)
16	"	"	" (2.0)
17	"	"	" (4.0)
18	"	"	" (8.0)
19	OL	4.05	HPr (0.125)
20	"	"	" (0.25)
21	"	"	" (0.5)
22	"	"	" (1.0)
23	"	"	" (2.0)
24	"	"	" (4.0)
25	OL	2.78	HnBu (0.0625)
26	"	"	" (0.125)
27	"	"	" (0.25)
28	"	"	" (1.0)
29	"	"	" (4.0)
30	"	"	" (8.0)

RESULTS AND DISCUSSION

Biomass Growth in the Anaerobic Filters

The performances of both filters are presented in Fig. 2 as functions of the organic loading rate (OLR). As can be seen the MAF operated properly till 24 kg COD/m^3d while the maximum applicable OLR to the thermophilic anaerobic filter (TAF) was 10-12 kg COD/m^3d.

Biomass distribution in the MAF, which occupied more than 90 % of its useful volume at the end of operation, was always more homogeneous than in the TAF. The profiles of total biomass distribution in the TAF when working at the maximum OLR are presented in Fig. 3. As can be seen, the percentage volume occupied by the biomass and its distribution in this reactor remains practically invariable, during a period of more than 100 days. The estimated biofilm thickness along the filter, always higher than 1 mm, allowed us to suppose that filter is active at all the different levels, as was stated in previous papers (Carozzi, 1988).

The purged biomass from the external settler and the VSS in the effluents from both reactors are presented in Fig. 4a and 4b respectively. The biomass washout from both filters increases as the OLR increases. However, a significant modification was observed when the MAF operated at values higher than 10-12 kg COD/m^3d. The VSS of effluent was practically constant and independent of OLR or HRT (hydraulic retention time). This modification in the behaviour of the MAF occurred simultaneously with an important change in the biomass characteristics; while thermophilic digester biomass maintained its original disperse aspect with poor settling properties, the mesophilic sludge presented a high content of big flocs settling

quickly. This fact can be observed in the settling assay shown in Fig. 5, carried out by adding samples of 25 mL of mesophilic and thermophilic sludges to a 250 mL graduated cylinder full of clear water and taking 2 photographs 10 and 30 s later.

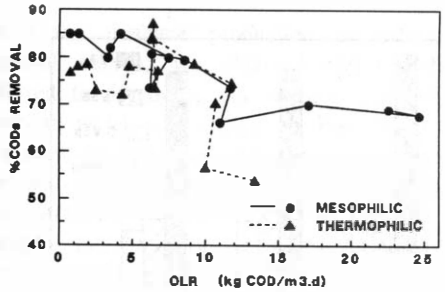

Fig. 2. Efficiency of the MAF and the TAF at several OLR.

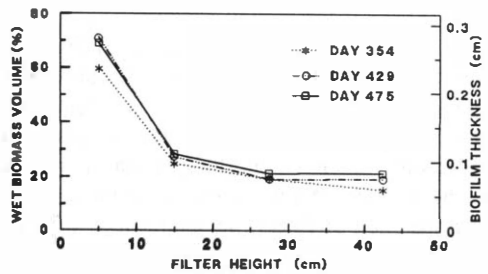

Fig. 3. Biomass distribution in the TAF during the period working at the maximum OLR.

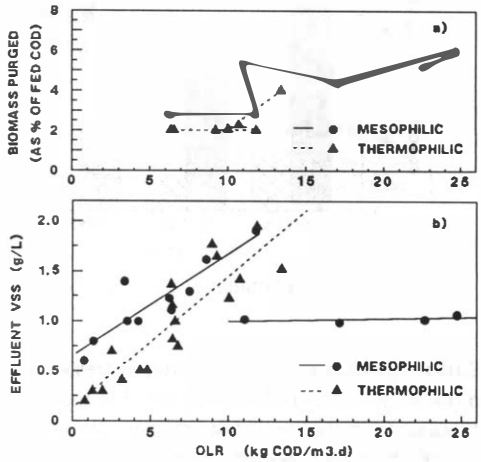

Fig. 4. Biomass from the external settler (a) and VSS in the effluent (b) for MAF and TAF.

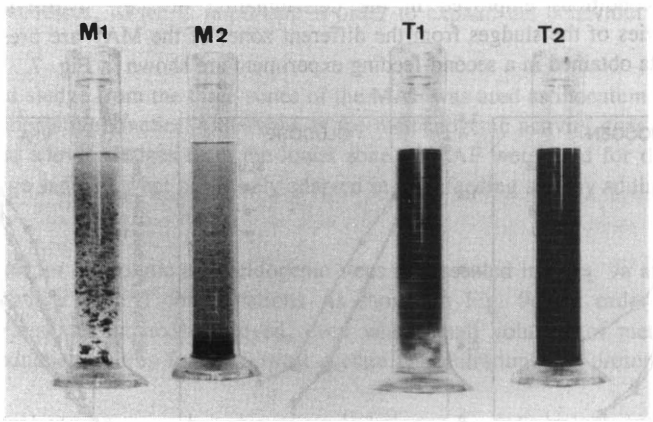

Fig. 5. Settling assays of the mesophilic (M) and thermophilic (T) sludges at 10 s (M1, T1) and 30 s (M2, T2).

Biomass Content and Characteristics at the End of Operation

Figs. 6a and 6b allow us to compare overall SS and VSS contents of fresh sludges and their distribution from both filters.

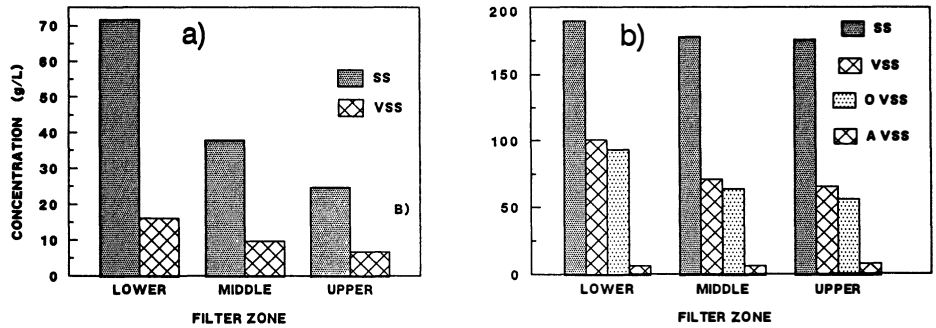

Fig. 6. Biomass content and distribution in the TAF (6a) and the MAF (6b) at the end of operation.

Some important differences between fresh sludges in the MAF and the TAF were observed. a) While most of biomass in the TAF was attached to the support, the MAF retained its biomass mainly occluded. b) The biomass from the mesophilic reactor contained an important fraction of biodegradable solids while thermophilic biomass seemed to be more mineralized. It was clearly observed after 5 days of stabilization by batch digestion, because while the content of SS and VSS in thermophilic sludge remains practically constant, a percent solids removal of 50-53% for SS and 40-50% for VSS was observed in the mesophilic sludge. This is in agreement with the fact that during the operation of filters, the degradation of VSS of influent (mostly protein) was always higher in the TAF.

Zonal Methanogenic and Non-methanogenic Activity of Mesophilic Sludges

The experimental conditions employed in the determination of the hydrolytic, fermentative and methanogenic activities of the sludges from the different zones of the MAF are presented in Table 3 and the experimental data obtained in a second-feeding experiment are shown in Fig. 7.

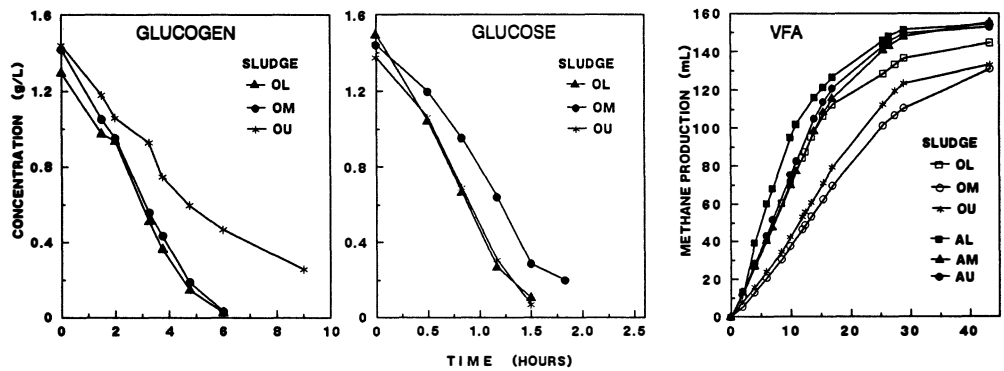

Fig.7. Batch assays for specific activities determination of mesophilic sludges.

A latent phase during the degradation of glucogen and glucose was observed during the first-feeding experiments, probably because the sludges were not well adapted to the high substrate concentrations. However no latent phase was observed during the methanogenic assays, except in the HnBu acid degradation.

From the methane production or substrate removal curves, we obtained the activities referred to glucogen, glucose and total VFA removal which are shown in Fig. 8. It is important to point out that these values were obtained by using different sludge concentrations. In spite of that, we are able to compare them because, as was determined in previous studies (Soto, 1990), the use of sludge concentrations between 1.5 and 5 g VSS/L in these assays doesn't influence the final result.

As can be observed, the hydrolytic and fermentative activities decrease from the lower to the upper zone of MAF, while the specific activity corresponding to VFA methanization remains almost constant along the filter. In that case the specific activity of attached biomass is slightly lower than the occluded biomass.

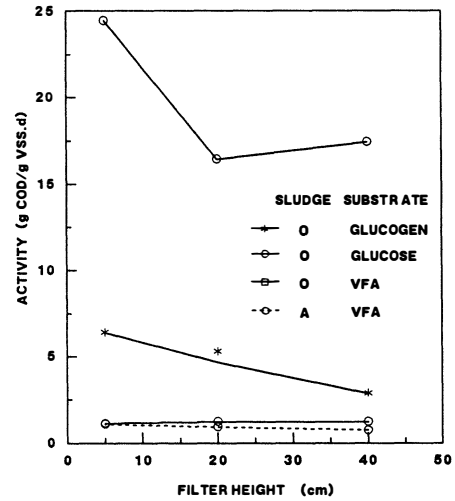

Fig. 8. Specific activities for mesophilic sludges.

Substrate Removal Kinetics for Mesophilic Sludge

The determination of the hydrolytic, fermentative and VFA removal rates at several substrate concentrations (assay conditions are presented in Table 4) allowed us to determine the kinetic model and parameters of these processes, which is important in order to explain the behaviour and performance of MAF.

A mixture of occluded sludge from the three zones of the MAF was used as inoculum in the determination of hydrolytic and fermentative kinetics. Otherwise as the methanogenic activity does not depend on filter position, as was stated above, sludges from the lower zone of MAF were used for the VFA degradation experiments. All sludge samples were previously adapted in a prefeeding step by adding the corresponding substrate.

The substrate evolution for hydrolytic and acidogenic steps is presented in Figs. 9a and 9b. The methane production from acetate at several concentrations is shown in Fig. 9c. In order to demonstrate the accuracy of the experimental method employed, even when small volumes of methane are produced, detailed methane production curves for the lowest acetate concentrations are presented, as example, in Fig. 9d.

The calculated values of the specific activities vs. substrate concentration for the different steps of the process are presented in Figs. 10a and 10b. A very general equation (Eq. 1) assuming a possible inhibition-by-substrate effect was considered to fit the experimental results. The obtained values of the model parameters allow a comparison of the behaviour of the different stages.

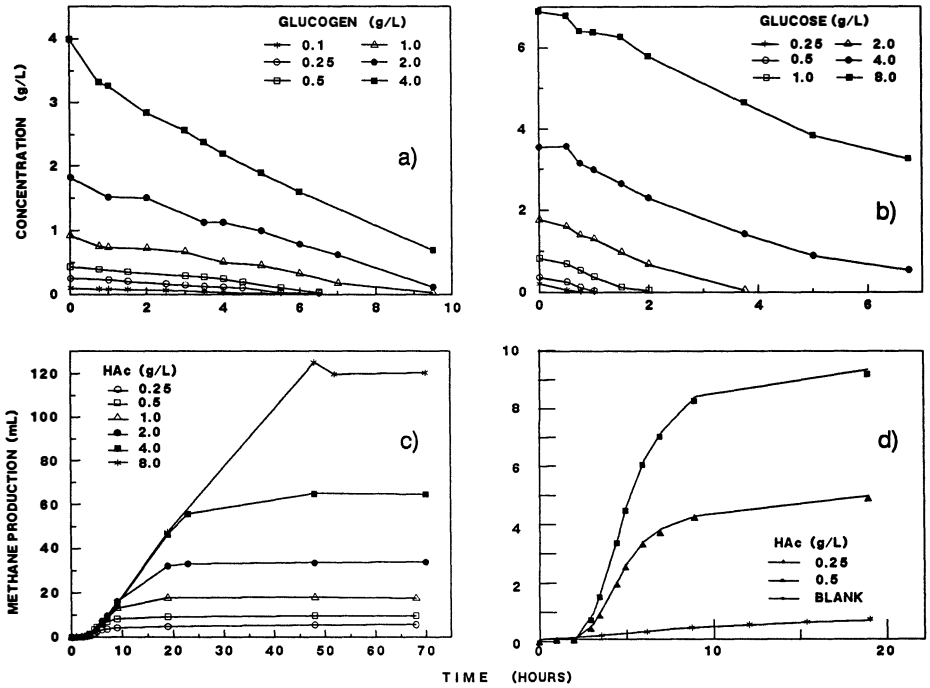

Fig. 9. Substrate removal (a, b) and methane production (c, d) during the kinetic assays.

$$A_c = A_{cm}\, S / (K_s + S + K_i\, S^n) \qquad \text{(Eq. 1)}$$

where A_c (g substrate removed / g VSSd) is the activity (determined as the quotient of the observed reaction rate and the VSS concentration), A_{cm} the maximum activity (g substrate removed / g VSSd) and K_s (g/L) and K_i (L/g) the saturation and inhibition-by-substrate constants, respectively.

The glucogen degradation kinetics clearly corresponds to a first-order model :

$$Ac = (A_{cm} / K_s)\, S \qquad \text{(Eq. 2)}$$

which indicates that the velocity process is controlled by the substrate concentration ($K_s >> S$, and $K_s >> K_i S^2$). The slope of the straight line representing A_m/K_s takes a value of 0.876 L/g VSSd.

An iterative optimization method was used to determine the kinetic parameters corresponding to each remaining process which are presented in Table 5. The solid lines in the Figs. 10a,b represent the calculated values of the activity by the Eq. 1.

As can be seen, a slight substrate inhibition process take place during the degradation of VFAs, while the Monod model represents quite precisely the behaviour of the fermentative step. On the other hand, the hydrolytic step rate is slower than the fermentative one and faster than the VFA methanization rate.

Table 5. Calculated Values of the Different Parameters for Kinetic Models.

Substrate	Parameters			
	n	A_{cm}	K_s	K_i
Glucose	-	13.59	0.287	0
HnBu	2	0.065	0.025	0.15
HPr	2	0.35	0.55	0.20
HAc	2	1.25	0.68	0.05

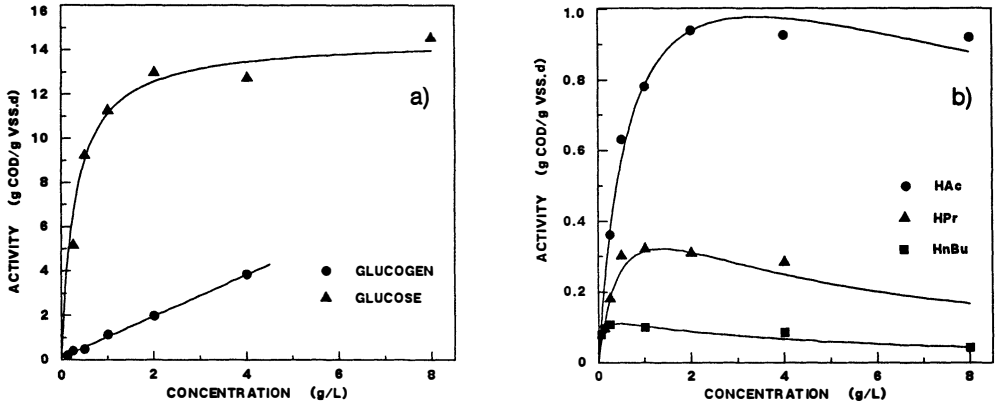

Fig. 10. Modelling of substrate removal kinetic for different degradation steps.

Although a first analysis of these figures could indicate a kinetic control of the process by the methanogenic step, it has to be taken into account that the foreseen glucogen and glucose concentrations in a steady state efficient operation would be lower than the ones employed in these experiments, so giving smaller velocities specially for the hydrolytic step.

MAF AND TAF PERFORMANCES AND CHARACTERISTICS OF BIOMASS

We will try to explain the different performances of both filters regarding the results obtained in the previous sections concerned with the retention capacity and the kinetic characteristics of sludges.

Several periods have been observed along the operation of both (MAF and TAF) equipments. During the first month of operation the MAF performance was higher than the TAF probably because of quicker adaptation of mesophilic sludges to the high salinity of the wastewater (Soto, 1990). Later, when the same degree of adaptation was reached in both filters, the substrate was better degraded in the TAF (Lema *et al.*, 1988), at operating OLR lower than 10-12 kg COD/m^3d. From then the efficiency of the TAF decreased very quickly because of difficulty of the equipment to retain the biomass. There is another important point explaining this fact. As the H_2S production remains constant because the sulphate is completely degraded, its concentration would be more important as the biogas production decreases. So a higher toxic effect due to H_2S is expected as the efficiency of the process becomes lower.

The better performance of the MAF is likely explained because of its higher capacity of biomass retention. In fact, at the end of operation the biomass content of the MAF was much higher than the TAF, which agrees with that hypothesis.

The MAF biomass presents a higher fermentative specific activity than activities in the other steps. Although the HnBu degradation rate is especially slow, it would not affect the efficiency of the MAF because its usual concentration in the effluent was always lower than 0.1 g/L.

It is interesting to observe two aspects that can be explained from the kinetic results (lower glucogen hydrolysis and lower acetate methanization). a) When MAF was operated at OLR higher than 6 kg COD/m^3d, the effluent contained normally steady state levels of glucogen (0.2-0.4 g/L) and HAc (0.5-1.5 g/L). b) During overloading experiences, glucogen and acetate accumulation was observed (Lema *et al.*, 1987b).

ACKNOWLEDGEMENTS

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REFERENCES

APHA-AWWA-WPCF. (1985). Standard Methods for the Examination of Water and Wastewater, Washington.

Carozzi, A. (1988) Reactores anaerobios de lecho fijo. Proc. 4th Seminar D.A.A.R. Valladolid (Spain), 23-25 Nov., 139-169.

Dewalle, F.B. and Chian, E.S.K. (1976). Kinetic of substrate removal in a completely mixed anaerobic filter. Biotechnol. Bioengng., 28, 1275-1295.

Ehlinger, F., Audic, J.M. and Faup, G.M. (1987). Relationship between the concentration of acetate in the feed and the composition of a biofilm in an anaerobic filter. Environm. Technol. Lett., 8, 197-207.

Kennedy, K.J. and Guiot, S.R. (1986). Anaerobic up-flow bed-filter - development and application. Wat. Sci. Technol. 18(12), 712-86.

Lema, J.M.; Soto, M.; Veiga, M.C. and Méndez, R. (1987a). Mesophilic and thermophilic anaerobic treatment of wastewaters from industrial processing of mussel. in "Biomass for Energy and Industry". Elsevier Appl. Sci. Pu., 872-877.

Lema, J.M.; Méndez, R, and Soto, M. (1987b). Stability studies of thermophilic and mesophilic anaerobic filters treating very high saline wastewater. Proc. 4th Mediterranean Congress Chem. Engng. 2, 770-771.

Lema, J.M.; Soto, M.; Méndez, R. and Blázquez, R. (1988). Comparison of mesophilic and thermophilic anaerobic filters treating very high saline wastewaters. 5th International Symposium on Anaerobic Digestion. Bologna, Italy. May 22-26, (Posters) 547-549

Méndez, R.; Lema, J.M.; Blázquez, R.; M. Pan, M. and Forjan, C. (1989). Characterization, digestibility and anaerobic treatment of leachates from old and young landfills. Wat.Sci.Technol. 21 (12), 145-155.

Soto, M.; Veiga, M.C.; Méndez, R. and Lema, J.M. (1989). Semi-micro C.O.D. determination method for high-salinity wastewater. Environm. Technol. Lett., 10, 541-548.

Soto, M. (1990). Depuraçom de efluentes residuais da indústria de processado de produtos mariños mediante um filtro anaeróbio. PhD. Thesis. Univ. of Santiago de Compostela, Spain.

Weiland, P. (1987). Development of anaerobic filters for treatment of high strength agro-industrial wastewaters. Bioprocess Engng. 2, 39-47.

Wilkie, A.; Reynolds, P.J.; O'Kelly, N. and Colleran, E. (1984). Supporting matrix and packing effects in anaerobic filters. Proc. 2nd Int. Conf. on Fixed Film Biological Process. Washington D.C. July 10-12.

Young, J.C. and Yang, B.S. (1989). Design considerations for full scale anaerobic filters. Journal WPCF, 61(9), 1576-1587.