

Biodegradability of slaughterhouse wastewater with high blood content under anaerobic and aerobic conditions

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Abstract: In this work, the biodegradability of wastewater from a slaughterhouse located in Keşan, Turkey, was studied under aerobic and anaerobic conditions. A very high total COD content of 7230 mg dm⁻³ was found, due to an inefficient blood recovery system. Low BOD₅/COD ratio, high organic nitrogen and soluble COD contents, were in accordance with a high blood content. A respirometry test for COD fractionation showed a very low readily biodegradable fraction (S_S) of 2%, a rapidly hydrolysable fraction (S_H) of 51%, a slowly hydrolysable fraction (X_S) of 33% and an inert fraction of 6%. Kinetic analysis revealed that hydrolysis rates were much slower than these of domestic sewage. The results underlined the need for an anaerobic stage prior to aerobic treatment. Tests with an anaerobic batch reactor indicated efficient COD degradation, up to around 80% removal. Further anaerobic degradation of the remaining COD was much slower and resulted in the build up of inert COD compounds generated as part of the metabolic activities in the anaerobic reactor. Accordingly, it is suggested that an appropriate combination of anaerobic and aerobic reactors would have to limit anaerobic degradation to around 80% of the tCOD and an effluent concentration above 1000 mg dm⁻³, for the optimum operation of the following aerobic stage.

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Keywords: Characterisation; COD fractionation; hydrolysis; modelling; residual COD; respirometry; slaughterhouse; aerobic–anaerobic treatment

NOTATION

b_H	Endogenous decay coefficient for active biomass (T ⁻¹)
C_S	Biodegradable COD (M COD L ⁻³)
C_T	Total COD (M COD L ⁻³)
f_{ES}	Fraction of endogenous mass converted into soluble inert products
f_{EX}	Inert fraction of biomass
k_{hS}, k_{hX}	Hydrolysis rate constants (T ⁻¹)
K_S	Half saturation coefficient (M COD L ⁻³)
K_{XS}, K_{XX}	Saturation coefficients for particulate COD (M COD(M cell COD) ⁻¹)
S_H	Soluble slowly biodegradable COD (M COD L ⁻³)
S_I	Soluble inert COD (M COD L ⁻³)
S_O	Oxygen concentration (M O ₂ L ⁻³)
S_P	Soluble residual product (M COD L ⁻³)
S_S	Readily biodegradable COD (M COD L ⁻³)
S_T	Soluble COD (M COD L ⁻³)

X_H	Active heterotrophic biomass (M cell COD L ⁻³)
X_I	Particulate inert COD (M COD L ⁻³)
X_P	Particulate residual product (M COD L ⁻³)
X_S	Particulate slowly biodegradable COD (M COD L ⁻³)
Y_H	Heterotrophic yield coefficient (M cell COD (M COD) ⁻¹)
μ_{Hmax}	Maximum heterotrophic growth rate (T ⁻¹)

1 INTRODUCTION

Accurate information on wastewater characteristics is a key factor in the design of adequate treatment facilities. In recent years, rational design of wastewater treatment plants has relied mostly on process model-

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ling. To provide a general basis for design and operation, it is necessary to provide a rational description of related processes in terms of microbial kinetics, emphasising the required wastewater characterisation for the assessment of biological treatability. Although this rational approach is now adopted for domestic wastewater treatment plants, it is still largely overlooked in the treatment of industrial wastewaters.¹ Besides, the high variability of wastewater composition and, therefore, the different biodegradability characteristics of the effluents of each industrial category make characterisation essential for an appropriate design of a treatment plant. In fact, variations in wastewater composition are very high even in the same type of industry (such as slaughterhouses), depending on the production procedures, by-products' recovery and cleaning procedures. This characterisation should involve differentiation of COD fractions with different biodegradation patterns.

On the other hand, the traditional activated sludge process is likely to exhibit serious difficulties in direct treatment of strong wastewaters such as slaughterhouse effluent. These difficulties are not only limited to high aeration costs and high levels of waste sludge produced, but also involve oxygen transfer problems. For this reason, anaerobic pretreatment is an efficient and cost-effective solution for this kind of wastewater. Thus, optimum the design of a treatment plant for such a specific type of wastewater as slaughterhouse effluent requires adequate information on the level of biodegradation that can be achieved using aerobic and anaerobic processes.

In this context, the objective of this study was defined as the experimental evaluation of the biodegradation of slaughterhouse effluent under anaerobic and aerobic conditions. It involved conventional characterisation of the wastewater, COD fractionation, respirometric assessment of biodegradation under aerobic conditions and batch experiments on the COD removal potential of a dual anaerobic/aerobic system with emphasis on the impact of inert organics and generation of residual microbial products.

2 MATERIALS AND METHODS

2.1 The slaughterhouse plant

The wastewater investigated in this study was obtained from a medium-size slaughterhouse plant in the city of Keşan (Turkey) with an average daily capacity of 70 cows and 70 lambs. Blood, skin, intestine and stomach were recovered, and its contents were dry-emptied. The processing in the plant was largely labour-intensive and blood recovery, at the time the samples were taken, was inadequate. However, although the COD concentration in the wastewater was very high, the overall daily organic load was at the average level for this type of activity because water use was significantly lower as compared with similar but highly automated plants (Table 1). There were basically two

wastewater streams; the main one came from the main line after blood recovery and the second one, which represented around 15% of the total flow, came from the adjacent halls where by-products were handled.

2.2 Sampling and analytical methods

For wastewater characterisation, a composite sample was collected for the two wastewater streams, of which aliquots were taken every 2 h during the production and final cleaning periods. Samples from both streams were mixed in the laboratory, in proportion to the corresponding flow rates (85–15%), before analysis for significant parameters. Analyses for conventional characterisation were performed in accordance with standard methods.² The soluble fraction was defined as the filtrate obtained after filtering the sample using cellulose acetate, 0.45 µm, membrane filters. Oxygen uptake rate (OUR) measurements were conducted with a Manotherm RA-1000 continuous respirometer, equipped with a PC control. The readily biodegradable COD fraction was determined according to the method proposed in the literature.³ A nitrification inhibitor was added during the test to avoid interferences in oxygen consumption by nitrifiers. Biomass acclimated to the slaughterhouse wastewater was grown and maintained in an aerated fill and draw reactor of 5 dm³ volume, operated with a food to microorganism (F/M) ratio of 0.2 gCOD (gVSS)⁻¹ and a sludge age of 15 days for 5 weeks. The acclimated biomass was used in the COD fractionation experiments and in the respirometric evaluation of the anaerobic treatment effluent.

2.3 Data analysis

Evaluation of the experimental data was performed by means of model simulation, using the AQUASIM computer program developed by the Swiss Federal Institute for Environmental Science and Technology.^{4,5} Activated Sludge Model No 1 – ASM1,⁶ modified for endogenous decay, was used in the study for the mechanistic interpretation of the OUR data.⁷ A matrix representation of the model is given in Table 2.

2.4 Anaerobic batch reactors

A dm³ anaerobic batch reactor was set at 30–35 °C in order to estimate the viability of an anaerobic pretreatment step for the highly-loaded wastewater. A high biomass concentration of 1300 mg dm⁻³ and an initial tCOD of 1500 mg dm⁻³ were set to optimise operation under laboratory conditions. The alkalinity was maintained at a level high enough to avoid any

Table 1. Observed range of water usage and wastewater COD load

	Water use (dm ³ kg ⁻¹ livestock)	COD load (g kg ⁻¹ livestock)
Keşan slaughterhouse	0.8–1.3	2.4–3.8
Johns ¹⁰	0.8–29	8.6–18
WPCD ⁸	6.5	1.5–14

Table 2. Process kinetic and stoichiometry for carbon removal

Process	Component							Process rate (ML ⁻³ T ⁻¹)
	1	2	3	4	5	6	7	
	S _S	S _H	X _S	X _H	X _P	S _P	S _O	
Growth	$-\frac{1}{Y_H}$			1			$-\frac{(1 - Y_H)}{Y_H}$	$\mu_{Hmax} \frac{S_S}{(K_S + S_S)} X_H$
Rapid hydrolysis	1	-1						$k_{HS} \frac{S_H/X_H}{(K_{XS} + S_H/X_H)} X_H$
Slow hydrolysis	1		-1					$k_{HX} \frac{X_S/X_H}{(K_{XX} + X_S/X_H)} X_H$
Decay				-1	f_{EX}	f_{ES}	$-(1 - f_{EX} - f_{ES})$	$b_H X_H$
Parameter (ML ⁻³)	COD	COD	COD	Cell COD	COD	COD	O ₂	

possible acid inhibition of the anaerobic process. Seed granular anaerobic sludge was obtained from an upflow anaerobic sludge blanket (UASB) reactor treating distillery wastewater. Any possible oxygen interference was avoided in both reactors by periodical nitrogen gas bubbling, keeping always a closed nitrogen-saturated atmosphere.

Samples of decanted supernatant from the anaerobic reactor were taken periodically in order to follow the evolution of COD degradation with time. The same samples were also employed in COD fractionation tests with a respirometer so as to determine the evolution of the aerobic biodegradability of the anaerobic effluent.

3 RESULTS AND DISCUSSION

3.1 Wastewater characterisation

Wastewater characteristics based on the analysis of the composite sample from the mixed streams (main and adjacent halls) are shown in Table 3, together with similar data from the literature.⁸⁻¹⁰

The high COD concentration of the wastewater was due to an important volume of blood reaching the main line of the wastewater. This fact had a great influence on the rest of the parameters and the nature of wastewater, for example the BOD₅/COD ratio of

0.44 was below the normal biodegradability of domestic wastewaters (0.60 BOD₅/COD). Also, the soluble fraction was very high (77%) and the COD/total Kjeldahl nitrogen (TKN) ratio was 10.5, with a high organic nitrogen content of 90%, indicating that most of the organic matter consisted of protein from the blood.

The oil and grease content was also quite high due to the handling of the intestines (250 mg dm⁻³). The suspended solids came mainly from the adjacent halls in which some of the intestine and stomach contents, such as straw, reach the water stream. The stream accounting for 15% of the total wastewater flow had a very high particulate fraction of 53% and only 18% of this fraction was composed of settleable solids. Finally, the high chloride content was due to the skin-salting process.

3.2 Respirometry tests

Respirometric evaluation of the wastewater sample was carried out both for the determination of major COD fractions in the wastewater and model simulation and calibration of the experimental OUR data obtained for the identification of the most appropriate kinetic coefficients.

Experimental assessment of the significant COD fractions in the wastewater was carried out using basic

Table 3. General characterisation of slaughterhouse wastewater

Parameter (mg dm ⁻³)	This study	WPCD ⁸	Tritt and Schuchard ⁹	Johns ¹⁰
Total COD	7230		1000–6000	1400–11000
Soluble COD	5500			780–10000
Total BOD ₅	3180	1420–2000	1000–4000	490–4600
Soluble BOD ₅	2480			
TSS	910	320–610		220–600
VSS	850			
TKN-N	690	100–240	250–700	110–700
NH ₃ -N	67		200–300	30–300
Total phosphate	3.3		80–120	
Ortho-phosphate	1.3			
Chloride	2450			
Grease and oil	250	200–240		50–200
pH	7.5		6.5–10	

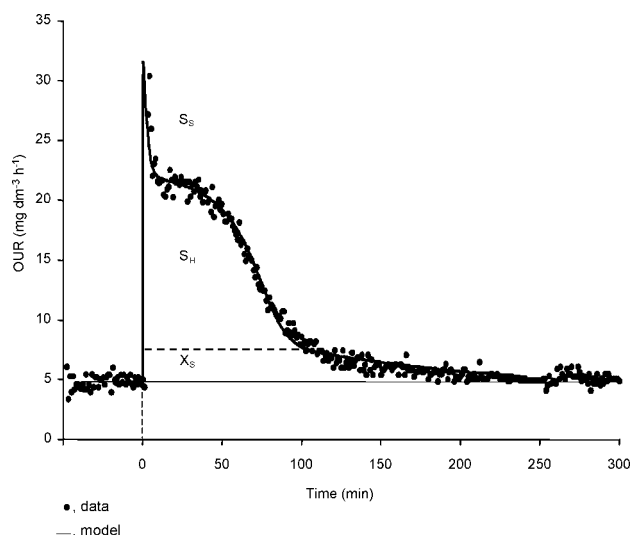


Figure 1. OUR profile of the wastewater sample for $F/M=0.1 \text{ gCOD gVSS}^{-1}$.

respirometry, from the OUR profiles obtained with the composite sample at two F/M ratios of 0.1 and $0.28 \text{ gCOD g}^{-1} \text{ VSS}^{-1}$. The test reactors were seeded with activated sludge from an acclimatised fill and draw reactor. The MLVSS concentration in the reactor was maintained around 1000 mg dm^{-3} . The tests were carried out at room temperatures between 24 and 27°C .

For the evaluation, biodegradable organic matter was assumed to contain three basic components: the readily biodegradable substrate, S_S , the rapidly hydrolysable fraction, S_H , and the slowly hydrolysable substrate, X_S . The COD fractions were calculated from the area below the OUR profile. The inflexion points on the curve were assumed to be clear indications for successive changes in the rate of utilisation of different biodegradable COD fractions. A Y_H coefficient of 0.68 d^{-1} , previously suggested for this type of wastewater, was adopted for the calculations.¹¹ The inert COD was calculated from the differences between the measured tCOD of the wastewater and the total area calculated, as proposed in the literature.¹²

The calculated values of the COD fractions were used with slight adjustments for model calibration of the experimental data for the estimation of kinetic parameters. Estimation of COD fractions and model simulation of the OUR curve for an F/M ratio of $0.10 \text{ gCOD gVSS}^{-1}$ are illustrated in Fig 1.

Table 4 shows the results of the COD fractionation for the wastewater tested at two different F/M ratios together with similar data reported in the literature for meat processing and poultry slaughterhouses wastewaters.^{11,13} It should be noted that tests carried out at two different initial F/M ratios yielded close results from a practical standpoint. In fact, they have predicted the total biodegradable COD content with around 4% approximation, which has reflected on the total inert COD levels.

One of the most significant aspects was the very low readily biodegradable fraction (less than 3% of the total COD), which was even lower than the values already reported in the literature. Another interesting point was related to the slowly hydrolysable fraction, X_S , which is usually associated with particulate matter.¹⁴ This assumption is consistent with the data in the literature, in which the particulate matter of the wastewater ($C_T - S_T$) is approximately the sum of X_S and X_I . However, for the high blood content of this slaughterhouse wastewater, this assumption was not true because of the relatively low particulate matter fraction of $0.23 \text{ gCOD (gCOD)}^{-1}$. Consequently the X_S fraction must be presumably considered to be composed of particulate matter and filterable high molecular weight proteins.

Model calibration was carried out for appropriate values of kinetic coefficients yielding best fit with experimentally determined OUR curves. COD fractions ascertained for the wastewater, with two significant hydrolysable compounds S_H and X_S dictated the adoption of a dual hydrolysis model previously proposed for domestic sewage and a number of industrial wastewaters.¹⁻¹⁵ A total of seven coefficients were tested (namely, μ_{Hmax} , K_S , k_{hS} , K_{XS} , k_{hX} , K_{XX} and b_H). Defaults values as defined in ASM1 were adopted for the other parameters. Evaluation was made again on the composite sample at two F/M values, and also, on two similar OUR data obtained from a stronger individual sample with a tCOD of 13000 mg dm^{-3} (OUR tests 1a and 1b in Table 5).

Values of the kinetic coefficients displayed in Table 5 are, in general, quite reproducible and in accordance with the values reported in the literature. The temperature differences explain most of the variations in the values of μ_{Hmax} , k_{hS} and k_{hX} for the OUR tests of the same sample at different F/M ratios. As has been mentioned before, μ_{Hmax} and K_S values show greater variations than the rest of the parameters. The K_S

Table 4. Biodegradable COD fractions of meat processing wastewaters

Wastewater	$C_T \text{ (mg dm}^{-3}\text{)}$	$S_T \text{ (mg dm}^{-3}\text{)}$	S_S/C_T	S_H/C_T	X_S/C_T	$(S_S+X_S)/C_T$
This study						
Sample 2a $F/M=0.10 \text{ gCOD gVSS}^{-1}$	7230	5500	0.02	0.63	0.27	0.08
Sample 2b $F/M=0.27 \text{ gCOD gVSS}^{-1}$	7360	5480	0.02	0.56	0.38	0.04
Average	7290	5490	0.02	0.59	0.33	0.06
Meat processing ³	2600	1140	0.15	0.28	0.44	0.13
Poultry processing ¹	2490	1770	0.11	0.46	0.32	0.12

Table 5. Kinetic parameters for slaughterhouse wastewater

	<i>F/M</i>	<i>T</i> (°C)	μ_{Hmax} (day ⁻¹)	K_S (g m ⁻³)	k_{hS} (day ⁻¹)	K_{XS} (g m ⁻³)	k_{hX} (day ⁻¹)	K_{XX} (g m ⁻³)	b_H (day ⁻¹)
This study									
Individual sample									
(Sample No: 1)									
1a	0.08	24	3.8	1.5	1.4	0.013	0.8	0.07	0.20
1b	0.29	26	4.3	1.2	1.6	0.010	0.8	0.04	0.25
Composite sample									
(Sample No: 2)									
2a	0.10	26	4.2	3	1.6	0.008	0.6	0.02	0.20
2b	0.27	27	4.5	3	1.8	0.008	0.8	0.04	0.20
Poultry ¹¹	–	20	3.9	14	3.3	0.07	2.3	0.6	0.20
Meat ¹³	–	15	4.2	30	1.4 ^a	0.13 ^a			0.10

^a The OUR profile allowed only a single hydrolysable fraction for modelling.

values in the range of 1.2–3.0 mg dm⁻³ found from the model fit to the first sudden drop of the OUR profile (Fig 1) are low compared with values associated with poultry and meat processing wastewaters.^{11,13} They are however reasonable in view of the fact that even lower K_S values of around 0.6 mg dm⁻³ were previously reported.¹⁶ An accurate estimation of the growth parameters would require specific experimental setups but, as long as the readily biodegradable fraction hardly provides a significant contribution to the total COD, the interest should be focused on the hydrolysis constants.

The values in Table 5 provide a clear justification for the adoption of a dual hydrolysis model, as the rate coefficients defining hydrolysis of rapidly and slowly hydrolysable compounds are markedly different. Rapid hydrolysis is characterised by a k_{hS} value in the range of 1.4–1.8 d⁻¹. This rate is lower than that associated with domestic sewage,¹² a common property for a number of industrial wastewaters.¹ Slowly hydrolysable COD, X_S , was characterised by a much slower hydrolysis rate coefficient of around 0.6–0.8 d⁻¹. This significantly slower hydrolysis rate makes it necessary to consider S_H and X_S as separate model components in the evaluation of the electron acceptor utilisation potential of the wastewater.

3.3 Anaerobic biodegradability

As previously mentioned, the slaughterhouse generates a strong wastewater with a tCOD content of more

than 7000 mg dm⁻³. This level is too high for direct application of an aerobic treatment, in terms of excessive cost of aeration and sludge disposal involved and requires an anaerobic pretreatment stage.

In this context, an anaerobic batch test was started by diluting the composite wastewater sample down to a COD level of 1500 mg dm⁻³; therefore, the results are applicable by using a dilution factor of 4.86 v v⁻¹. The test was continued for 22 days. The time evolution of tCOD, as well as the removal efficiency of the anaerobic batch reactor are outlined in Table 6 and illustrated in Fig 2. The data indicated that a high COD degradation rate of 0.12 kg COD m⁻³ d⁻¹ could be sustained in the anaerobic batch reactor up to a COD concentration of 250–300 mg dm⁻³, after a biodegradation period of 8 days. A significant decrease in the removal rate was observed with further treatment. This implied that the effective COD removal was limited to around 80%. Consequently, at full scale, a continuous anaerobic reactor fed with the raw wastewater would be expected to produce an effluent with a remaining COD concentration of around 1500 mg dm⁻³ at 80% removal, a too high level for direct disposal, while an aerobic system would be able to reduce the COD content down to its inert fraction, initially present and generated during the course of biochemical reactions. Therefore, the optimum treatment configuration for a highly loaded wastewater such as the slaughterhouse effluent would involve a sequence of anaerobic and aerobic units.

Table 6. Evolution of the distribution of COD fractions during anaerobic treatment

Treatment period (days)	COD fractions												
	Total COD (C_T)		Biodegradable COD (C_S)			Readily biodegradable COD (S_S)		Rapidly hydrolysable COD (S_H)		Slowly hydrolysable COD (X_S)		Inert COD ($S_I + X_I$)	
	mg dm ⁻³	Removal (%)	mg dm ⁻³	Removal (%)	C_S/C_T	mg dm ⁻³	S_S/C_T	mg dm ⁻³	S_H/C_T	mg dm ⁻³	X_S/C_T	mg dm ⁻³	$(S_I + X_I)/C_T$
0	7290	–	6840	–	94	140	0.02	4300	0.59	2400	0.33	450	0.06
5	2780	65	2450	64	92	310	0.11	980	0.35	1160	0.42	330	0.12
7	1680	77	1330	81	79	135	0.08	490	0.29	705	0.42	350	0.21
12	960	87	110	98	11	10	0.01	40	0.04	60	0.06	850	0.89
22	610	90	50	99	8	–	–	15	0.02	35	0.06	560	0.92

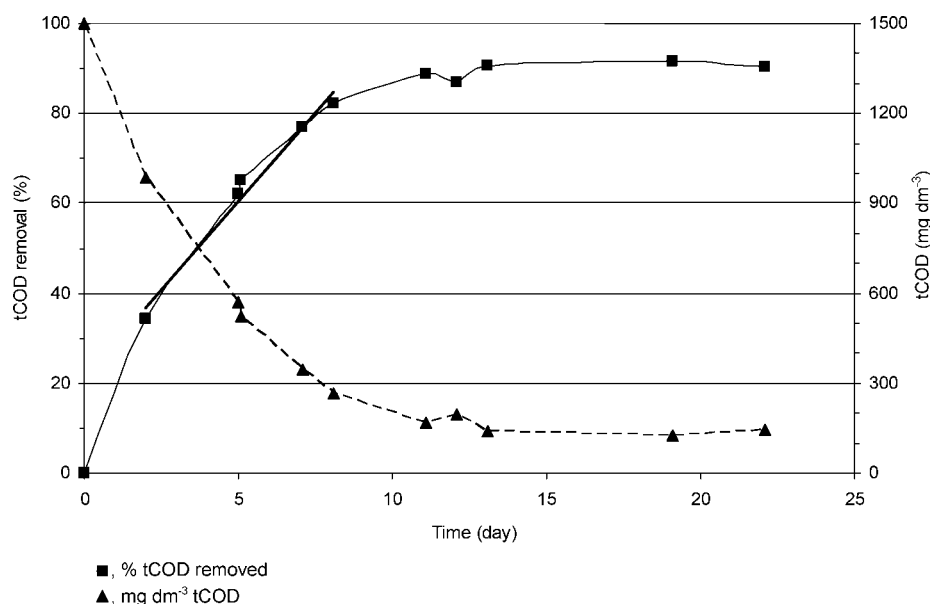


Figure 2. COD biodegradation in the anaerobic batch reactor.

Accordingly, respirometric tests were carried out on samples taken from the anaerobic reactor at different time intervals ranging from 2 to 22 days, in order to determine the extent of further COD reduction that can be achieved under aerobic conditions. The OUR profiles obtained are given in Fig 3. These OUR profiles were used to evaluate, as before, the remaining total biodegradable COD and the composition of the anaerobic effluent in terms of significant COD fractions, prior to aerobic treatment. The change induced on COD fractions during the course of anaerobic treatment is outlined in Table 6 and schematically presented in Fig 4.

A significant observation derived from the OUR profiles is the almost total removal of the biodegradable COD before 10 days. In fact, 8 days of anaerobic degradation results in total depletion of biodegradable COD, while the tCOD removal appears to be limited at 87%, due to build up of inert fractions. This observation highlights a common mistake of extending biological (anaerobic/aerobic) treatment with the hope of improving COD removal, but the addition reaction time always remains ineffective as it is bound to deal with inert organic compounds alone.

Further inspection of the data presented in Table 6 gives the indication of a degradation rate clearly limited by the hydrolysis of slowly biodegradable compounds, resulting during the preliminary phases of the treatment (first 5 days) in an increase of the readily biodegradable COD, probably due to generation of short-chain fatty acids inside the reactor. Moreover, there was a net increase in the inert COD with time because of the reaction products of the anaerobic process, mainly the particulate ones. This fraction practically dominated the anaerobic effluent as it represented around 90% of the tCOD after 10 days.

The evolution of the COD fractions indicated that the anaerobic process decreased the overall organic

load, especially the S_H , which was the most important one in the raw wastewater with high blood content. From these results, an appropriate combination of anaerobic and aerobic reactors for this kind of wastewater would imply anaerobic degradation up to 80% of the tCOD and effluent concentration above 1000 mg dm^{-3} to avoid anaerobic kinetic limitation. The slaughterhouse wastewater pretreatment could be carried out in an oriented-media fixed film system,¹⁷ in order to avoid the instability of suspended biomass reactors like Upflow Anaerobic Sludge Blanket (UASB) or Expanded Granular Sludge Bed (EGSB) in the presence of high grease concentrations and the filter-clogging problems of the random media reactors.^{18,19}

4 CONCLUSIONS

The following can be outlined as the concluding remarks derived from the experimental observations of this study:

The slaughterhouse operation generates a strong effluent of variable character, depending on the degree of blood recovery. The average quality of the generated wastewater could be characterised as having a total COD of around 7300 mg dm^{-3} , highly soluble in nature, a low BOD_5/COD ratio of 0.44, a significant total N (TKN) concentration of 690 mg dm^{-3} and a total P concentration of only 3.3 mg dm^{-3} , very much below the level that would be required to sustain biological treatment.

Respirometric evaluation of the wastewater revealed a similar COD composition in terms of different COD fractions, supporting conventional characterisation. Around 50% of the COD consisted of soluble slowly biodegradable fraction (S_H) in agreement with the low BOD_5/COD ratio, with a minor readily biodegradable COD content of only 2% and a total inert COD of 6%.

Model evaluation of the respirometric analysis

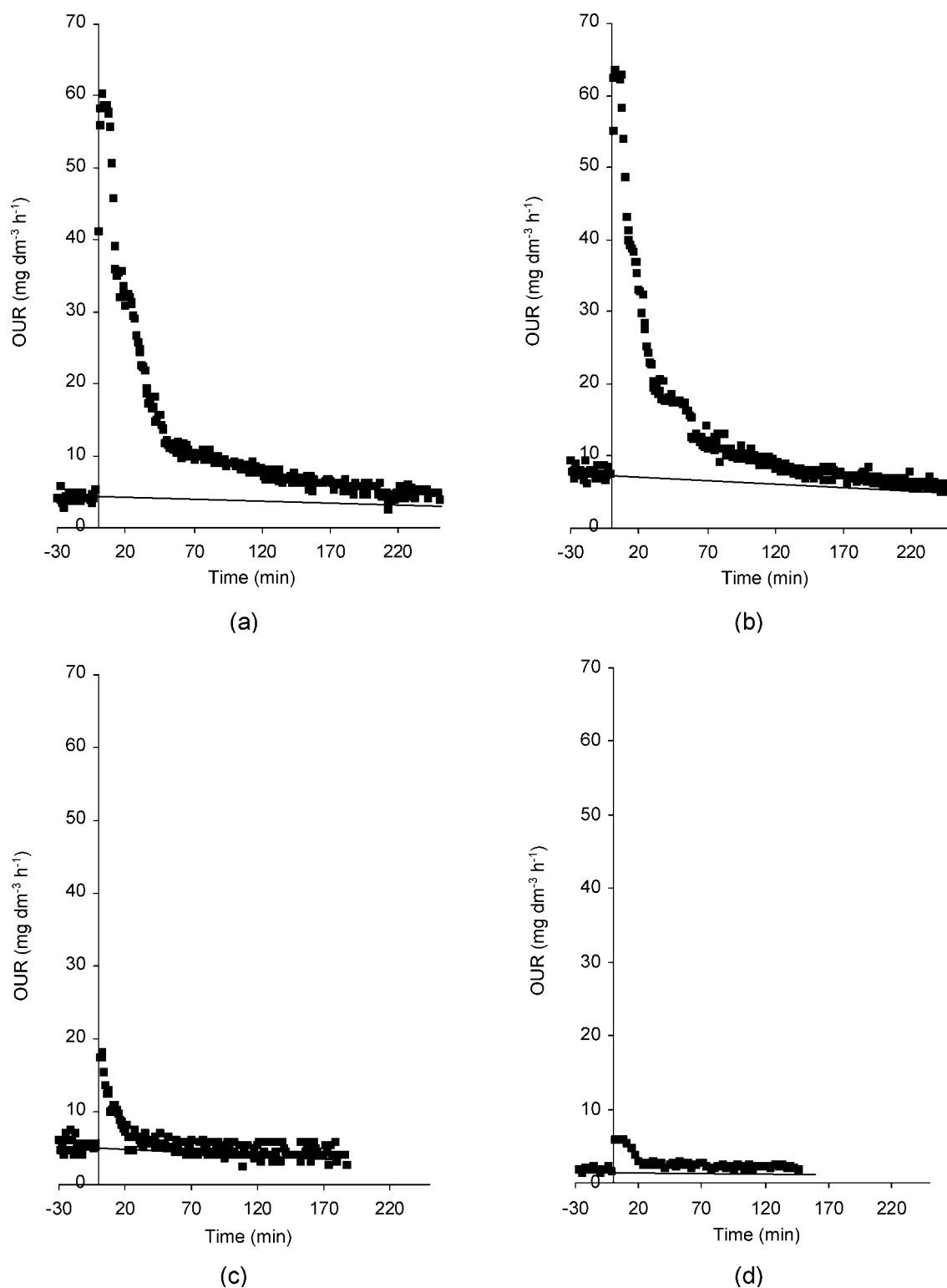


Figure 3. Evolution of the OUR profiles associated with the anaerobic supernatant (a=5 days, b=7 days, c=12 days, d=22 days).

yielded heterotrophic growth kinetics compatible with domestic sewage. Markedly different rate coefficients for rapidly and slowly hydrolysable compounds necessitated the adoption of a dual hydrolysis kinetics. The rate for the hydrolysis of soluble compounds was computed as $1.4\text{--}1.8\text{d}^{-1}$. The same rate constant for more slowly hydrolysable compounds was evaluated to remain in the range of $0.6\text{--}0.8\text{d}^{-1}$.

The wastewater responded well to anaerobic treatment with a COD removal of around 80%. Further anaerobic degradation of the remaining COD was much slower and resulted in the build up of inert COD compounds generated as part of the metabolic activities in the anaerobic reactor. Respirometric analysis of the anaerobic reactor supernatant at different intervals provided useful information on the

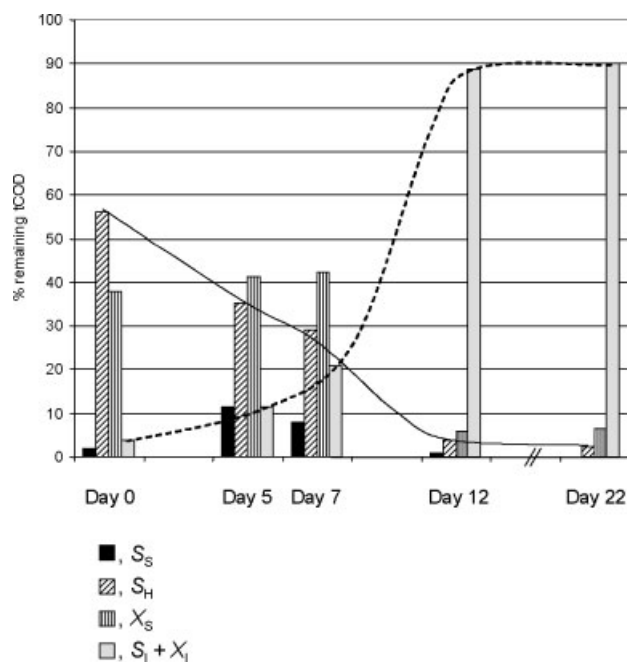


Figure 4. Change in COD fractionation during anaerobic treatment.

evolution of COD fractionation expected efficiency of the aerobic treatment following the anaerobic phase. The results suggest that an appropriate combination of anaerobic and aerobic reactors would have to limit anaerobic degradation to around 80% of the tCOD and an effluent concentration above 1000 mg dm^{-3} , for the optimum operation of the following aerobic stage.

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