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ScienceDirect
Journal of Hazardous Materials 149 (2007) 700–706
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

Application of acidogenic fixed-bed reactor prior to anaerobic membrane bioreactor for sustainable slaughterhouse wastewater treatment

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Received 7 October 2006; received in revised form 8 April 2007; accepted 10 April 2007
Available online 19 April 2007

Abstract

High rate anaerobic treatment systems such as anaerobic membrane bioreactors (AMBR) are less popular for slaughterhouse wastewater due to the presence of high fat oil and suspended matters in the effluent. This affects the performance and efficiency of the treatment system. In this work, AMBR has been tried for slaughterhouse wastewater treatment. After the start up period, the reactor was operated with an average organic loading rate (OLR) of 4.37 kg TCOD m^{-3} d^{-1} with gradual increase to an average of 13.27 kg TCOD m^{-3} d^{-1}. At stable conditions, the treatment efficiency was high with an average COD and BOD_5 reduction of 93.7 and 93.96%, respectively. However, a reduction in the AMBR performance was shown with the increase of the OLR to 16.32 kg TCOD m^{-3} d^{-1}. The removal efficiencies of SCOD and BOD_5 were drastically decreased to below 53.6 and 73.3%, respectively. The decrease of the AMBR performance was due to the accumulation of VFAs. Thus, a new integrated system composed of a FBR for the acidogenesis step followed by the AMBR for methanogenesis step was developed. At high ORL, the integrated system improved the performance of the anaerobic digestion and it successfully overcame the VFA accumulation problem in the AMBR. The anaerobic treatment led to a total removal of all tested pathogens. Thus, the microbiological quality of treated wastewater fits largely with WHO guidelines.

Keywords: Slaughterhouse wastewater; Anaerobic membrane bioreactor; Fixed bed reactor

1. Introduction

The slaughterhouse industry poses a significant environmental impact by discharging effluents to receiving water containing high concentration of biodegradable organic matter [1]. Slaughterhouses generate a large volume of effluents. The consumption of water per slaughtered animal varies according to the animal and the process employed in each industry, and ranges from 1 to 8.3 m^3 [2]. Most of this amount is discarded as wastewater, with volumes from 0.4 to 3.1 m^3 per slaughtered animal being reported in the literature [3,4].

Slaughterhouse wastewaters composition is strong compared to domestic wastewater. The main contributors of organic load to these effluents are paunch, faecal, fat and lard indigested food, blood, suspended material, urine, loose meat, soluble proteins, excrement, and particles [5,6].

In wastewater treatment, biological processes are mainly used for the removal of organic pollution [7]. However, aerobic processes are not regarded as a suitable treatment option because of high-energy requirements for aeration, limitations in liquid-phase oxygen transfer rates and large quantities of sludge production.

In anaerobic degradation, enzymes hydrolysed complex organics such as: polysaccharides, proteins and lipids to sugars, amino acids and fatty acids. These intermediate products are then degraded by acidogens, forming volatile fatty acids, which are further degraded by acetogens, forming acetate, carbon dioxide and hydrogen. Acidogens grow relatively faster and are less sensitive to pH variation than acetogens/methanogens [8]. This usually results in the accumulation of organic acids and lowering of pH, leading to the suppression of methanogenic activities and in some cases, even process failure [9]. Instability or failure of single-phase methanogenic reactors has been widely reported for a variety of wastewaters, especially under high loading conditions [10].

Abbreviations: AMBR, anaerobic membrane bioreactor; BOD5, biological oxygen demand; FBR, fixed bed reactor; HRT, hydraulic retention time; MPN, most probable number; OLR, organic loading rate; SCOD, soluble chemical oxygen demand; SS, suspended solids; TCOD, total chemical oxygen demand; TSS, total suspended solids; VFAs, volatile fatty acids; VSS, volatile suspended solids

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doi:10.1016/j.jhazmat.2007.04.031

The use of two-phase anaerobic systems was originally conceptualized for the purpose of optimizing the environmental conditions for the different anaerobic process, i.e. hydrolysis and acidogenesis in the first phase and acetogenesis and methanogenesis in the second phase. Each system can be operated at its optimal condition [9]. Consequently the occurrence of an imbalance between the different groups of anaerobic bacteria, which may take place in one-phase system, could be prevented [11,12].

However, despite the advantages of two-phase separation, anaerobic digesters are single pass reactors without selective solids recycle which lead to the active biomass washout. Membrane bioreactor process, an effective and efficient membrane application for advanced wastewater treatment, usually combines biological wastewater treatment with microfiltration or ultrafiltration process to treat wastewater biologically and to separate biomass physically from mixed liquor in an integrated step. The membrane bioreactor has demonstrated its superiority in improving effluent quality over the past 20 years, and has been extended to advanced wastewater treatment due to stringent wastewater effluent regulations and the continuous development of membrane technology [13].

Recent research studies indicate feasibility of this process to treat domestic effluents as well [14,15]. The membrane bioreactor processes can be especially suitable for reuse and recycling of wastewater owing to their high-quality and disinfected effluents [16].

However, membrane bioreactor technology is currently facing some research and development challenges. The main challenge for membrane bioreactors has been fouling of membrane units [17–20]. Membrane fouling is the result of adsorption of organic matter, precipitation of inorganic matter, and adhesion of microbial cells to the membrane surface [18].

Membrane bioreactors have been widely adopted for high-strength industrial wastewaters treatment [21]. AMBRs were extensively applied in wastewater treatment field [11,22,23,17,14,15]. However, few numbers of studies performed with AMBR and real slaughterhouse wastewater were realised [23].

This article is focused on the application of a cross-flow anaerobic membrane bioreactor coupled to a fixed bed reactor for the treatment of slaughterhouse wastewater originated from the municipal slaughterhouse located in Sfax region, Tunisia.

2. Material and methods

2.1. Slaughterhouse wastewater

The wastewater used in this study was collected from the municipal slaughterhouse of Sfax region (Tunisia). This plant processes 700 head of bovine and 4500 head of sheep monthly and it generates solid residues and wastewaters. Part of the solid residues was manually recuperated in big baskets. The non-recuperated solid residues and the wastewater are discharged in sewage of the municipal wastewater treatment plant. The wastewater studied was collected after a primary screening of solids larger than 2 mm. In the laboratory, the wastewater was screened again to remove solids larger than 0.5 mm. After that, it was stored at −20 °C before introduced to the reactor by a peristaltic pump. Storage at a low temperature was necessary to reduce the microbial activity and maintain the characteristics of wastewater, since the environmental temperature was high and the time between collection and usage was long. Since the number of slaughtered animals varied considerably because of fluctuations in market demand, the composition of the wastewater also varied. The main characteristics of the wastewater are presented in Table 1. The slaughterhouse wastewater had an average TCOD concentration of approximately 15,880 mg l^{-1}, which about 60–75% was in soluble form and 25–40% in particulate matter.

Table 1
Composition of the raw slaughterhouse wastewater and the permeate during the experimental period at one-stage treatment (AMBR)

Parameters	Unit	Feed min–max	Permeate min–max
pH	–	7.53–7.7	7.36–7.67
Conductivity	mS cm^{-1}	1.928–3.32	1.26–4.63
TCOD	mg l^{-1}	7148–20400	–
SCOD	mg l^{-1}	5440–15500	265–1980
VFA	mg l^{-1}	26–131	91–480
BOD_5	mg l^{-1}	3501–8030	114–900
Fat	mg l^{-1}	233–310	0
Protein	mg l^{-1}	1890–3210	0

2.2. Experimental apparatus

2.2.1. Anaerobic membrane bioreactor

The experimental set-up was constructed within the frame of the INCO-MED project "ICA3-CT-1999-00013 MBR recycling" and it was installed in "Centre de Biotechnologie de Sfax, Tunisia". The schematic diagram of the experimental set-up is shown in Fig. 1. The jet flow anaerobic bioreactor (4) was constructed of Plexiglas and has a total volume of 100 l and a working volume of 50 l. The temperature was maintained constant at 37 °C by circulating water through the water jacket of the reactor. The bioreactor is fed via peristaltic pump P2 from the acidified slaughterhouse wastewater reservoir. The influent was supplied through the nozzle (13) into the jet flow module. Nozzle is co-axially located at the top of an inner tube (12), this created a down flow in the inner tube and an up flow between the inner tube and the reactor wall. This circulation of the liquid allows a perfect homogenization of the medium. The reactor was coupled via a multistage centrifugal pump Lowara SV805 (2–3 kW, Q_{max} = 10–12 m^3/min at 5–6 bars, and frequency controlled by a Stöber FBS/FDS) to a TECHNOCON GmbH ultrafiltration system composed by a membrane module Stork (Friesland BV, 3.048 m long). The membrane, which was Stork WFFX 0281, had 1 m^2 area, and 100 kDa cut-off. The cross-flow velocity and the trans-membrane pressure were fixed at values of 3 m s^{-1} and 1 bar, respectively. A gas meter (Ritter, TG05) was used for measuring the biogas production (14).

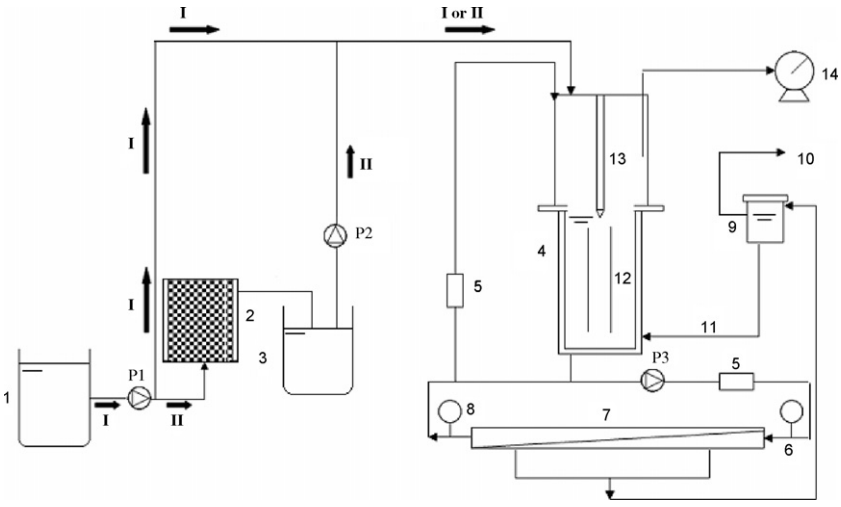

Fig. 1. Schematic diagram of the experimental process installed in Sfax, Tunisia: (I) application of AMBR for slaughterhouse wastewater treatment; (II) application of acidogenic FBR prior to AMBR for slaughterhouse wastewater treatment; (1) raw slaughterhouse wastewater reservoir; (P1 and P2) peristaltic feed pumps; (2) fixed bed reactor (acidogenic step); (3) acidified slaughterhouse wastewater reservoir; (4) jet flow anaerobic reactor (methanogenic step); (P3) circulation pump; (5) flow meter; (6) manometer; (7) ultrafiltration membrane; (8) manometer; (9) permeate tank; (10) permeate discharged in the sewage system; (11) permeate recycling; (12) inner tube; (13) nozzle; (14) gas flow meter.

2.2.2. Acidogenic fixed bed reactor (FBR)

The 25 l FBR (2) was filled with Hiflow ring. This reactor was arranged vertically in order to avoid reactor plugging due to the high solids concentration of the slaughterhouse wastewater [24]. The reactor was inoculated from a 50 l anoxic reactor operating with the same type of substrate and fed daily with raw slaughterhouse wastewater. The FBR was kept at room temperature.

2.3. Analytical methods

COD was determined according to standard method [25]. Five-day biological oxygen demand (BOD_5) was determined by the manometric method with a respirometer (BSB-Controller Model 620 T (WTW)). Total soluble proteins were determined according to the Bradford method [26]. Total Kjeldahl nitrogen was determined according to standard method [27].

Total solids (TS), volatile solids (VS), total suspended solids (TSS) and volatile suspended solids (VSS) were determined according to the standard methods [28]. To get the gas composition, gas samples were taken with a syringe from the tank of biogas and analysed by a gas chromatograph (Model: IGC11 of DELSI) equipped with a thermal conductivity detector. Volatile fatty acids (VFA) were analysed by a gas chromatograph (SHIMADZU GC-9A) equipped with a flame ionisation detector (SHIMADZU CR 6A). The conductivity and the pH were determined using a conductivimeter model CONSORT C 831 and a pH meter model Metrohm 744, respectively.

2.4. Microbial estimation

Total coliforms (TC), faecal coliforms (FC) and faecal streptococci (FS) were estimated according to water standards methods [29,30]. MPN determination of *Salmonella* (S) was carried out by modified method of Yanko et al. [31].

2.5. The MPN method for bacterial abundance

The most-probable-number technique (MPN, APHA) [32] was used for the enumeration of hydrolytic and methanogenic bacteria. The enumeration was performed in Hungate tubes containing 10 ml basal medium supplemented with 20 mM glucose for hydrolytic bacteria and 20 mM acetate for methanogenic bacteria. Decimal dilution series up to 10^{-12} were made in triplicate. Inoculated cultures were left at 37 °C for 2 weeks and measuring substrate degradation assessed growth bacteria. MPN values were calculated from McGrady statistical tables and expressed as cells ml^{-1} culture medium.

The basal medium used for bacteria enumeration was prepared using anaerobic techniques as described by Hungate [33] and modified for use with syringes [34,35]. This synthetic medium contained (per litre of bi-distilled water): 0.5 g yeast extract; 1 g NH_4Cl; 0.2 g $MgCl_2{\cdot}6H_2O$; 0.1 g KCl; 0.1 g $CaCl_2{\cdot}2H_2O$; 0.6 g NaCl; 0.25 g cysteine·HCl and 1 mg resazurin. The medium was supplemented with 1.5 ml trace element solution [36]. The pH was adjusted to 7 with 10 M KOH solution. The medium boiled under a stream of O_2-free N_2 gas and cooled to room temperature. 9-ml aliquots were dispensed into 20-ml Hungate tubes and subsequently sterilized by autoclaving at 121 °C for 20 min. Prior to culture inoculation, 0.2 ml of 5% (w/v) $NaHCO_3$ and 0.2 ml of 2.5% (w/v) $Na_2S{\cdot}9H_2O$ and 0.2 ml of glucose or acetate from sterile stock solutions.

3. Results and discussion

3.1. Methanisation performance

3.1.1. Start-up:

OLR (kg TCOD m^{-3} d^{-1}), biomass, SCOD and TCOD concentrations in the raw slaughterhouse wastewater and permeate

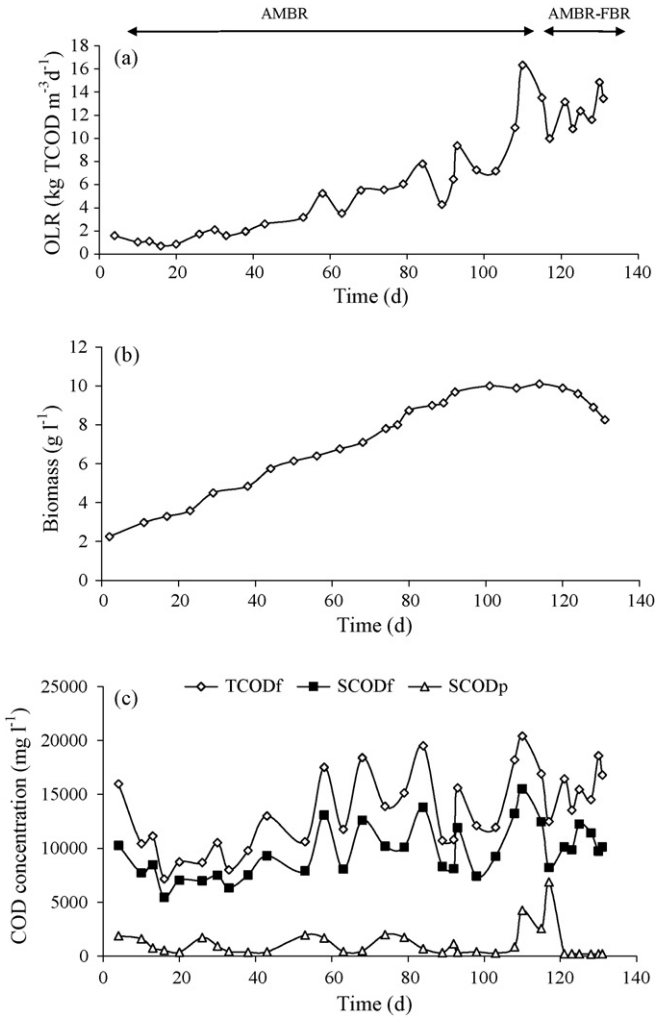

Fig. 2. Organic loading rate introduced in the AMBR during slaughterhouse wastewater methanisation (a); biomass concentration in the AMBR (b); TCOD, SCOD concentrations of the feed and SCOD concentration in the permeate (c).

SCOD concentrations, during the experiment are presented in Fig. 2. During the start-up period, the organic loading rate was progressively increased from 1.59 to 2.6 kg TCOD m^{-3} d^{-1} over a 40-day period (Fig. 2a). The increase of biomass concentration in the AMBR was observed during this period of time. After about 44 days, volatile suspended solids (VSS) concentration was 5.49 g l^{-1} (Fig. 2b).

During the first few days of start-up, the average SCOD of the raw slaughterhouse wastewater was 7648 mg l^{-1}, this value decreased to 359 mg l^{-1} in the permeate on day 38.

Soluble COD remained below 400 mg l^{-1} in the permeate during the rest of the start-up period (Fig. 2c). Thus, it can be concluded that, the anaerobic bacteria was acclimatized to the slaughterhouse wastewater. Indeed, start-up is the period during which the anaerobic bacteria are being acclimatized to new environmental conditions and substrate. A 40-day start-up was reported for a mesophilic anaerobic contact reactor treating slaughterhouse wastewater [37]. Borja et al. [38,39] also reported a 40-day start-up for an FBR treating slaughterhouse wastewater containing mainly soluble organics.

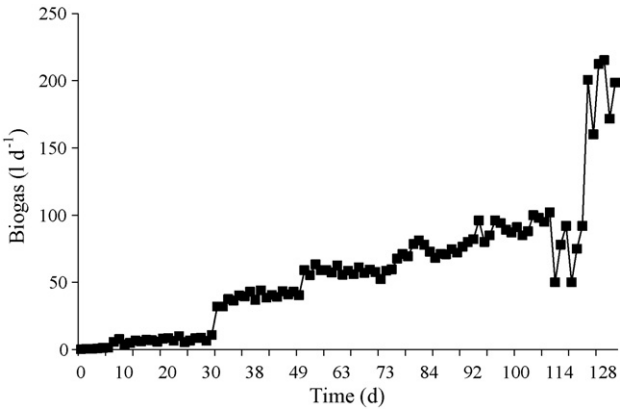

Fig. 3. Biogas production during slaughterhouse wastewater methanisation.

After the start-up period, the OLR introduced into the reactor was increased in order to study its influence on process efficiency.

3.1.2. Operation

In order to study the influence of OLR on process efficiency, values between 3.18 and 16.32 kg TCOD m^{-3} d^{-1} were applied. Between day 53 and 108, the average OLR was increased from 4.37 to 8.23 kg TCOD m^{-3} d^{-1} (Fig. 2a). Effluent SCOD remained below 400 mg l^{-1} in most effluent samples (Fig. 2c). However, at the beginning of each phase, when OLR increased, there was a decrease in the removal efficiency but the system recovered shortly and adapted to the new conditions with time.

Gas production increased progressively with the increase of OLR to reach a maximum value of 1021 d^{-1} at an HRT of 1.66 d (Fig. 3). The performance of the AMBR is summarized in Table 2. It shows the methane yield (l CH_4 per g of $SCOD_{removed}$), the average permeate SCOD concentration (SCODp) and the average COD removal efficiency (% COD) for the different average feed concentration (SCODf) used, the corresponding hydraulic retention times, the operation period and the average OLRs. The methane yield which ranged between 0.2 and 0.31 l CH_4 g $TCOD^{-1}_{removed}$ gives evidence that at stable conditions, the suspended organic matter was not simply retained by the membrane filtration but it was biologically degraded in the reactor.

Permeate quality indicated that suspended solids, fats and protein were completely removed (Table 1).

The volatile acidity was monitored in the reactor (Fig. 4), in permeate and in the raw slaughterhouse wastewater (Table 1). The VFAs concentrations in the reactor were ranged between 62 and 378 mg VFA l^{-1}, until day 108. Low VFAs concentration in the reactor during this experimental period suggested that methanization of the SCOD was achieved.

Between day 110 and 117, the bioreactor showed a drastically decrease of its performance. At day 110, the high loading rate of 16.32 kg TCOD m^{-3} d^{-1} (HRT = 1.25 days) had overloaded the system. Indeed, the increase of the OLR may have an important harmful effect on anaerobic biological processes, causing destabilization of the microbial populations. This leads to VFA accumulation (Fig. 4) (At day 117, more than 3000 mg l^{-1} of

Table 2
Summary of the AMBR performance at stable conditions

Operation period (d)	HRT (d)	OLR (kg TCOD m^{-3} d)	SCODf ($g\,l^{-1}$)	SCODp ($g\,l^{-1}$)	COD removal (%)	Yield l CH_4 g $SCOD^{-1}_{removed}$
One-phase anaerobic system						
46–73	3.33	4.37 ± 0.3	12.33 ± 2.31	0.445 ± 0.02	96.4 ± 0.75	0.31 ± 0.004
74–91	2.5	5.92 ± 1.28	12.43 ± 3.18	1.175 ± 0.32	90.6 ± 2.65	0.29 ± 0.005
92–109	1.66	8.23 ± 2.5	10.174 ± 3.31	0.338 ± 0.06	94 ± 2.12	0.2 ± 0.002
110–117	1.25	13.27 ± 2.6	12.05 ± 1.72	4.556 ± 1.35	62 ± 1.9	0.13 ± 0.006
Two-phase anaerobic system						
118–131	1.25	12.7 ± 1.71	10.58 ± 0.99	0.196 ± 0.004	98.75 ± 0.44	0.33 ± 0.001

VFA was measured in the reactor) that can acidify the reactor (the pH effluent taken from the reactor decreased to 6.5) and therefore inhibits methanogenic bacteria.

Borja et al. [40] detected VFA accumulation and an important removal efficiency decrease due to the inhibition of the digestion process at an influent slaughterhouse wastewater COD concentration of 29,000 mg l^{-1} in a down-flow fixed bed reactor. Moreover, biogas production has been shown to decline up to 50 l d^{-1} with increasing loading rate to an average value of 13.27 kg COD m^{-3} d^{-1}. The decrease of biogas production can be attributed not only to the inhibition of the methanogenic bacteria but also to the faster growing acidogenic bacteria at higher organic loading rates compared to methanogenic bacteria. The removal efficiencies of SCOD and BOD_5 were drastically decreased to below 53.6 and 73.3%, respectively.

Thus, to give the system the time to recuperate, the feeding of the reactor was turned off completely and a new setup, whose main feature is a two-phase digestion process, was developed. An anaerobic fixed bed reactor was selected for the acidogenesis due to its simplicity, its stability at higher loading rates and safety of operation. The pH-value was kept low to suppress a methanogenic reaction (Table 3). The effluent of the FBR was used to feed the AMBR after being cleared of particulates including detached anaerobic bacteria cells by filtration with a filter made of nylon net followed by a decantation in a settler tank.

Table 3 shows the important process parameters of the plant. VFAs composition in the anoxic FBR is given in Fig. 5. At an average OLR of 12.7 kg TCOD m^{-3} d^{-1}, the mean VFA concentration in the acidified effluent was 2.524 g VFA l^{-1}, in which

Table 3
The average process parameters of the plant

	Acidogenic step		Méthanogenic step	
	FBR	Effluent FBR	MBR	Perméat
pH	7.6	5.2	7.76	7.6
TCOD influent ($g\,l^{-1}$)	15.88	–	–	–
SCOD effluent ($g\,l^{-1}$)	–	12.23	–	0.196
SCOD influent ($g\,l^{-1}$)	–	–	12.23	–
TSS at day 131(reactor) ($g\,l^{-1}$)	–	–	8.257	–
Total VFA ($g\,l^{-1}$)	–	2.524	0.262	0.165

acetic acid (51.23%) and propionic acid (19.36%) were the main products of the total VFAs. Additionally, the anaerobic membrane bioreactor VFA concentration reached an average of 262 mg l^{-1} (Table 3) and the COD effluent concentrations of the most samples were less than 200 mg l^{-1} (Fig. 2c). Furthermore, in the combined process, biogas production increased progressively with increasing the OLR to reach a maximum value of 215 l d^{-1} at an HRT of 1.25 days and the methane yield increased to an average of 0.311 l CH_4 g $COD^{-1}_{removed}$.

Therefore, the two stage anaerobic system successfully overcame the problem of VFA accumulation and showed a significant improvement in process efficiency as measured by COD removal (98.75% in average) and biogas conversion.

3.2. Microbiology and biomass concentration

The results for *Salmonella*, total and feacal coliform and feacal *Streptococci*, as measured in the influent, in the reactor and in permeate are presented in Table 4. Total coliform, feacal col-

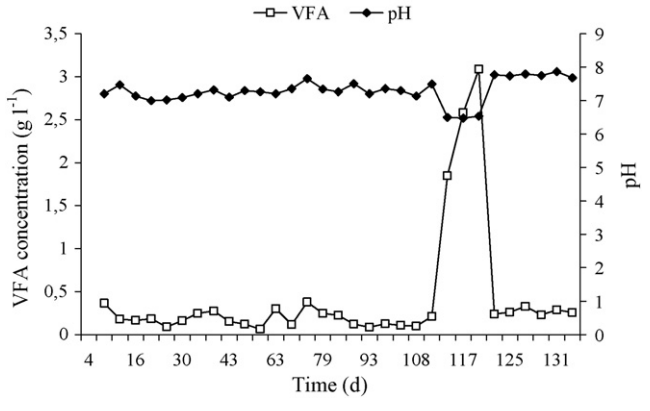

Fig. 4. Variation of pH and VFA concentration in the anaerobic membrane bioreactor.

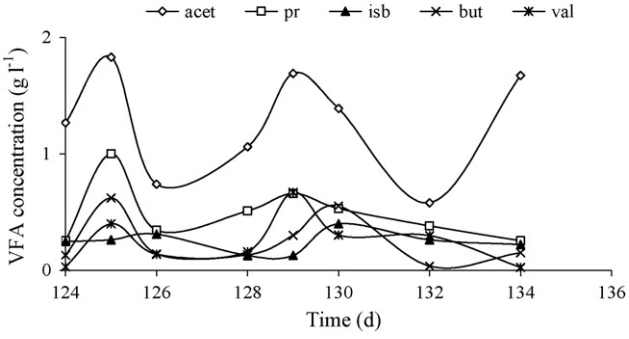

Fig. 5. VFA concentrations in the fixed bed reactor.

Table 4
Microbiological characteristics of raw and permeate wastewater

	Unit	Raw wastewater	Permeate
Total bacteria	CFU ml^{-1}	6×10^8 to 25×10^9	0
Total coliform	CFU ml^{-1}	20×10^7 to 32×10^8	0
Feacal coliform	CFU ml^{-1}	7×10^7 to 21×10^8	0
Streptococci	MPN ml^{-1}	1.1×10^3 to 2.1×10^3	0
Salmonella	–	+	–

iform, and feacal *Streptococci* are all below detection limit in the reactor (data not shown). These bacteria are completely removed in permeate. *Salmonella* are also not detected in both reactor and permeate.

Fig. 2b shows the evolution of the sludge concentration in the bioreactor. Despite the use of a pump for recycling the biomass in the AMBR, after the start-up period, the sludge concentration increased with time reaching at the day 114 a value of 10.1 g VSS l^{-1}. The increase in sludge concentration was due to the biomass recycling in the reactor and the efficient anaerobic operating conditions (pH, temperature and loading rate) for the anaerobic bacteria. However, several reports in literature pointed out the negative effects of pumping on the activity of methanogenic sludge and they showed that 50% of the activity is lost at a pumping rate of 20 cycles [41].

The use of the integrated acidogenic fixed-bed reactor-anaerobic membrane bioreactor for slaughterhouse wastewater treatment caused a slightly decrease of the biomass in the AMBR from 10.1 to 8.257 g VSS l^{-1}. This was possible because the overall reaction was divided into two steps and therefore, only methanogenic bacteria were necessary for the reaction in AMBR.

The metabolic stages involved in the production of methane from wastes are hydrolysis, acidogenesis, acetogenesis and methanogenesis. The numbers and types of microorganisms present in digesters are likely to depend upon the type of digester, its operating conditions and the waste composition. Hydrolytic and acidogenic stages may be combined in the anaerobic acidogenic bacteria. In the anaerobic membrane bioreactor sludge there are 31 10^9 MPN ml^{-1} of hydrolytic bacteria. Methanogens are present in AMBR biomass at population up to 45 10^5 MPN ml^{-1}. Limited ranges of substrates are used by the methanogens, acetate and $H_2 + CO_2$ being the most important substrates in anaerobic digestion. Microscopic observation of the biomass showed that the density of the methanogens increased slightly during the study (data not shown).

3.3. Filtration performance

The profile of permeate flux versus time at constant cross-flow velocity (3 m s^{-1}), is shown in Fig. 6. Three phases of flux decline were observed: firstly, a rapid exponential decline from 20 to 8 l h^{-1} m^{-2} after the first day, when the VSS concentration in the AMBR was 2.25 g l^{-1} (data not shown), then a gradual reduction and finally a steady state flux with an average value of 2.22 l h^{-1} m^{-2}. Between days 12 and 80, when the permeate flux reached steady state, the biomass concentration increased progressively to 10.1 g l^{-1}. The decline in permeate flux can be explained by pores clogging and the cake formation during the filtration.

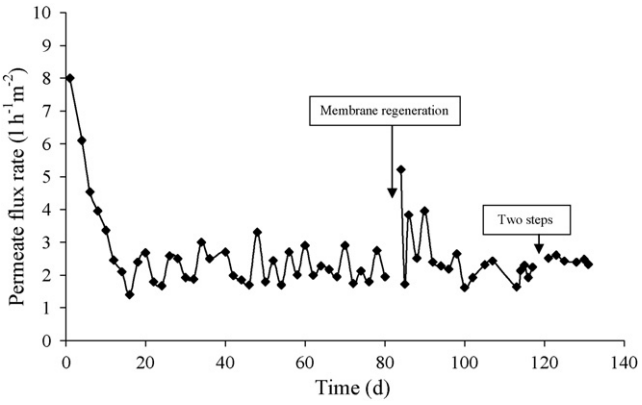

Fig. 6. Flux variation during slaughterhouse wastewater treatment.

To improve the flux rate, a cleaning cycle of the membrane was operated at the day 81. A slight increase in permeate flux rate was observed and then it reached the same average flux rate shown at the steady state.

In order to decrease the membrane fouling and to improve the AMBR performance at high organic loading rate, slaughterhouse wastewater was treated in two-phase step (acidogenesis, methanogenesis). Fig. 6 shows that the flux rates showed a slight increase to an average of 2.46 l h^{-1} m^{-2}. The hydrodynamic operation conditions of the membrane filtration were similar to the single step mode (TMP = 1 bar, V_s = 3 m s^{-1}).

4. Conclusions

The following conclusions can be reached from the results obtained in this work. The AMBR proved to be efficient for the treatment of slaughterhouse wastewater when operated with an average organic loading rate less than 13.27 kg TCOD m^{-3} d^{-1}. The increase of the OLR introduced in the reactor (16.32 kg TCOD m^{-3} d^{-1}) caused a drastic decrease of the AMBR performance. This is believed to be due to the accumulation of fermentation intermediates in the form of VFAs at high OLR. The two-stage anaerobic treatment system of the anaerobic filter as acidogenic reactor and the AMBR as methanogenic reactor successfully overcame the problem and showed a significant improvement in process efficiency as measured by COD removal and biogas conversion. As to microbiological characterisation, the permeate quality was free from all microorganisms and it was conform to the microbial WHO standards.

Acknowledgements

Thanks to financial support provided by the "Ministère de l'Enseignement Supérieur, de la Recherche Scientifique et de la Technologie of Tunisia under « Contrats Programme du Laboratoire des Bioprocédés ».

References

[1] M. Kobya, E. Senturk, M. Bayramoglu, Treatment of poultry slaughterhouse wastewaters by electrocoagulation, J. Hazard. Mater. 133 (2006) 172–262.

[2] C.E.T. Caixeta, M.C. Cammarota, A.M.F. Xavier, Slaughterhouse wastewater treatment: evaluation of a new three-phase separation system in a UASB reactor, Bioresour. Technol. 81 (2002) 61–69.

[3] Z. Wang, C.J. Banks, Evaluation of a two stage anaerobic digester for the treatment of mixed abattoir wastes, Process Biochem. 38 (2003) 1267–1273.

[4] W.P. Tritt, F. Schuchardt, Materials flow and possibilities of treating liquid and solid wastes from slaughterhouses in Germany, Bioresour. Technol. 41 (1992) 235–245.

[5] N.Z. Al-Mutairi, M.F. Hamoda, I. Al-Ghusain, Coagulant selection and sludge conditioning in a slaughterhouse wastewater treatment plant, Bioresour. Technol. 95 (2004) 115–119.

[6] N.Z. Al-Mutairi, Coagulant toxicity and effectiveness in a slaughterhouse wastewater treatment plant, Ecotoxicol. Environ. Saf. 65 (2006) 74–83.

[7] G.S. Mittal, Treatment of wastewater from abattoirs before land application—a review, Bioresour. Technol. 97 (2006) 1119–1135.

[8] A. Cohen, A.M. Breure, J.G. van Andel, A. van Deursen, Influence of phase separation on the anaerobic digestion of glucose-I maximum COD-turnover rate during continuous operation, Water Res. 14 (1980) 1439–1448.

[9] H.Q. Yu, H.H.P. Fang, Acidification of mid-and high-strength dairy wastewater, Water Res. 35 (2001) 3697–3705.

[10] S. Ghosh, K. Buoy, L. Dressel, T. Miller, G. Wilcox, D. Loos, Pilot-and full-scale two-phase anaerobic digestion of municipal sludge, Water Environ. Res. 67 (1995) 206–214.

[11] A. Saddoud, I. Hassaïri, S. Sayadi, Anaerobic membrane reactor with phase separation for the treatment of cheese whey, Bioresour. Technol. 98 (2007) 2102–2108.

[12] S. Ghosh, Pilot-scale demonstration of two-phase anaerobic digestion of activated sludge, Water Sci. Technol. 23 (1991) 1179–1188.

[13] T. Stephenson, S. Judd, B. Jefferson, K. Brindle, Membrane Bioreactors for Wastewater Treatment, IWA Publishing, London, 2000.

[14] A. Saddoud, M. Ellouze, A. Dhouib, S. Sayadi, A comparative study on the anaerobic membrane bioreactor performance during the treatment of domestic wastewaters of various origins, Environ. Technol. 27 (2006) 991–999.

[15] A. Saddoud, M. Ellouze, A. Dhouib, S. Sayadi, Anaerobic membrane bioreactor treatment of domestic wastewater in Tunisia, Desalination 207 (2007) 205–215.

[16] N. Cicek, H. Winnen, M.T. Suidan, B.E. Wrenn, V. Urbain, J. Manem, Effectiveness of the membrane bioreactor in the biodegradation of high molecular weight compounds, Water Res. 32 (1998) 1553–1563.

[17] Y. He, P. Xu, C. Li, B. Zhang, High-concentration food wastewater treatment by an anaerobic membrane bioreactor, Water Res. 39 (2005) 4110–4118.

[18] K.H. Choo, C.H. Lee, Membrane fouling mechanisms in the membrane-coupled anaerobic bioreactor, Water Res. 30 (1996) 1771–1780.

[19] S. Elmaleh, L. Abdelmoumni, Cross-flow filtration of an anaerobic methanogenic suspension, J. Membr. Sci. 131 (1997) 261–274.

[20] K.H. Choo, I.J. Kang, S.H. Yoon, H. Park, J.H. Kim, S. Adiya, C.H. Lee, Approaches to membrane fouling control in anaerobic membrane bioreactors, Water Sci. Technol. 41 (2000) 363–371.

[21] P. Sutton, Membrane bioreactors for industrial wastewater treatment: the state-of-the-art based on full scale commercial applications, in: Proceedings of 2003 Annual Symposium of Water Environment Federation, Los Angeles, 11–15 October, 2003.

[22] F.I. Hai, K. Yamamoto, K. Fukushi, Performance of newly developed hollow fiber module with spacer in integrated anaerobic–aerobic fungi reactor treating textile wastewater, Desalination 199 (2006) 305–307.

[23] W. Fuchs, H. Binder, G. Mavrias, R. Braun, Anaerobic treatment of wastewater with high organic content using a stirred tank reactor coupled with a membrane filtration unit, Water Res. 37 (2003) 902–908.

[24] R. Del Pozo, V. Diez, S. Beltran, Anaerobic pre-treatment of slaughterhouse wastewater using fixed-film reactors, Bioresour. Technol. 71 (2000) 143–149.

[25] R.J. Knechtel, A more economical method for the determination of chemical oxygen demand, Water Pollut. Control. (1978) 25–29.

[26] M.M. Bradford, A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dyebinding, Anal. Biochem. 72 (1976) 248–254.

[27] J. Kjeldahl, A new method for the determination of nitrogen in organic matter, Z. Anal. Chem. 22 (1883) 366.

[28] APHA, Standard Methods for the Examination of Water and Wastewater, eighteenth ed., American Public Health Association, Washington, DC, 1992.

[29] ISO 4832, Microbiology—General Guidance for the Enumeration of Coliforms—Colony Count Technique, International Organization for Standards, Geneva, 1991.

[30] AFNOR NF T90-411, Recherche et dénombrement des streptocoques du groupe D, *Méthode générale par ensemencement en milieu liquide* (*NPP*), AFNOR, Paris, 1989.

[31] W.A. Yanko, A.S. Walker, J.L. Jackson, L.L. Libao, A.L. Gracia, Enumerating Salmonella in biosolids for compliance with pathogens regulations, Water Environ. Res. 67 (1995) 364–370.

[32] APHA, Standard Methods for the Examination of Water and Wastewater, seventeenth ed., American Public Health Association, Washington, DC, 1989.

[33] R.E. Hungate, A roll-tube method for the cultivation of strict anaerobes, Meth. Microbiol. 136 (1969) 194–198.

[34] J.M. Macy, J.E. Snellen, R.E. Hungate, Use of syringe methods for anerobiosis, Am. J. Clin. Nutr. 25 (1972) 1318–1323.

[35] T.L. Miller, M.J. Wollin, A serum bottle modification of the Hungate technique for cultivating obligate anaerobes, Appl. Microbiol. 27 (1974) 985–987.

[36] D. Imhoff-Stuckle, N. Pfennig, Studies on the spectrometric determination of DNA hybridization from renaturation rates, Syst. Appl. Microbiol. 4 (1983) 184–192.

[37] C.R. Kostyshyn, W.A. Bonkoski, J.E. Sointio, Anaerobic treatment of a beef processing plant wastewater: a case history, in: Proceedings of the 42nd Industrial Waste Conference, 1988, pp. 673–692.

[38] R. Borja, C.J. Banks, Z. Wang, Performance and kinetics of an upflow anaerobic sludge blanket (UASB) reactor treating slaughterhouse wastewater, J. Environ. Sci. Health A29 (1994) 2063–2085 (Part A).

[39] R. Borja, C.J. Banks, Z. Wang, Stability and performance of an anaerobic downflow filter treating slaughterhouse wastewater under transient changes in process parameters, Biotechnol. Appl. Biochem. 20 (1994) 371–383.

[40] R. Borja, Ch. Banks, A. Martin, Influence of the organic volumetric loading rate on soluble chemical oxygen demand removal in a down-flow fixed-bed reactor treating abattoir wastewater, J. Chem. Technol. Biotechnol. 64 (1995) 361–366.

[41] M. Brockmann, C.F. Seyfried, Sludge activity under the conditions of crossflow microfiltration, Water Sci. Technol. 35 (1997) 173–181.