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Improvement by Micro-Aeration of Anaerobic Digestion of Slaughterhouse Wastewater At 38°C

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ABSTRACT: The anaerobic digestion of slaughterhouse wastewaters, used as a model of fluids with high TSS content, was improved by application of micro-aeration of the influent. Digestibility of the total suspended solids (TSS) was improved to prevent their accumulation in the sludge and protect methanogens from their inhibition effect. This was ensured by a controlled dissolution of oxygen in the influent, prior to the anaerobic digestion, by continuous aeration with air at a rate of 15 ml/min, corresponding to 0.05 vvm (volume of air per volume of feeding solution per minute). The methane production yields were of almost 350 and 200 ml CH_4 per removed g of COD_{sol} and COD_{tot}, respectively. The micro-aeration of the influent also lead to application of higher organic loads to the treatment system, besides the high methane production yields. These results were obtained at 38°C, which is not common and suitable to anaerobic digestion, but appropriate for enhanced hydrolytic enzymes activities. It was clearly shown, that the improvement of methane production yields was associated to dissolution of TSS. These findings are of great importance from the practical point of view, and technically evidence the emerging idea of tolerance of low aeration in anaerobic technologies.

KEYWORDS: Anaerobic, Micro-aerobic, Slaughterhouse wastewater, digestion, Methanogenic.

I. INTRODUCTION

Slaughterhouses wastewaters are characterized by variations in characteristics and quantities [12]. This is, mainly, attributed to differences in processing, animal species and wastewater management [13]. These wastewaters are considered complex, due to almost 40 % - 50% of organic matter as insoluble and slowly biodegradable suspended solids [13]. Similarly, total COD of the slaughterhouses wastewaters includes 40% to 50% insoluble COD [13]. These solids are originated from the lipids and proteins present in the wastewater and also include lignocellulosic substances and bacterial cells if manure is mixed with the wastewater [3]. The suspende.d solids are therefore composed of polymeric substances that should first be hydrolyzed through liquefaction process to become soluble, before their biological consumption [6]. Consequently, the slaughterhouses wastewaters are comparable to municipal wastewaters and dilute manure effluents, regarding the slowly biodegradability of their suspended solids [12]. These suspended solids are considered responsible of the low methanogenic capacity of the treatment system of such wastewaters, due to accumulation and entrapment of non-biomass coarse suspended solids form the wastewater in the sludge, resulting in a dilution of the active biomass causing thus a decrease in the methanogens counts [14]. In addition, the entrapment of the cells biomass by a film of increasing thickness would hamper the supply of the substrate to the cells present in the sludge aggregate, leading to deterioration of the specific methanogenic activity. This phenomenon was observed with municipal wastewater [14]. Similarly, it was shown that the poor biodegradability of these organic solids in the treatment systems, results in less complete liquefaction of the biodegradable fraction of the solids, and consequently the deterioration of the specific activity of methanogens in the sludge [4, 5, 14].

Liquefaction of the biopolymers occurs through the action of exoenzymes secreted by the hydrolytic bacteria. Accordingly, theses hydrolytic enzymes identified in anaerobic digesters as well as from rumen populations are

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lipolytic, proteolytic and cellulolytic ones [5]. In the anaerobic digestion of wastewaters containing high amounts of insoluble substrates, such as manure and piggery wastes, the liquefaction step, frequently, is reported as being the rate limiting step in the overall process [5]. The rate of liquefaction is dependent on the chemical composition of the substrate but also the environmental factors such as, particularly, the temperature.

Anaerobic systems were developed and actually, are operational in many countries for the treatment of slaughterhouses wastewaters [1, 3, 4, 5]. The UASB technology was particularly shown the most efficient technology [3]. But, all the successful systems were based on treating low suspended solids slaughterhouses wastewaters [16]. In Qatar, the content of suspended solids in such slaughterhouses wastewaters is very high, especially due to the processing systems, with limited use of water (personal communication from Mawashi Company of Slaughtering in Qatar). It could represent an appropriate slaughterhouses wastewater for studying the effect of high solid contents on the anaerobic digestion.

Previous reported literature showed that a limited aeration enhanced a lot the methane yield in anaerobic digestion [6, 7, 8, 9, 10], predicting the existence of an optimum oxygenation level corresponding to a maximum methane yield [10]. A few other studies also suggested that limited aeration can lead to enhanced methane generation [9, 10, 14]. Pirt and Lee [15] reported that traces of oxygen enhanced the anaerobic digestion of algal biomass in batch mode reactors. Similarly, it was reported that about a 20% increase in methane production was noticed at low oxygen fluxes in a chemostat provided with headspace oxygen [14, 15]. This was mostly due to increase of the rate of the hydrolysis of the biodegradable suspended solids and their liquefaction by exoenzymes, produced by the first step of the anaerobic digestion step, which is mainly aerobic [10].

Here, the objective of this work is to investigate the enhancement of the anaerobic digestion of slaughterhouses wastewaters containing high contents of organic solids, by micro-aeration of the anaerobic system, leading to a high methane yield and high rate anaerobic digestion. The temperature of the systems was fixed at 38°C, corresponding to the average temperature of most of hydrolytic enzymes, knowing that optimal temperatures for the overall anaerobic digestion process are much lower . The choice of the temperature of 38°C may be favorable to increase the rate of enzymatic hydrolysis of the suspended solids.

II. MATERIAL AND METHODS

II.1. Wastewater samples

Wastewater samples were taken from a local slaughterhouse company (Almawashi slaughterhouse) in Doha (Qatar). For their characterization, wastewaters were freshly sampled once or twice a week, and immediately analyzed without any conservation time. The stock wastewater used in the experiments was characterized and stored in 2000 ml bottles at -20°C. Before use, each bottle was defrosted, conserved at +4°C and used for feeding the reactors as described in the feeding procedure. The homogeneity of the stock solution was ensured by agitation to ensure reproducible amounts of total suspended solids in the samples.

The stock wastewater contained 7466 mg/l Total COD (COD_{tot}), 4271 mg/l Soluble COD (COD_{sol}) and 3643 mg/l Total Suspended Solids (TSS). The complete composition is detailed in Table 1.

II.2. Anaerobic sludge

The sludge used in all the experiments came from an auto-digested sludge, sampled from the slaughterhouse reservoir. This reservoir collects wastewaters of the slaughter and then delivers the wastewaters to the sewer system after a short sedimentation time of almost 1h. The sludge was sampled almost 2 m in depth, from the bottom of the reservoir. It contained 160 g/l Total Suspended Solids (TSS) and 122 g/l Volatile Suspended Solids (VSS).

II.3. Reactors and batch experiments

Digesters of a total volume of 1 l were used. 4 digesters (bottles of 18 cm height and 10 cm diameter) were fixed in a water bath set at 38°C. The working volume of all the reactors is 525 ml, including 250 ml sludge, at time zero of the experiments. The initial reactor medium (sludge + wastewater) contained 76.2 g/l TSS corresponding to 58.1 g/l VS. All batch reactor experiments were carried out at 38°C and at an initial pH of 7.2. The anaerobic digesters are gently manually stirred once a day. The aerobic digester was magnetically stirred at 100 rpm.

Sludge in each reactor was kept almost 2 weeks at the corresponding condition to ensure adaptation of the biomass and reaching the steady state, before starting counting methane production and measuring CODs.

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The reactors were continuously fed with the stock wastewater, using a pre-calibrated peristaltic pump, as following:
- OLR (Organic Loading Rate) of 1 g COD/l.d: 70 ml stock wastewater per day, corresponding to a HRT (Hydraulic Retention Time) of 7.5 d.
- OLR (Organic Loading Rate) of 2 g COD/l.d: 140 ml stock wastewater per day, corresponding to a HRT (Hydraulic Retention Time) of 3.75 d.
- OLR (Organic Loading Rate) of 3 g COD/l.d: 211 ml stock wastewater per day, corresponding to a HRT (Hydraulic Retention Time) of 1.87 d.
- OLR (Organic Loading Rate) of 4 g COD/l.d: 280 ml stock wastewater per day, corresponding to a HRT (Hydraulic Retention Time) of 0.94 d.

A diagram of the setup used for the anaerobic, micro-aerobic and aerobic batch reactors is presented in Fig.1. The outcoming biogas passed through a 1.5% (w/v) NaOH solution for trapping CO_2, NH_3, H_2, NO_2 and H_2S gases if any. The volume of methane produced was determined using a liquid displacement system, based on inversed graduated cylinder. Only methane passes through the solution and equivalent volume is pushed out of the cylinder.

The batch reactor used for strict anaerobic digestion was fed with the stock wastewater without any aeration of the feeding solution. The 2 reactors used at micro-aeration conditions were fed with the stock wastewater from the feeding solutions which were continuously aerated with air at approximate rates of 15 ml min^{-1} and 45 ml/min respectively. These rates corresponded to 0.05 vvm and 0.15 vvm (volume of air per volume of feeding solution per minute). The air was pumped into the feeding solutions through a pre-calibrated air flow regulator.

The batch reactor used for the aerobic digestion was fed with the stock wastewater without any aeration of the feeding solution. This reactor was continuously fed with air at a rate of almost 500 ml/min corresponding to 1 vvm. The air was pumped into the reactor through a pre-calibrated air flow regulator. Air was dispersed in the reactor using an air diffusing stone.

The volume of the feeding solutions was 100 ml, 200 ml, 250 ml and 300 ml respectively for OLR of 1 g COD/l.d, 2 g COD/l.d, 3 g COD/l.d and 4 g COD/l.d. The aeration rates of the feeding solutions were calculated on the basis of these volumes.

II.4. Chemical analyses

The measurements of COD, suspended solids and volatile solids were carried out according to standard methods [2]. Samples for soluble COD (COD_{sol}) were centrifuged at almost 4000 rpm for 15 min prior to being filtered through a washed 0.45 µm retention cellulose nitrate membrane filter (Whatman Ltd, Piscataway, NJ, USA). Suspended solids were measured after evaporation at 103°C, when the weight was constant (generally after 24 h evaporation). The volatile matter was measured after burning the dry solids in a furnace set at 550°C.

III. RESULTS

III.1. Importance of TSS in slaughterhouses wastewaters

Table 1 shows the composition and characteristics of wastewaters of the slaughterhouse in Doha (Qatar). During almost 5 months (November 2012-April 2013), samples were sampled and analyzed twice a week. Table 1 gives the lowest and the highest values of each parameter. It is clear that this wastewater is characterized by strong fluctuations in concentrations of the main components, from single to double or even triple with some components, such as TSS which in turn represent 33 to 46% of the total dry matter. Similarly, COD in TSS represent 32 to 44% of the total COD. In addition, the analyses show that only 84 to 95% of COD are exhibited by the total dry matter. This means that TSS are important to consider in any treatment process of the total wastewater. The stock wastewater used in the digestion experiments was selected to be representative of the strongest composition. This would allow to clearly show the role of the TSS in the anaerobic digestion of the slaughterhouses wastewaters. This feeding stock solution contained 3643 mg/l TSS, 7466 mg/l COD_{tot}, 4271 mg/l COD_{sol}. Indeed, TSS represent 42.8 % of the total COD.

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Table 1; Characteristics of the slaughterhouses wastewaters in Qatar and the stock wastewater used in the current work

Parameters	*Crude Wastewater*		*Stock Wastewater*
	*Low**	*High***	
TSS (mg/l)	1282	3733	3643
TDM (mg/l)	3822	8051	7901
SDM (mg/l)	2540	4318	4301
pH	6.5	7.2	6.9
COD_{Tot} (mg/l)	3220	7679	7466
COD_{TSS} (mg/l)	1037	3371	3304
COD_{sol}(mg/l)	2183	4308	4271
(TSS/TDM)100	33.5	46.4	46.1
(COD_{TSS}/COD_{Tot})100	32.2	43.9	44.2
(COD_{Tot} /TDM)100	84.2	95.4	94.5
(COD_{sol} /TDM)100	57.1	53.5	54.1

*: The lowest values obtained in the samples
**: The highest values obtained in the samples

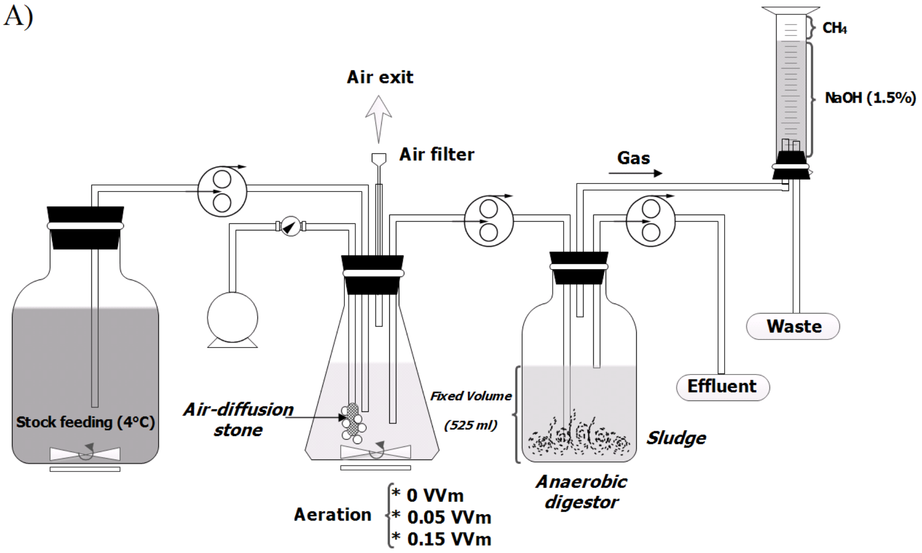
III.2. Effect of oxygen of the digestion of slaughterhouses wastewaters

The experimental procedure described in Fig.1, was designed to perform digestion of the stock feeding wastewater, at strict anaerobic, aerobic and 2 micro-aerobic conditions. The micro-aerobic conditions were ensured by low and high aeration of the feeding solutions before feeding the anaerobic digesters. They are named, Low-micro-aerobic and High-micro-aerobic, respectively.

III.2.1 *Anaerobic and micro-aerobic digestion of slaughterhouses wastewaters*

Fig. 2 shows results obtained at strict anaerobic and Low-micro-aerobic conditions. At strict anaerobic conditions, it is clear that the digestion of such wastewater is high at an optimal OLR of 3 g/l.d, with an efficiency of almost 98%, considering COD_{sol} and 95% considering COD_{tot}. In contrast, the methane production yields were very different, of 167 ml and 295 ml CH_4 per removal of 1 g of COD_{total} and COD_{sol}, respectively. In addition, Fig. 3 shows that TSS and VS continuously increased in the anaerobic sludge. This means that part of the TSS of the influent were not completely digested, but accumulated in the sludge. This is well evidenced from Fig. 2, where the OLR was increased to 4 g COD/l.d, by which the efficiency of digestion was dramatically decreased as well as methane yields.

A)



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B)

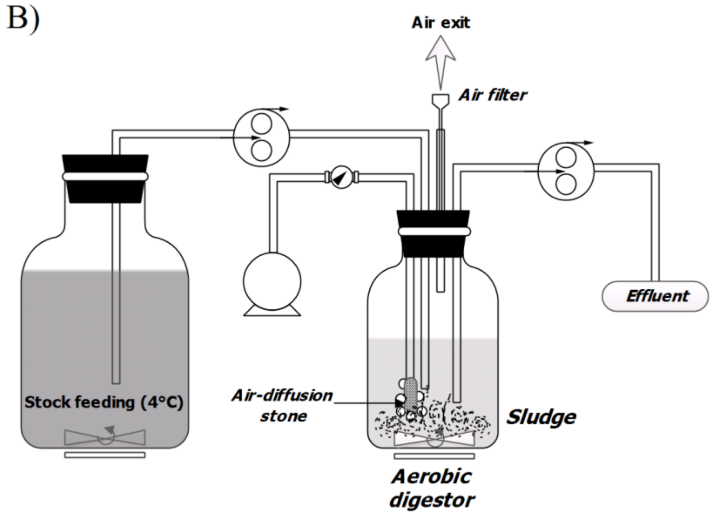
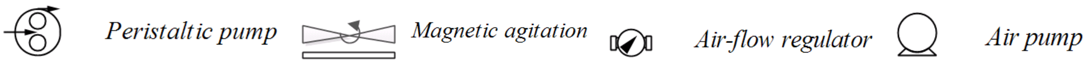
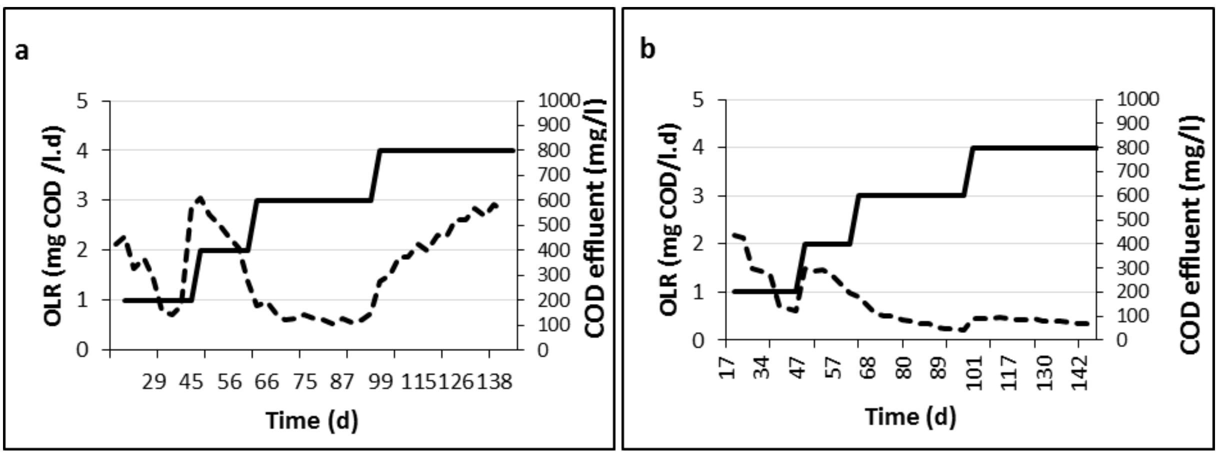


Fig. 1. Diagram of the setup used for the anaerobic, micro-aerobic and aerobic batch reactors.(*A*) Setup used for the strict anaerobic and micro-aerobic digestion of Qatari slaughterhouse wastewater. (*B*) Setup used for the aerobic digestion of Qatari slaughterhouse wastewater.



When developing an hybrid technology based on the anaerobic treatment for methane production and pre-aeration for creation of a micro-aerobic conditions within the anaerobic sludge, results of Fig. 2 clearly show that the efficiency of treatment was higher than 98% even with OLR of 4 g COD/l.d, considering both COD_{tot} and COD_{sol}. The methane production yields were of almost 350 and 200 ml CH_4 per removed g of COD_{sol} and COD_{tot}, respectively. They are much higher than those obtained at strict anaerobic conditions (Fig. 2). The methane yield of 350 ml/g COD_{sol} is close to the theoretical yield of 365 ml/g removed COD, obtained with complex wastewaters.



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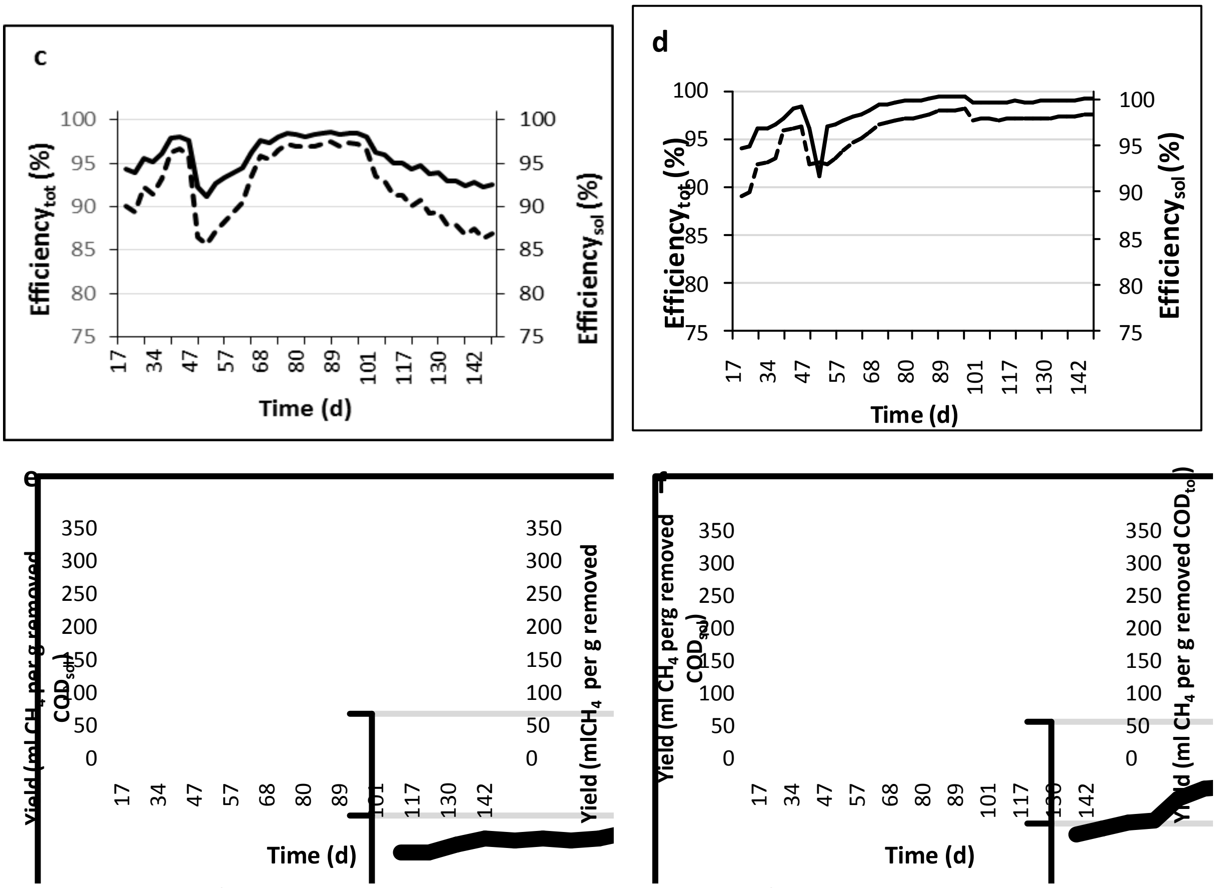

Fig. 2. Digestion of the Qatari slaughterhouse wastewaters at strict anaerobic (a, c, f) and Low-micro-aerobic (b, d, e) conditions.
a, b: COD in the effluent with increasing the OLR: (_______) OLR; (------) $COD_{effluent}$
c, d: Efficiency of the treatments considering the soluble COD (COD_{sol}) or the total COD (COD_{tot}) in the feeding solution: (_______) $Efficiency_{tot}$; (------) $Efficiency_{sol}$
e, f: Methane production yields considering the soluble COD (COD_{sol}) or the total COD (COD_{tot}) in the feeding solution: _______Yield (ml CH_4 per g removed COD_{sol}),(------) Yield (ml CH_4 per g removed COD_{tot})

In addition, Fig. 3 shows that the increase in TSS and VS in the sludge may only be explained by the accumulation of microbial biomass, which is theoretically of almost 20% of the removed COD, due to bacterial growth.

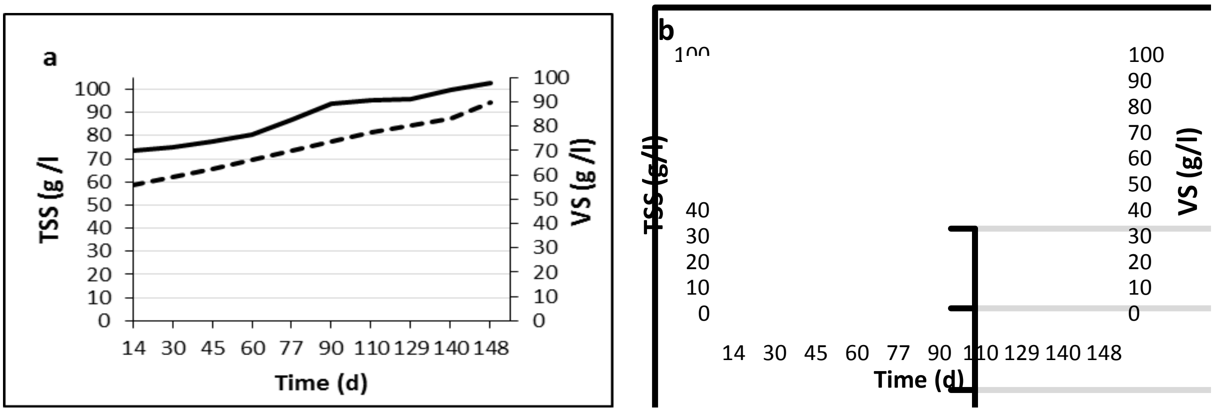

Fig.3. Total Suspended Solids (TSS) and Volatile Solids (VS) in the sludge during the digestion of slaughterhouse wastewater at strict anaerobic (a) and low- micro-aerobic conditions. (_______)TSS, (------) VS

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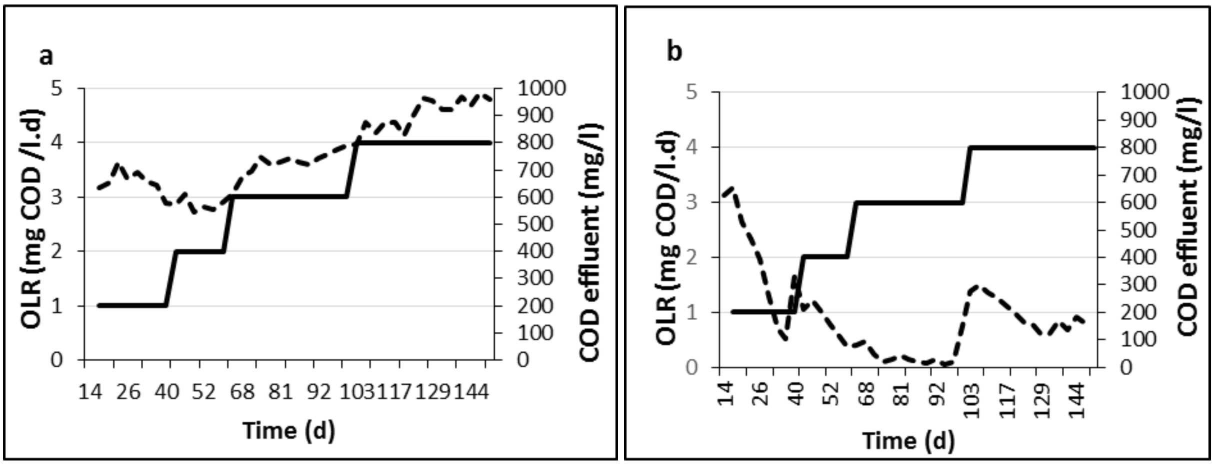
III.2.2. Effect of oxygen on anaerobic digestion of TSS in slaughterhouses wastewaters

Fig.4 shows results obtained aerobically and at the named high-micro-aeration condition ensured with high aeration of the feeding solution of the anaerobic digester. It is obviously clear that the anaerobic digestion of the highly oxygenated influent was very poor, with continuous decrease of the efficiencies and the methane production yields, calculated based on the COD_{sol} and COD_{tot}. This was accompanied with accumulation of TSS and VS in the anaerobic sludge (Fig. 5).

IV. DISCUSSION

Anaerobic digestion is a biological process in which organic materials are mainly converted into methane and carbon dioxide in the absence of oxygen. The degradation process is not a sequence of independent reactions, but it is characterized by a complex of mutual interactions between different microbial species. These processes can be grouped into four stages which proceed consecutively during the anaerobic digestion of complex material via hydrolysis, acid genesis, acetogenesis and methanogenesis. The overall composition including soluble, suspended solids as well as the chemical and biological oxygen demands is the basis of any successful digestion process. Wastewaters for the Qatari slaughterhouse were shown characterized with high COD contents, representing a serious source of pollution, but an appropriate wastewater to study the effect of high solid contents on the anaerobic digestion. In addition, the characterization showed that almost 44% of the COD are associated to suspended solids. Also, 84 to 95% of the COD are in the total dry matter. This means that TSS are important to consider in any treatment process of the total wastewater. This wastewater characteristics could represent an interesting model to investigate the most important limitation in anaerobic processes which is the concomitant digestion of dissolved and solid pollutants. At strict anaerobic conditions, an optimal OLR of 3 g/l.d may be applied, with an efficiency of almost 98%, considering COD_{sol} and 95% considering COD_{tot}. In contrast, the high efficiency of COD_{tot} removed is not translated into methane because the TSS of the influent were not completely digested, but accumulated in the sludge. This observation was more clear pronounced with higher OLR. The low methanogenic capacity of the treatment system is then due to the accumulation and entrapment of non-biomass coarse suspended solids form the wastewater in the sludge, resulting in a dilution of the active biomass causing thus a decrease in the methanogens counts as previously evidenced [5].



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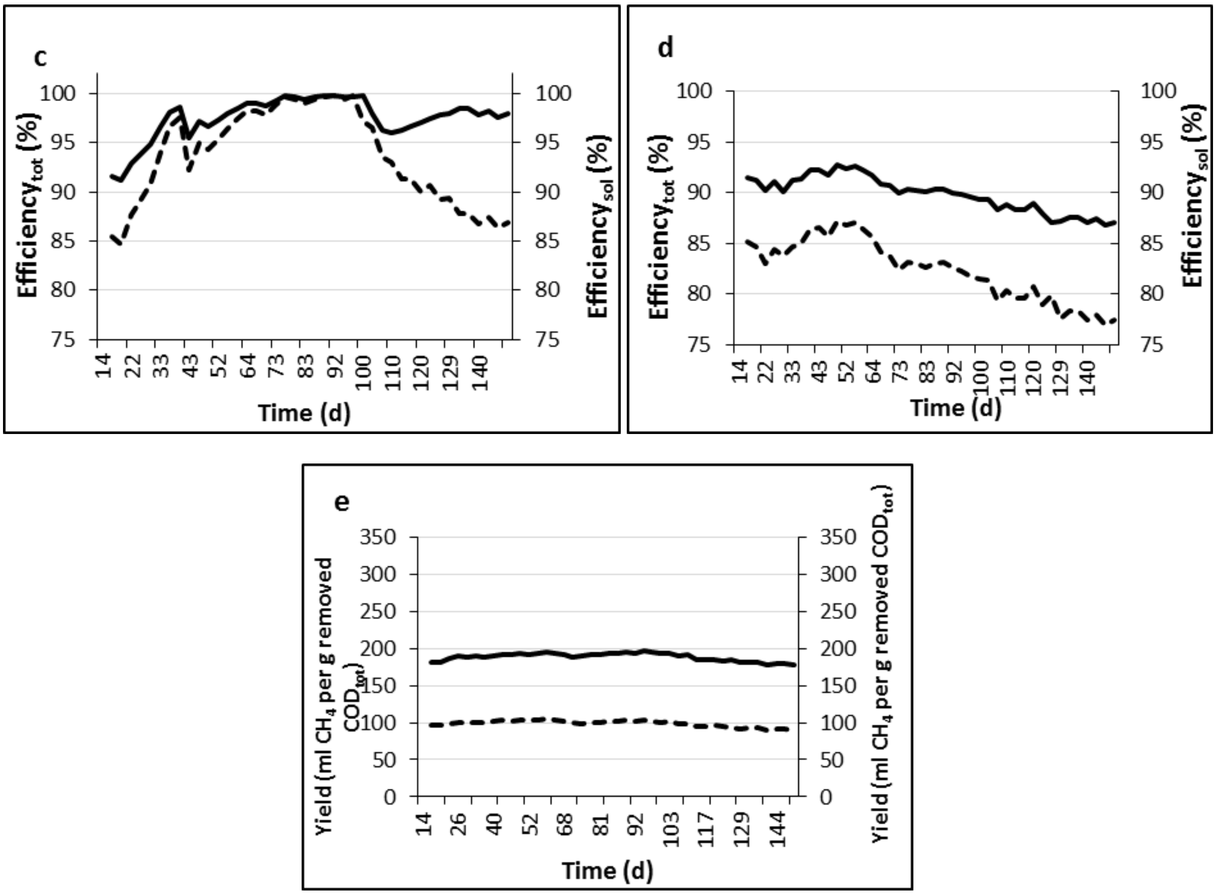

Fig. 4. Digestion of the Qatari slaughterhouse wastewaters at high microaerabic (a, c, f) and aerobic (b, d) conditions.
a, b: COD in the effluent with increasing the OLR: (______) OLR; (------) $COD_{effluent}$
c, d: Efficiency of the treatments considering the soluble COD (COD_{sol}) or the total COD (COD_{tot}) in the feeding solution: (______) $Efficiency_{tot}$; (------) $Efficiency_{sol}$
e: Methane production yields considering the soluble COD (COD_{sol}) or the total COD (COD_{tot}) in the feeding solution: ______Yield (ml CH_4 per g removed COD_{sol}),(------) Yield (ml CH_4 per g removed COD_{tot})

Inhibition of the anaerobic digestion may be explained by a potential toxic effect of dissolved oxygen in the influent, exceeding tolerance limits of methanogens. In contrast, the aerobic digestion of the influent was as expected, with high efficiencies of almost 99% considering soluble total COD, at OLR of 3 g COD/l.d. This high digestion was accompanied with stable TSS and VS in the aerobic sludge (Fig. 5), leading to the conclusion that the TSS were not accumulated in the reactor. Higher aeration is needed to ensure the same efficiencies at higher OLR.

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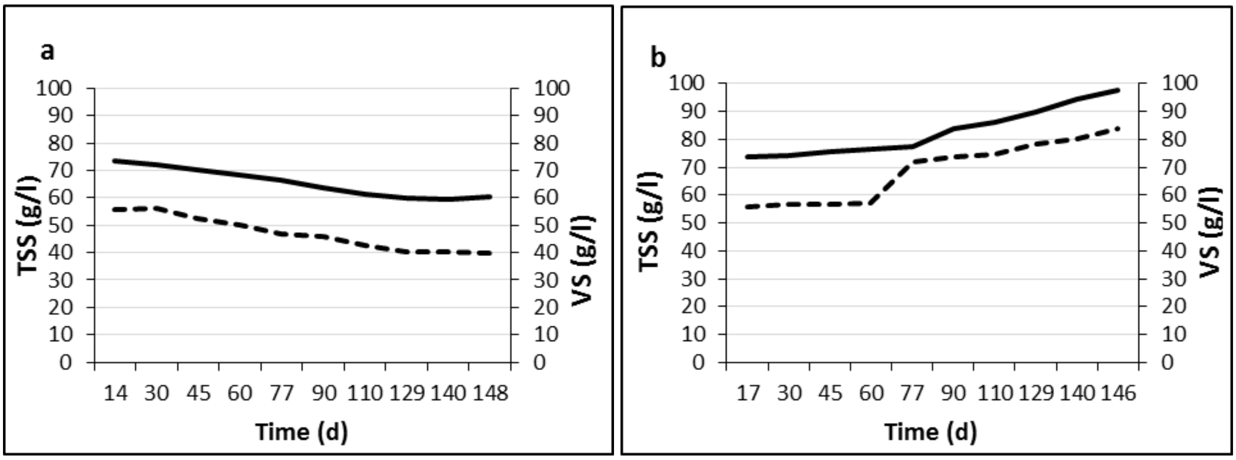

Fig.5. Total Suspended Solids (TSS) and Volatile Solids (VS) in the sludge during the digestion of slaughterhouse wastewater at high micro-aerabic (a) and aerobic conditions (b). (———)TSS, (------) VS

But, this was expected since the latters used an anaerobic filter technology, which is more appropriate for wastewaters with low TSS. Here, we used an anaerobic sludge semi-continuously stirred, in which the effect of TSS is less pronounced. In addition, the entrapment of the cell biomass by a film of increasing thickness, would hamper the supply of the substrate to the cells present in the sludge aggregate, leading to deterioration of the specific methanogenic activity [5]. This is in addition to transfer limitations due to formation of agglomerates of microbial biomass.

In order to evidence this observation, the highly oxygenated influent was used to feed the strict anaerobic system. The digestion was very poor, with continuous decrease of the efficiencies and the methane production yields, calculated based on the COD_{sol} and COD_{tot}. This was accompanied with accumulation of TSS and VS in the anaerobic sludge. Consequently, the inhibition of the anaerobic digestion may be explained by an additional potential toxic effect of dissolved oxygen in the influent, exceeding tolerance limits of methanogens. In contrast, the aerobic digestion of the influent was as expected, with high efficiencies of almost 99% considering soluble total COD, without accumulation of TSS and VS in the aerobic sludge, leading to the conclusion that the TSS were not accumulated in the reactor. Hydrolysis of TSS is highly improved with oxygen supply in the reactor. The combination of all our results, could lead to the conclusion that oxygen has an important impact in biogas generation which can either be positive or negative based on several parameters, including the appropriate redox potential for the acclimatized microbial population, TSS hydrolysis rate, biomass concentration in the sludge, hydraulic retention time and the organic loading rate.

The overall results of the experimental procedure described in Fig.1 clearly showed that controlled micro-aeration could enhance the anaerobic digestion of the complex slaughterhouses wastewaters containing high TSS. The micro-aeration of the influent, prevents accumulation of the TSS in the anaerobic sludge. With high aeration of the influent, dissolved oxygen inhibits methanogens, as expected. Indeed, it is quite natural that some amount of oxygen can reach anaerobic digesters unintentionally [11, 10] as the reactors are operated with feeding influents, especially through interactions with the surroundings such as mixing. Most anaerobic digesters are therefore subjected to minute and varying aerobic loading conditions. The possible effects of such aeration are neither extensively quantified nor handled in standard anaerobic models of digestion of slaughterhouses wastewaters. In the present work, it is clearly shown that a great improvement of the anaerobic digestion of slaughterhouses wastewaters containing 44% TSS may be ensured by micro-aeration. Such a condition ensured degradation and solubilisation of the TSS, avoiding their accumulation in the sludge. This led to prevent inhibition of the methanogenics, normally occurring with the entrapment of the cells biomass by a film of increasing thickness. The consequence of such entrapment is the limited supply of the substrate to the bacteria present in the sludge [14]. Digestion of TSS at micro-aerobic conditions may explain the high methane production yield by avoiding deterioration of the specific methanogenic activity.

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Similar results were reported by Botheju et al. [5] who observed positive response due to oxygenation in a range of oxygenation loads (0 – 16%) in anaerobic batch bioreactors fed with starch and predicted the existence of an optimum oxygenation level corresponding to a maximum methane yield [10, 11].

These results are important from the practical point of view, considering the high efficiency of the anaerobic digestion of slaughterhouse wastewaters with high methane production yields using a relatively simple technology that does not require overly expensive or complex modifications to anaerobic digestion systems. In addition, the temperature of almost 38°C is a positive factor in this case, increasing the rate of enzymatic hydrolysis of TSS by exoenzymes.

V. CONCLUSION

Effects of micro-aeration on anaerobic digestion of slaughterhouses wastewaters was investigated under 38°C. At controlled level of micro-aeration, solubilisation of TSS was enhanced, avoiding their accumulation in the anaerobic sludge and subsequent anaerobes inhibition. Higher OLR was applied and methane yields were enhanced. Interestingly, dissolution of required oxygen is not inside the anaerobic digester, but in the feeding influent preventing risk of accidental macro-aeration of the anaerobic sludge and thus definitive loose of methanogens. Continuous aeration of the feeding wastewater with 0.05 vvm is adequate to ensure high improvement of the anaerobic digestion of TSS.

VI. ACKNOWLEDGMENTS

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