

## Two-Stage Anaerobic Digestion Process Combining Pre-digestion with Methanization عملية معالجة حماة الصرف الصحي بمرحلتين مترنة بعملية انتاج الميثان

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**Abstract**

Anaerobic digestion of a mixture of manure and sewage sludge by combination of the biological pre-digestion at 73°C with methanization at 55°C was studied with regard to the biogas process efficiency, energy balance and sanitation effect. Performance of the two-stage digestion was compared with the conventional, one-stage moderate thermophilic digestion at 55°C and mesophilic digestion at 37°C, respectively. The best performance was achieved by the two-stage treatment both in terms of Volatile Solids (VS) removal and biogas yield. Up to 60% of VS was removed and a specific methane yield of 300 ml CH<sub>4</sub> gVS<sup>-1</sup> was achieved. Sanitation effect was measured as inactivation of the indicators of bacterial pathogens - the faecal enterococci and spores of *Clostridium perfringens*. Elimination of faecal enterococci to non-detectable level occurred only during treatments at 73°C and 55°C. Numbers of *Clostridium perfringens* spores were reduced solely by the combined 73°C/55°C treatment.

**Key words:** Biogas, Anaerobic, Methanization, two-stage, sewage and manure

**الملخص**

مزيج من فضلات الحيوان مع حماة الصرف الصحي تمت معالجته بالطريقة اللاهوائية وبمرحلتين، حيث اشتملت المرحلة الاولى معالجة المزيج اللاهوائية وبدرجة حرارة 73 منوية مع عملية تكوين الميثان وبدرجة حرارة 55 منوية مع الاخذ بنظر الاعتبار انتاجية الغاز الحيوي وتوازن الطاقة وكذلك تأثير الصرف الصحي. لقد تمت مقارنة هذه الطريقة بطريقة المعالجة اللاهوائية بحرارة 55 منوية ومعتدلة الحرارة 37 منوية. لقد اثبتت النتائج ان الافضلية كانت للمعالجة بدرجة الحرارة 55/73 منوية من حيث انتاج الغاز الحيوي وكذلك التخلص من المواد الصلبة المتطرية اضافة الى صحة الناتج النهائي حيويا. اذ ان عدد بكتيريا المكورات المغوية البرازية وسبورات الكلوستريديوم بيرفرينكز انخفض عددها الى ما دون الكشف. لقد تمت ازالة 60 % من المواد الصلبة المتطرية بطريقة المعالجة 55/73 منوية اضافة الى وصول كمية الغاز الحيوي عليه الى 300 ملييلتر.

**كلمات مفتاحية:** غاز حيوي. لاهوائي، متننة، خطوتين، حماة الصرف الصحي

**Introduction**

It is well known that the sewage sludge and animal manure are the main sources of organic waste in Iraq. The energy produced by anaerobic digestion (AD) of these two types of wastes could be increased significantly, if the waste available in the country was treated by anaerobic digestion [1].

It is well known that the two-stage AD system consists of a hydrolytic-acidogenic stage followed by the methanogenic stage. AD process can be processed at mesophilic or thermophilic temperatures. Due to its stability and lower use of energy, mesophilic AD is used widely comparing to the thermophilic AD. Nevertheless, both systems achieve a reduction of pathogens [2, 3, 4, 5]. Bendixen recorded, a decrease of pathogens from the waste stream by 2 and 4 log units during mesophilic and thermophilic AD respectively [6, 7, 8, 9].

The advantages of AD systems include: (i) increased specific activity of methanogens leading in a higher methane product (ii) more stability with better pH control; (ii) more reduction of VS and (iv) high potential for removing pathogens [6, 7, 8, 9]. Meanwhile, the disadvantages of AD systems include: (i) elimination of inter dependent nutrient, which is required for the methane forming bacteria;(ii) inhibition of acid-forming bacteria resulted by the hydrogen built-up (iii) somehow complex process and (iv) costs are high [10,11].

The diversity within the group of thermophilic hydrolytic bacteria facilitate to develop a two stage digestion system, where the temperature in the first stage is optimal for the activity of extremely thermophilic hydrolyzing bacteria, while the temperature of the second stage will be kept at maximally 55°C, ensuring the right conditions for active methanogenesis [12, 13, 14]. In a recently published study [9] we already characterized a two-stage reactor system showing improved digestion efficiency when operated at 68°/58°/55°C [4, 9].

The microbial adaptation to the new operational conditions is affecting the transition of AD systems from mesophilic to thermophilic conditions. In addition to the presence of different groups of microorganisms which are capable of performing their functions at certain temperatures [15, 16, 17, 18 ].

The aims of this study were to compare the process performance and the sanitation effect of the two-stage, 73°C/55°C combining Pre-digestion system and the conventional one-stage reactors operated at 55°C and 37°C, respectively.

### Materials and Methods

#### Reactor Experiments Set-up

Three anaerobic continuing stirred-tank reactors (CSTR), setup for digestion were used in this study. A mesophilic, one-stage reactor system, designated R-37°C, had an active volume of 3.6L and was operated at 37°C. A moderate thermophilic, one-stage system operated at 55°C, R1-55°C, had an active volume of 3.0L. The two-stage system consisting of a pre-digestion reactor with 0.4 Lactive volume, operated at 73°C (R-73°C), and connected in series with a 55°C digester, R2-55°C, having an active volume of 2.6L .

The substrate in the reactor R-73°C was stirred constantly with a magnetic bar, while the content of the other reactors was mixed intermittently by a propeller installed inside the reactors. The reactors were fed three times per day, ensuring a minimum guaranteed retention time of 8 hours for the biomass inside the digesters. The same volumetric loading was applied to all three systems, resulting in the hydraulic retention time (HRT) of 2, 13, 15 and 20 days for the reactors R-73°C, R2-55°C, R1-55°C and R-37°C, respectively. The reactors were operated for a period of four months and the performance was assessed under the steady state conditions.

#### Batch Test

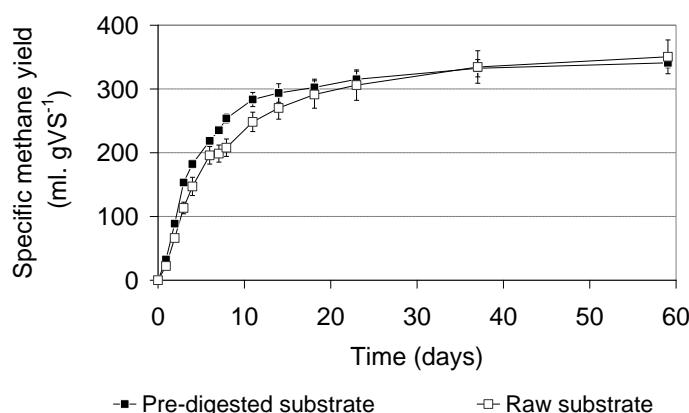
Development of methane from substrates tested was followed by batch incubation. The 118-ml vials were filled with substrate in an amount corresponding to 1g of Volatile Solids content (VS), and 20ml of inoculums from R1-55°C was added. Controls were omitted for the substrate. The vials were incubated at 55°C for two months. Experiment was done in triplicate.

#### Analyses

Analysis of Total Solids content (TS), VS and Total Nitrogen (N)were performed according to standard methods [19]. The samples were analysed in duplicates. The numbers of *Clostridium perfringens* spores were estimated by a three-tube MPN method using iron-milk medium [20].

#### Results and Discussion

Analysis of the mixture of cattle manure, cattle manure and sewage sludge showed that the substrate contained: 51g TS.l<sup>-1</sup>; 37gVS.l<sup>-1</sup>; 4g Total N.l<sup>-1</sup> and the total Volatile fatty acids (VFA) corresponded to 9.5g Ac.l<sup>-1</sup>. The ultimate methane potentials of the raw substrate and substrate pre-treated at 73°C were found to be similar, 350ml CH<sub>4</sub>.gVS<sup>-1</sup> (Figure 1). The course of batch fermentation showed as well, that the extreme thermophilic pre-treatment had a positive effect on subsequent methanogenic conversion, since the rate and extent of the methane production during the first 14 days incubation was higher than with raw substrate.



**Fig.(1): Course of methane production from the raw substrate and substrate pre-digested in the 73°C reactor (R-73°C).**

Performance parameters of the continuously operated anaerobic reactors are summarized in Table (1). Anaerobic digestion in the one-stage mesophilic digester was characterized as a process in balance without significant accumulation of the VFA. The methane content in the biogas produced, and the pH in the digested waste had values typical for healthy processes operated with similar substrate. However, the 40% VS removal and the specific methane yield, 30% below the ultimate specific methane yield, indicated that the anaerobic degradation was to some extent limited. In comparison to the 37°C digester, the one-stage 55°C digester (R1-55°C) exhibited a better performance both in terms of VS removal and the methane production. A slightly elevated level of the VFA was detected during the digestion at 55°C. This phenomenon is typical for operation under moderate thermophilic conditions [21].

The performance parameters of the two-stage system showed clearly that the pre-hydrolysis and pre-digestion in reactor R-73°C had a stimulating effect for methanogenesis in subsequent reactor unit.

The two stage system and particularly the digester R2-55°C, exhibited the best performance with the highest biogas production rate, biogas yield, specific methane yield and VS removal. In comparison to the one-stage systems operated at 37°C and 55°C, the increase in biogas yield and specific methane yield was 22-23% and 13-15%, respectively Table (1).

In the 73°C/55°C reactor set-up the major production of methane occurred in R2-55°C. Even the biogas from R-73°C contained 20% of methane only 1% of the total, daily volumetric methane production was derived from this first process stage.

Suppression of methanogenesis in the current reactor set-up could be a result of distinct substrate composition, a 5°C increase of the operational temperature and the reduction of the HRT from three to two days. From the perspective of large scale operation, we regard the production in the current 73°C/55°C system for being more optimal, because an enhanced production of biogas with high content of methane was achieved in only one reactor unit.

**Table (1): Operational and performance parameters of the continuing stirred-tank reactors(CSTR)treating a mixture of manure and sewage sludge.**

Parameters	One stage		Two stage		
	R-37°C	R1-55°C	R-73°C	R2-55°C	73°C/55°C
Biogas production rate (l.l reactor vol <sup>-1</sup> .d <sup>-1</sup> )	0.7 (0.1)	1.0 (0.1)	0.3 (0.1)	1.1 (0.1)	-
Biogas yield (l.l influent <sup>-1</sup> )	13.1 (1.7)	14.2 (1.3)	0.7 (0.2)	16.1 (2.6)	-
Methane %	69.0 (2.0)	67.7 (1.5)	19.3 (1.7)	68.7 (1.8)	
Specific methane yield (ml.gVS <sup>-1</sup> )	246 (36)	261 (29)	4(1)	-	300 (45)
VS removal (%)	42.1(2.9)	52.2 (0.1)	n.d.		58.9 (7.5)
VFA (g Ac.l <sup>-1</sup> )	0.2 (0.1)	0.4 (0.1)	9.6 (1.3)	0.4 (0.1)	
Ph	7.7 (0.1)	7.8 (0.1)	7.3 (0.1)	7.8 (0.1)	

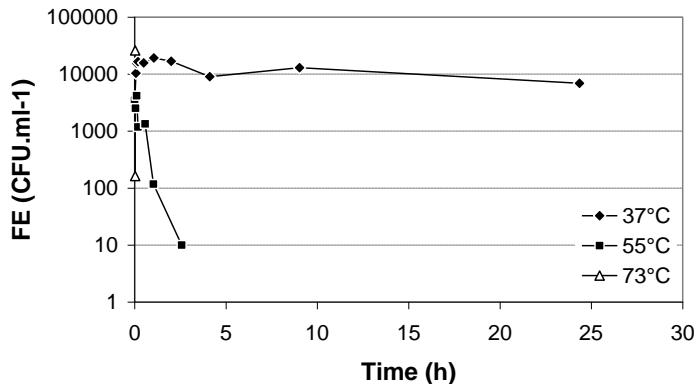
Results are given as means with SD in parentheses, number of replicates 3-17, n.d = not detectable.

The fate of indicators of bacterial pathogens was followed after feeding the raw substrate into the respective digesters. As it appears from Figure (2), no significant reduction in numbers of faecal enterococci was observed during mesophilic anaerobic digestion. On the other hand, treatment at the thermophilic temperatures was very efficient.

No faecal enterococci were detected after the waste was retained in R1-55°C for 4 hours, and after 5 min exposure of the waste to 73°C in the pre-digestion reactor.

The concentrations of *Clostridium perfringens* spores were determined in raw influents and after 8h of incubation in the respective reactor systems Table (2).

The 8 hours treatment in single stages, at 37°C, 55°C and 73°C, resulted in a reduction of spore numbers by 0.7, 1.5 and 1.6 log units, respectively.

**Fig.(2): Quantification of faecalenetrococci in reactors R-37°C, R1-55°C and R-73°C upon feeding with raw influent**

The combined treatment by 73°C/55°C-digestion reduced the numbers of spores by 2.8 log units. Spores of *Clostridium perfringens* are extremely thermostable and are efficiently inactivated only by autoclaving.

**Table (2): Quantification of spores of *C. perfringens* in influents and effluents of anaerobic digesters.**

Reactor	Influent	Waste digested for 8 hours
R-37°C	$2.3 \times 10^5$ ( $0.4 \times 10^5$ ; $12 \times 10^5$ )	$4.3 \times 10^4$ ( $0.7 \times 10^4$ ; $21 \times 10^4$ )
R1-55°C	$2.3 \times 10^6$ ( $0.4 \times 10^6$ ; $12 \times 10^6$ )	$4.3 \times 10^4$ ( $0.7 \times 10^4$ ; $21 \times 10^4$ )
R2-55°C	< $0.3 \times 10^4$ (n.d.)	$2.3 \times 10^2$ ( $0.4 \times 10^2$ ; $12 \times 10^2$ )
R-73°C	$1.5 \times 10^5$ ( $0.3 \times 10^5$ ; $4.4 \times 10^5$ )	$4.3 \times 10^3$ ( $0.7 \times 10^3$ ; $21 \times 10^3$ )

The combined treatment at 73°C/55°C did not completely eliminate the spores capable of growth, but it was found to be much more efficient than the 8 hours pasteurization of waste in the anaerobic environment of the 73°C bioreactor.

Based on our results the optimal incubation temperature was determined to be 55°C, due to a stable pH 7.3, high VFA production and VS reduction. At the one hand this pH indicates accumulation of VFA, and on the other hand it boosts the population of hydrolytic bacteria [22]. The spontaneous drop in pH under the chosen conditions is enough to suppress methanogenic activity [23, 24].

Relatively, in the two stage operation, high methane yields were reached through the optimized first-stage approach. Methane produced up to 68%, similarly to that found by previous authors [25, 26, 27]. The same authors [26], reported also methane yields, for various kinds of substrates and the results of the present work were also coherent with previous experience by Tenca *et al.* obtained with the same substrate [28, 29, 30].

The obtained data of this study give a robust contribution to demonstrate the general supremacy of the two-stage AD system, as compared to the one-stage approach. The method chosen in this work, i.e. to optimize methanogenesis, allowed over coming inhibition/ in efficiencies that could hide a general result, a shapened in previous experiences [33, 34, 35, 36]. Were, two- and one-stage gave the same results, even if a clear partial inhibition of the two-stage was observed [36]. In this work, with the same substrate used by Schievano *et al.* [36], the two-stage was demonstrated to be potentially (i.e. when both processes areoptimized) more productive than the one-stage.

In the present study the specific biogas production was relatively stable in the two-stage process. Nevertheless, a significant correlation was observed between substrate pre-treated at 73°C and the methane content. The observed values correspond well to data from Brooks *et al.*, who reported production of biogas with a methane content between 30 and 50 %. They argued that the one-stage fermentation is preferable, because of the lower operational costs and the elaborate and costly handling of the first-stage reactor. Never the less, this study showed that the two-stage reactor provides advantages. It was demonstrated that the first stage can be carried out stably without the need for additives like antifoaming agents and chemicals for pH adjustment. The pH in the one-stage and in the second stage of the two-stage fermenter was similar at  $7.5 \pm 0.2$  during the whole experiment which provides good conditions for methanogenic activity [37, 38].

The concentration and retention time of the resulting metabolites must also count in inducing changes in the fermentation environment and the whole efficiency of the fermentation, as already reported by Tenca *et al.*[28]. Here, VFA formation and VS were taken in to account as descriptive parameters and, generally, efficient methane productions corresponded to higher VFA/VS. On the other hand, to better explain the reasons for the differences in the optimum zone among different biomass types, a deeper look into the chemical composition of the organic matter and, also, into the speciation of the soluble/volatile metabolites (VFAs, phenols, amines, etc.)should be done in the future, as recently suggested by Schievano *et al.* [36].

### Conclusions

This study showed that the two-stage anaerobic digestion combining pre-digestion at the extremely thermophilic temperature of 73°C with methanization at 55°C, was superior to the conventional, mesophilic and moderate thermophilic one-stage processes. The improvement was found both in terms of reduction the amount of waste, substrate biodegradability, biogas productivity, power production potential and hygiene effect.

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