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Parameters affecting the formation of VFA during cow manure fermentation

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ABSTRACT
The aim of this study is to examine the relationship the effective parameters on acidification and solubility efficiency in manure co-fermentation process at different temperatures (20-55°C), HRT (2-10 day), pH (4-10) ve OLR (10-90 gCOD/l-d). The maximum acidification yield was found by applying 70 gCOD/l-day with pH 8 and 38°Cfor acidification yield of 193 gCOD/kgVS.The maximum solubility value was 308 gCOD/kgVS at pH 10 with same conditions. Acetic, butyric and propionic acid were primary fermentation products, while the maximum value was 70.3% at pH 10 as the acidification degree. When the trend analysis of acidification yield according to effective parameters was made, it was determined that the relationship between HRT and acidification yield at high concentrations is decreased compared to other parameters. When acidification yield was analyzed in principal component analysis; While OLR, pH and Temperature have a common effect, it has been determined that the HRT parameter had no effect on the process. To solubilization which is another investigation parameter, is possible to say that pH and OLR have some effect on.
Keywords: *Anaerobic, fermentation, manure,VFA, PCA, soluble COD*

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INTRODUCTION

Animal waste is one of the most important waste which caused by humans activity, generated worldwide. Daily produced amount of manure as the number of livestock and poultry breeding grows, increase. Also, With each passing day increase demanding of meat and dairy products accordingly increased manure amount cause environmental problems. The amount of the waste produced by this animal community makes it even more important considering environmental problems. In all the EU, about 1.3–1.8 billion tonnes (wet weight) of manure is manufactured each year's [1], at this the waste in nitrate vulnerable zones cause underground water pollution. Manure, which has many risks in terms of environment and human health, must be properly processed or treated. As well as being the hazardous waste has structural features that can be used as a renewable energy source and have a median calorific value of 5000 ± 1000 kcal/kg [2].If it is not treated or recycled properly, leads to green house gas emissions, also. If emissions from grazing and the applications of fertilizers to crops are included, European Environment Agency estimates that agriculture is the source of more than 93.3% of NH_3emissions in EU-28 [3]. Contribution in global warming by the emission of methane (CH_4) and nitrous oxide (N_2O) increase day by day. Mitigating the environmental effects of this kind type of waste is important research subject for many researcher . Anaerobic fermentation that has wide spread using for treating food, agricultural, livestock waste is attractive choice as an economical and eco-friendly (VFAs). Anaerobic fermentation is one of the most effective tools for renewable energy production. It uses to decomposition for biodegradable organic material by diverse microorganisms under oxygen-free conditions. Therein, VFAs are generally regarded as a better product than biogas owing to their high yield and wide application. Based on previous studies, VFAs have been successfully used for nutrient removal enhancement, biodegradable plastic production, biogas and polyhydroxyalkanoate biosynthesis [4]However, studies on the production of volatile fatty acids from manure by anaerobic fermentation systems have been carried out, but studies have limited. However, such wastes have an important advantage as maintain their structural integrity in biological processes such as anaerobic fermentation. this kind of waste which has lignocellulosic structure, is rate limiting at anaerobic processes. In order to improve the anaerobic fermentation efficiency and lignocellulose degradation rate, there are several treatment methods, consisting of physical, chemical and microbial treatments. Physical and chemical pretreatment methods including thermal treatment, microwave, sonication and chemical additions positively affect VFA production. These pre-treatments methods were used of organic matters solubilization by many researchers [5]. Due to this structure of wastes containing abundant organic material types such as lignocellulose, which are difficult to hydrolyze, significant difficulties are encountered in the production of short chain fatty acids in anaerobic fermentation. Reported that to removal of the matrix, many physical and chemical methods have been used. However, such studies have some disadvantages in terms of cost, application and environmental effects. The key for the success of the co-digestion system is the balanced substrate composition for mixed biomass. Nutrient balance, proper C:N ratio, and stable pH are prerequisites for good digester performance. Sorghum is one of several plant species as ‘‘energy crop’’ and has been used as co-substrate at anaerobic digester for a long time. Optimized, an anaerobic reactor will always be preferred for this kind waste with low cost operation and environmentally friendly final products. Therefore, product optimization studies for obtaining renewable energy sources such as short chain fatty acid or hydrogen in anaerobic fermentation reactors have gained importance in recent years. The key operational parameters for VFA production mainly is; temperature (T), hydraulic retention time (HRT), organic loading rate (OLR), pH, etc. In this study, fermentation of the cow manure with sorghum was carried out using anaerobic fermentor, effect of various operational and environmental parameters on VFA production was investigated.

MATERIAL AND METHODS

Manure characterization

In the anaerobic fermentation process, the manure of cow collected from veterinary faculty in Istanbul University-Cerrahpasa, have been used. The Manure used belongs to periods when animals are not using any vetmedicines and it has been taken as fresh as possible. Manure was collected in 20-L buckets and immediately transported to a -20 ºC store in the laboratory. The main characteristics of manure are shown in Table 1.

Table 1. Cow manure characterization

Characteristics	CM	Sorghum
TS (% dryweight)	16.20 ± 0.25	(%71.8)
VS (%TS)	76.13 ± 1.46	(%72.9)
pH	7,23 ± 0,06	7.53
TP g/kg (TS)	1.41 ± 0.08	3.4
C/N	22	47
C/H/N/S (%TS)	43/6/2/0.4	42/5.6/0.33/0.12
TN (% TS)	1.46	0.97
TC (%TS)	32.5	45.8

The C/N ratio of the fertilizer used in fermentation is 22, for sorghum this ratio is 47. By mixing both materials in appropriate proportions, 34.5, which is the value that will prevent ammonia inhibition in fermentation, was obtained.

Analytic methods

Samples which has slurry state were centrifuged at 11,000 rpm, and the soluble chemical oxygen demand (sCOD) concentration of the supernatant was measured. Chemical oxygen demand (COD) (5220 D-Closed Reflux, Colorimetric Method), and volatile suspended solids (VSS-2540 E. Fixed and Volatile Solids Ignited at 550°C) were analyzed according to Standard Methods[6]. Total nitrogen (TN) was determined by alkaline potassium persulfate digestion (SM 4500-N C). Soluble organics, 4 g sample was diluted with 40 ml deionized water, and was centrifuged at 9900rpm 4ºC for 10min was filtered through membrane filter. To the filtrate for analysis of soluble substances (etc. volatile fatty acids (VFAs), 3% phosphoric acid solution was added. VFA were measured using a HP Model 5890 Series II Gas Chromatograph (GC) (HP FFAP Column,10 m/530 mm/1mm) with nitrogen as a carrier gas. The sample was dried at 60ºC and ground in to powder before elemental analysis (Thermo-Scientific™ FLASH 2000 CHNS/O Analyzers, Massachusetts USA). For the purpose of production of emulsions and suspensions from big particulate in operations T 65 digital IKA Ultra-Turrax disperser was used.

Reactor operation

The process was carried out in a 4 dm^3 anaerobic stirred bioreactor with mechanical stirring batch mode. The reactor was fed in a batch. Mechanical stirrer was positioned in the middle of the reactor and driven by an electric engine rotation speed 20-30 rotations per minute. Predetermined temperature was ensured by heated water jacket. Cow manure slurry (50% water) was sieved on a 1-mm sieve (20-mesh) to separate the solid fraction of the manure. Twenty mesh Haver & Boecker sieves with 0.25 mm wire thickness and 1mm x 1mm mesh size manufactured in 304 chrome wire were used. Used stalks of sweet sorghum (Sorghum bicolor L) were chopped to>1 cm in size to enhance degradation during fermentation. Reactor was daily fed with different mixtures (wet weight basis), to obtain a suitable solid content as OLR. Before being added into reactor, manure was homogenized in a 30dm^3 plastic container by disperser. Fermentor was kept at a fixed working volume during the whole experimental period. At the first phase HRT were kept at desired values (HRT= 2 to 10 days, OLR=25 g TS/l-day, pH=10, temperature 38° C), second phase (HRT= 8 days, OLR=10-90 g TS/l-day, pH=10, temperature 38° C), third phase (HRT= 8 days, OLR=70 g TS/l-day, pH= 4-10, temperature 38° C), and the last phase (HRT= 8 days, OLR=70 g TS/l-day, pH=10, temperature 25-55° C) was realized. The first phase lasted 96 days, second and third lasted average 130 each one and last phase lasted 54 days.

Initial studies for fermentation

The environment and operating conditions of the reactor have been determined in accordance with fermentative bacteria. The pH of fermentation mixture was controlled at pH 10 by adding 4MNaOH and 4MHCl, since it has been reported that pH 10 is the most suitable condition for VFAs production during anaerobic sludge and the activity of methanogen could be inhibited completely[7]. Reactor was operated for nearly three HRTs reaching a steady state with stable daily VFA yield. Inoculum was not added because the microorganisms needed for the hydrolytic step was present in the cow manure.

Calculation

The solubilization yield was calculated as the difference between the final and initial soluble chemical oxygen demand (sCOD) in relation to the volatile solids (VS) fed. The same calculation for the acidification used too. Efficiency was calculated by the equations given below depending on both dissolved products and initial inputs[8].

$$Solubilization\ yield\ = \left(\frac{SCOD_f - SCOD_0}{VS_0}\right) \quad (1)$$

$$Acidification\ yield\ = \left(\frac{SCOD_f - SCOD_0}{VS_0}\right) \quad (2)$$

$SCOD_f$ and $SCOD_0$: soluble COD at the end and at the beginning (gr COD/L)

VFA_f and VFA_0: concentration at the end and at the beginning expressed in COD equivalents (gr COD/L)

VS_0: initial content of volatile solids (kg VS/L)

$$Acidification\ percentage = \frac{S_f}{S_i} \times 100 \quad (3)$$

S_f: COD equivalent of VFA produced,

S_i: COD value in influent

Statistical analysis

The experimental design was set up with four dependent and in dependent variable, while was fermented cow manure. Total VFA production, Acetic acid production, Acidification (as HAc) and Soluble COD yield and affected parameters were subjected to an analysis of variance. There is no correlation significantly different from 0 between the variables. At least one of the correlations between the variables is significantly different from 0.As the computed p-value is lower than the significance level alpha=0.05,there is a high level of significant difference. A principal component analysis was applied to significantly correlated variables. The criteria for selecting the principal components (PCs) were the percentage of total variance explained (>85%) and eigen values higher than 1 (λ>1).

RESULTS AND DISCUSSION

HRT Effect

Alkaline anaerobic fermentation of manure has been reported to be an effective method for VFA production inhibiting acid consumers [7].The fermentation step was applied to cow manure at 35°C, OLR 25 gr/L-day, pH 10 conditions. Meanwhile HRT increased step upto 10 days to evaluate the efficiency of the production of volatile fatty acids. at the same conditions fermentation process were applied for at least 3 HRTs and, in all cases, untils table steady state conditions were reached. In this stage, all nutrients entering the reactor were homogenized. Gradual increase of HRT kept acidification around 25% in low HRTs, while it increased by 20% from the eighth day to 63%. In the fermenter, where the acetic and butyric acid are dominant, the total acids increased to around 0.63 g / l on the eighth day, but the maximum acidity percentage decreased to around 0.43 g / l in the ten-day HRT. Dissolved COD values at the output also acted the same trend. Generally, in the studies per formed with cellulosic biomass, whether the gas production or fermentation, the HRT continuously increases the reactor performance in the first ten days and then either plateau or fall occurs. Although the study was batch, this effect could be easily seen in Figures 1 and 2.AlthoughHAc/HPr ratio is 2.74 in HRT 10, the amount of propionic acid has reached 11% in total acid. The high ratio of propionic acid in total at this pH compared to other acids suggests that it may have an effect on system performance.

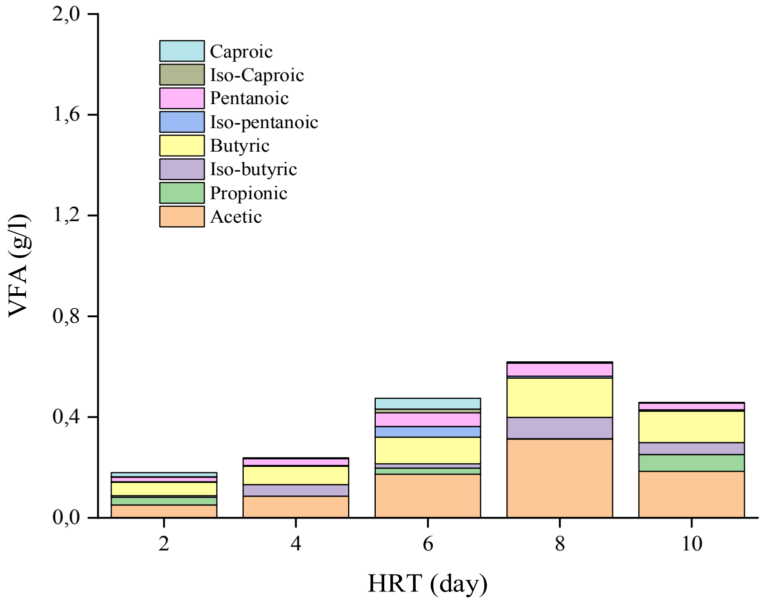


Figure1.VFA distribution by HRT

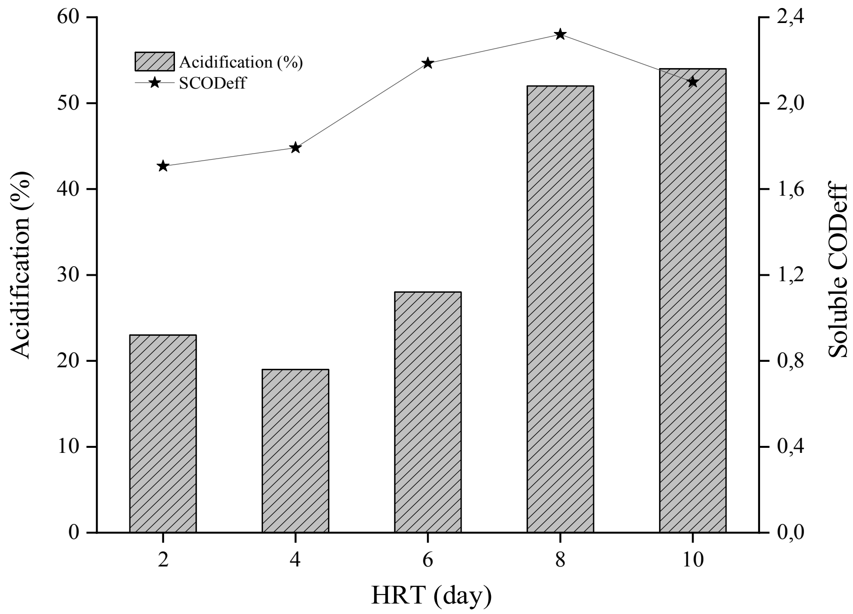


Figure2. Acidification (%) and COD yield

During this period, keeping the pH at 10 as an automatic control ensured alkaline fermentation. In the fermentation process, it is possible that the reason for increased solubility and acidification is the prolonged alkaline effect. Ammonia has an inhibition effect for sludge adapted at mesophilic temperature in slightly alkaline conditions (above 7.5)[9]. Although there are high alkaline conditions in any HRT, no ammonia effect was observed on fermentation. This reason that manure being brought to the proper level with the C / N ratio (34.5) with the sweet sorghum plant. In parallel with the formation of VFA, a high amount of 210 gCOD/kgVS dissolved COD (Eqn.1) was determined at the reactor (pH 10). When the acidification yield (Eqn.2) is compared in the two highest HRT; It was calculated as 279 gCOD/kgVS in HRT 8 and 210 gCOD/kgVS in HRT 10. Except for HRT 2 days, acetic acid and butyric acid were predominantly determined as fatty acids in all HRT's.

OLR Effect

The total solids were tested in the reactor in the range of 10-90 gTS/L and other parameters were kept constant at pH 10, HRT 8 days and temperature at 38 °C. In this process, parallel to the increase in OLR, the increase in VFA production, acidification percentage and dissolved COD values continued, but the OLR loading was terminated when the decrease in the increase rate. When the organic loading value increased from 30 gTS/L-d to 50 gTS/L-d, the acetic acid value increased from 0.20 gr/l to 0.26 g/l, but the acidification percentage increased from 39% to 65%.When VFA types are analyzed, in the system with reduced species diversity, an increase in efficiency in fermentation was observed (Figure 3).As a reason for this increase, it can be said that the content of the substrate in the particulate state turns in to dissolved organic compounds. It was determined to increase 1.56 times between the two values of the solubilization yield (Figure 4).High OLR means high methane formation and treatment capacity, but overloading can cause process in stability and collapse. Although the formation of propionic acid in the resulting conditions constitutes approximately 4.67% in the volatile fatty acids at 50 gTS/l-d, it was determined below 1% in higher organic matter loads. When viewed in high OLRs, the ratio of HAc/HPr at 70 gTS/l-d is 4.33, 90 gTS/l-d HAc/HPr rate was 1.52 and the amount of propionic acid in the total acid upto reached 21%. As a result, acidification also decreased significantly and decreased by 23% at 90 gTS/l-d. It is determined dissolved substance 177 gCOD/kgVS at the reactor with the decrease in VFA formation. Fatty acids such as acetic acid, butyric and iso-butyric acid, pentanoic (valeric acid) acid are pre dominant in all HRTs (except 2 days HRT) starting from OLR 50 grCOD/kgVS. In a study on conventional digesters, it states that isobutyric and isovaleric acid will cause errors or damage in the system when it exceeds 15 mg/l[10].The isobutyric acid and isovaleric acid (pentanoic acid) formed in these conditions (90 gTS/l-d) in the fermenter were determined at concentrations of 200 mg/l and 21 mg/l, respectively. Although structurally different (fermenter), it is possible to say that the stress factor occurs in the bacteria that occurs the system in this OLR. However, when equation 2 was used, the acidification efficiency was calculated as 177 gCOD/L at 70g TS/l-d OLR, which was the highest acidification yield at OLR.

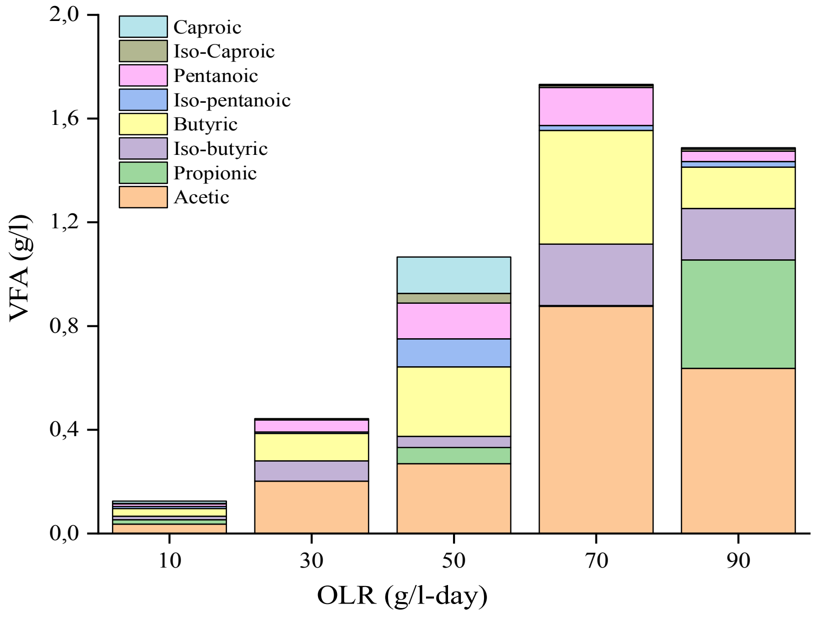


Figure3.VFA distribution in OLR

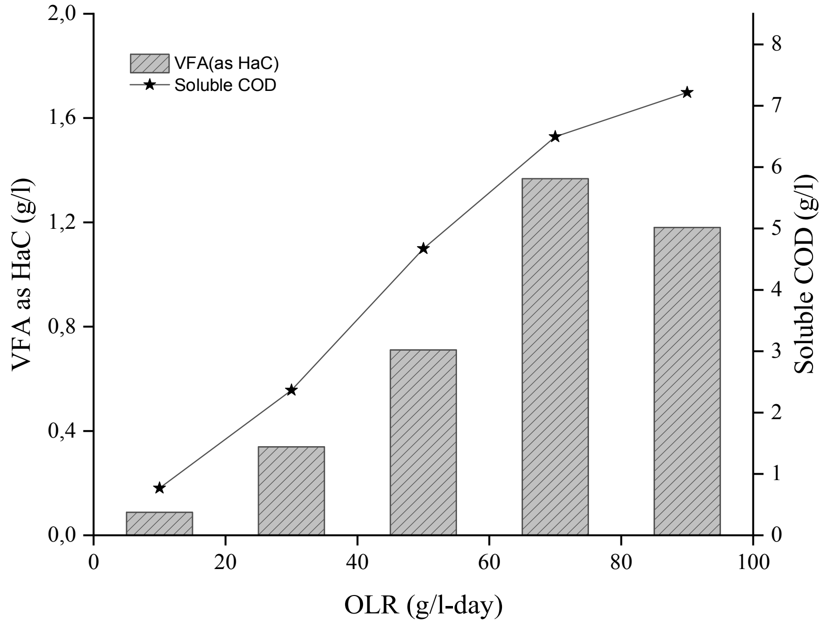


Figure4.VFA (as HaC) and sCOD

pH Effect

As stated by Liu *et al*, in studies on VFA producing systems, many of the acidogens can live at extremely pH (3-12) [11] . it is seen that many studies a wide range of pH (5-11) has been carried out using this advantage. Especially in studies with treatment sludges in alkaline conditions (pH 8-11), very successful results were obtained. In the study conducted in the fermentation reactor in the range of pH 4-10; At 70 gTS/l-d OLR, HRT 8 daysandthe temperature was 38°C. Acidification percentage at pH 4 remained at 11% and total acid production was determined as 197 mg/l as HAc. The acidification percentage at pH 6 and 7 is 45% and 40% respectively, and acetic acid at these pH values has been produced at 406 mg/land 354 mg/l, the reason for this drop in pH 7 is considered to be methanogenic activity. As of pH 8, acidification was climbed upto 64%, the maximum acidification percentage was 70% at pH 10. Acetic acid and butyric acid was the dominant acid species at this pH, isobutyric, propionic and pentanoic acid were the remaining acids. At this stage, iso-butyric acid and propionic acid concentrations are 379 mg/l and 236 mg/l, respectively, which can create stress and inhibition on microorganisms.

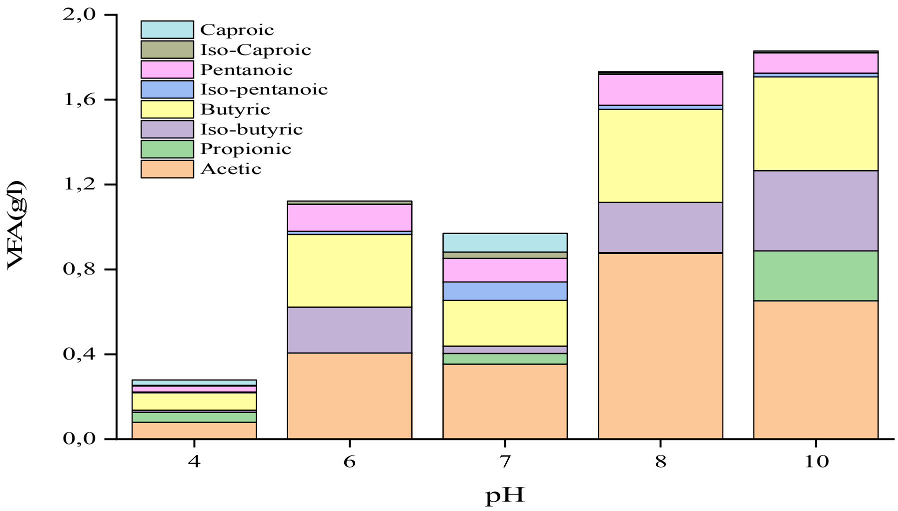


Figure 5.VFA distribution according to pH

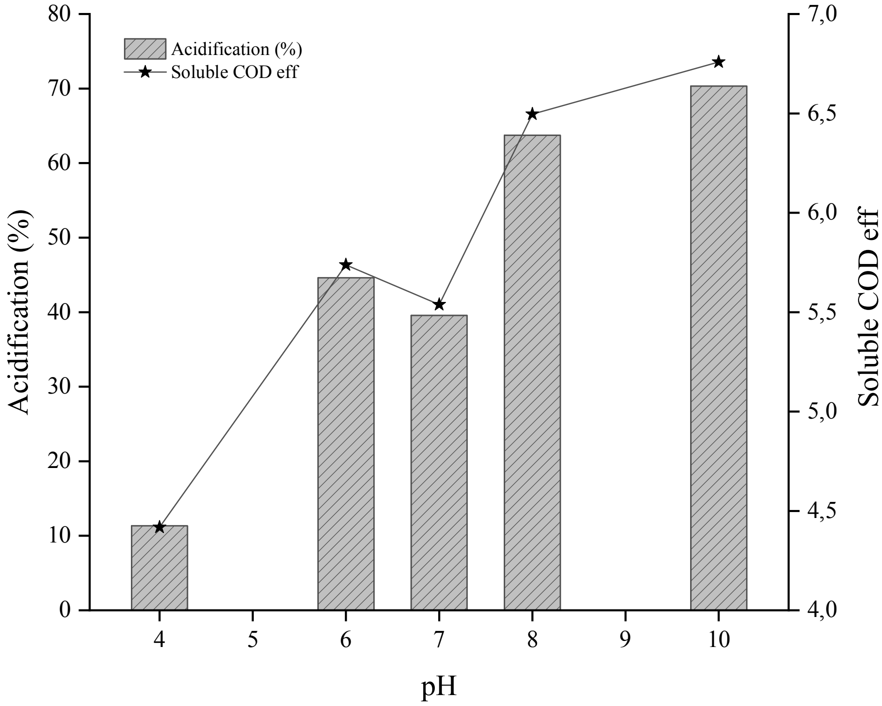


Figure 6. Acidification (%)and sCOD

Whiles COD value at the reactor was 6759 mg COD/l at pH 10, aceticacid has reached 31% in total acids and was determined as 652 mg /l. But there are the difference between pH 10 and 8 according to acidificaiton yield, at pH 8 it was calculated as 193 gCOD/kgVS. At pH 10, this value was determined to be 174 gCOD/kgVS. Apart fromthe VFA produced here, a lower conversion efficiency was calculated since different cellulosic ethanol, lactic etc. products were not evaluated as VFA.No chemical pre treatment has been applied to the feed to the system. however, in the studies performed, when NaOH was used in cellulosic content with the help of alkali pre-treatment processes, it caused a decrease in lignin between 24-55%. therefore, it has a high solubility efficiency and a low acidification efficiency [12]. At the same pH, the HAc/HPr ratio reached 2.76 and the amount of propionic acid in the total acidwas 11%. However, with the concentration of isobutyric acid reaching 8% in the total acid, it meansthatthere is a stress factor on the microorganism at pH 10, as in OLR.

Temperature Effect

Anaerobic treatment can take place at a mesophilic temperature of 25-40°C or a thermophilic temperature of 45-60°C[13].Although it has advantages such as high temperature hydrolysis, pathogen destruction, odor removal, whereas mesophilic operations have features such as less energy consumption, shock loads and less sensitivity to substances that cause inhibition. also, mesophilic temperature can accommodate a large microbial population capable of disrupting different types of organic matter. In this part of the study, the acidification percentage, acid conversion efficiency, dissolved organic matter conversion efficiency at different temperatures (20-38-55°C), at 70 gTS/l-d OLR, HRT 8 days, pH 10 were investigated separately. Acidification percentage 20°C, 38°Cand 55°C was calculated to be at 18.4%, 64.1% and 59.3%, respectively(Figure 7). At the end of the period, soluble COD was 6512 g COD/l at 35°Cand 55°Cwas 6321 gCOD/l (Figure 8). Acid conversion efficiency was determined as 165 grCOD/kgVS at mesophilic temperature and 141 grCOD/kgVS at thermophilic temperature. *Yu et al.,* stated that acid-forming enzyme activities in the thermophilic temperature range of 45 to 70°C are lower than mesophilic temperatures and that the increase in temperature does not matter in VFA production[14] .Here, the acidification yield was determined as 165 grCOD /kgVS at mesophilic temperature and 141 grCOD/kgVS at thermophilic temperature. At 20°C, these values are 33 grCOD/kg VS and the dissolved yield is 81 grCOD/kgVS respectively, which is quite low compared to other temperatures. As for the acid types; Acetic acid is the predominant VFA at 38°Cand 55°C, but butyric and isobutyric acid were identified as other dominant acids. Also, the rise of iso-bituric acid and propionic acids to 20% and 4.67% levels in 55°C causes us to say that stress occurs on the system at this temperature. Also, at this temperature, the HAc/HPr ratio was as high as 5.53.

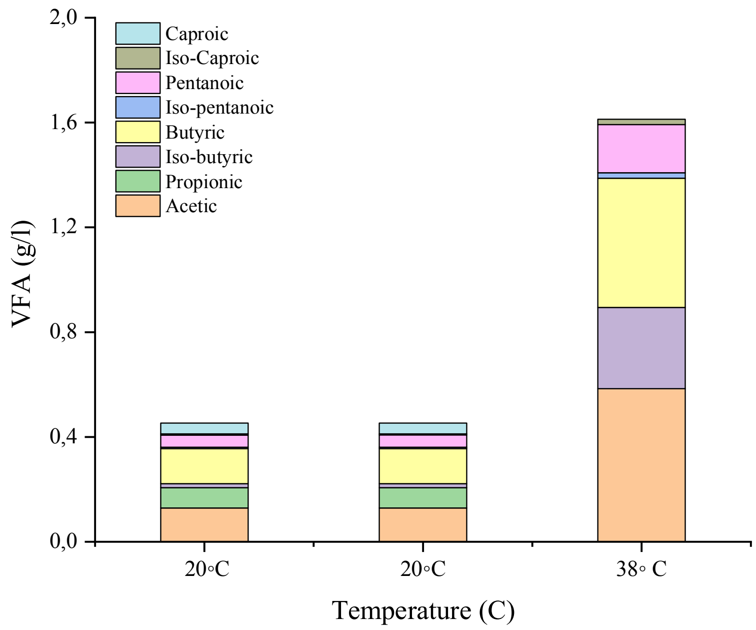


Figure 7.VFA distribution by temperature

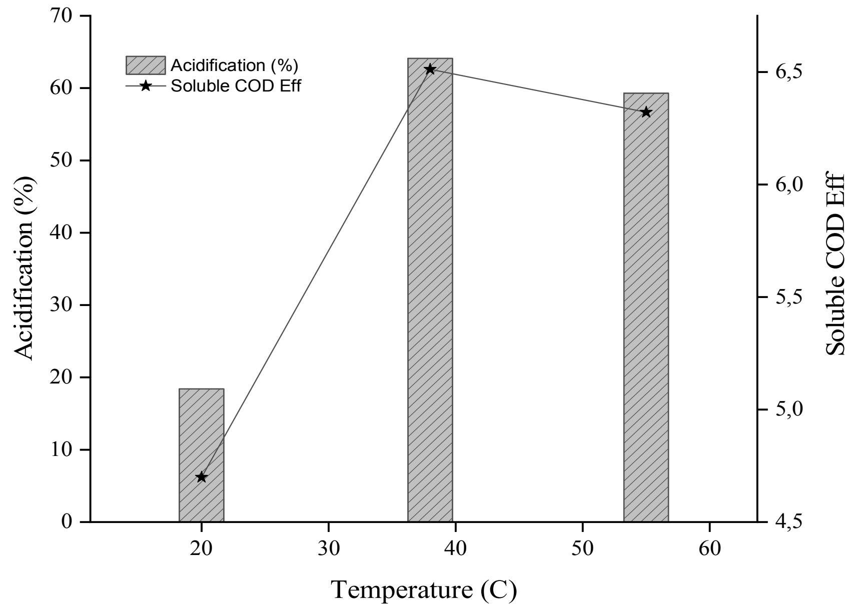
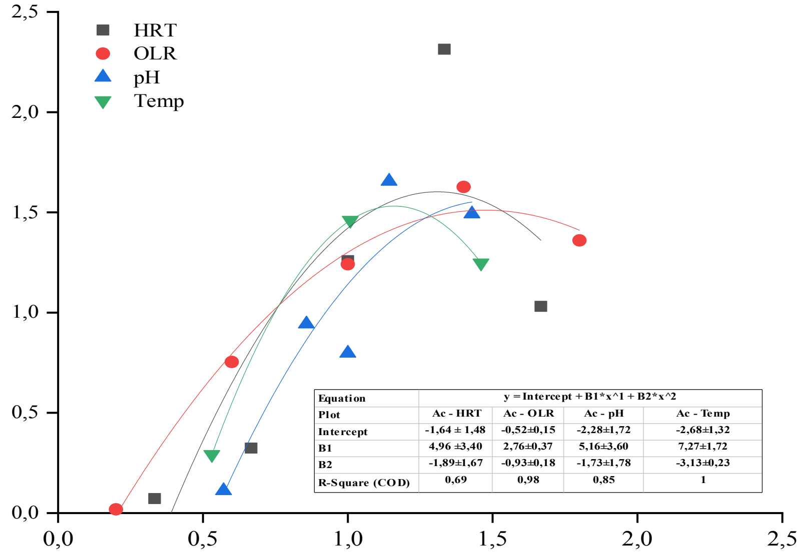


Figure 8. Acidification (%) and sCOD

Effective parameters' correlation

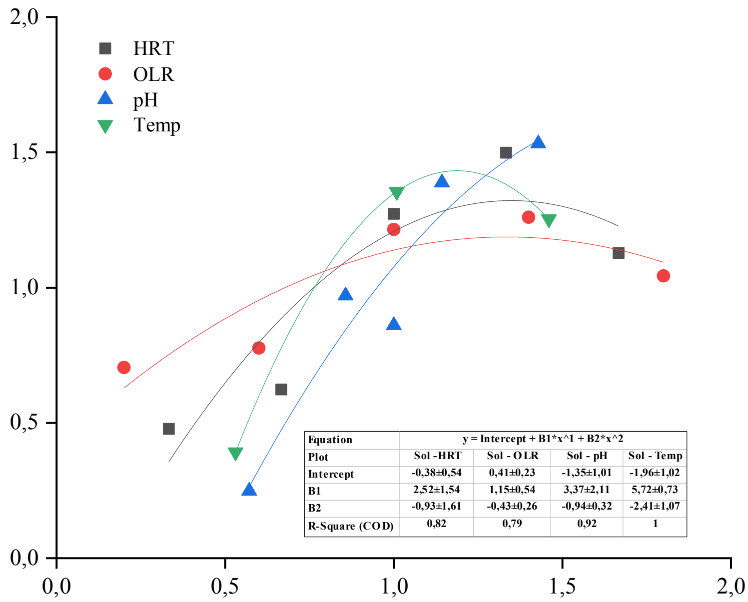
As a result of the optimization carried out the fermentation process, HRT 8 days at pH 10, temperature 38°C and OLR maximum 70 gCOD/l-d, while achieving the highest acidity percentage, a balanced system performance was achieved. The relationships between these effective operating and environmental parameters that affect the acidification percentage are also very important. In system optimizations, all these factors are evaluated based on the responses of the system. As can be seen in Figure 9, the reactions of acidification percentages with different values for each parameter was marked and their relations were examined through polynomal curves to display the trend. In the figure, while OLR, pH, Temperature show similar tendency of acidification at different values, HRT gives a different tendency to acidification at higher effect values than other parameters. The correlation coefficient of the polynomial is lower for this value compared to other parameters (R^2:0.69). Graphs were drawn by normalizing between values.



Equation	y = Intercept + B1*x^1 + B2*x^2			
Plot	Ac - HRT	Ac - OLR	Ac - pH	Ac - Temp
Intercept	-1,64 ± 1,48	-0,52±0,15	-2,28±1,72	-2,68±1,32
B1	4,96 ±3,40	2,76±0,37	5,16±3,60	7,27±1,72
B2	-1,89±1,67	-0,93±0,18	-1,73±1,78	-3,13±0,23
R-Square (COD)	0,69	0,98	0,85	1

Figure 9. Correlation of acidification percentage with effective parameters

However, the solubilization yield, another parameter used to evaluate system performance, was also evaluated (Figure 10). It can be said that OLR and HRT move in a similar trend among the effective parameters that take different values during the determination of dissolution efficiency, pH and temperature give a solubility efficiency independent of other parameters.



Equation	y = Intercept + B1*x^1 + B2*x^2			
Plot	Sol -HRT	Sol - OLR	Sol - pH	Sol - Temp
Intercept	-0,38±0,54	0,41±0,23	-1,35±1,01	-1,96±1,02
B1	2,52±1,54	1,15±0,54	3,37±2,11	5,72±0,73
B2	-0,93±1,61	-0,43±0,26	-0,94±0,32	-2,41±1,07
R-Square (COD)	0,82	0,79	0,92	1

Figure 10. Correlation of effective parameters in the solubilization yield

Principal component analysis (PCA)

PCA is one of the descriptive statistical methods used extensively in the evaluation of multiple data in the literature [15].98.8% of the total variable in the data was successfully explained by two main components. Figure 11 shows the relationship between key components and operating parameters. In principal component 1 (PC1), it explains 85.09% of the variability in the data and it has a positive correlation between the percentage of acidification, VFA (asHAc), Acetic acid formation, total VFA production, Dissolved COD and pH, OLR, temperature. In the upper right quadrant, there is a strong relationship between total acid production (TVFA) and HAc production, where optimum operating parameters with pH 10, pH8, OLR 70 g COD/kg VS and 38°C temperature can be seen. Dissolved COD and acidification do not have a strong correlation these parameters, however, as seen in Figure 9, HRT with other parameters do not show a similar trend, and HRT time in Figure 11 does not have a positive relationship with any of the acidification processes. similar way, soluble COD, it can be considered independent of HRT. It is appropriate to say that solubility is more related to pH and temperature than between long HRTs.

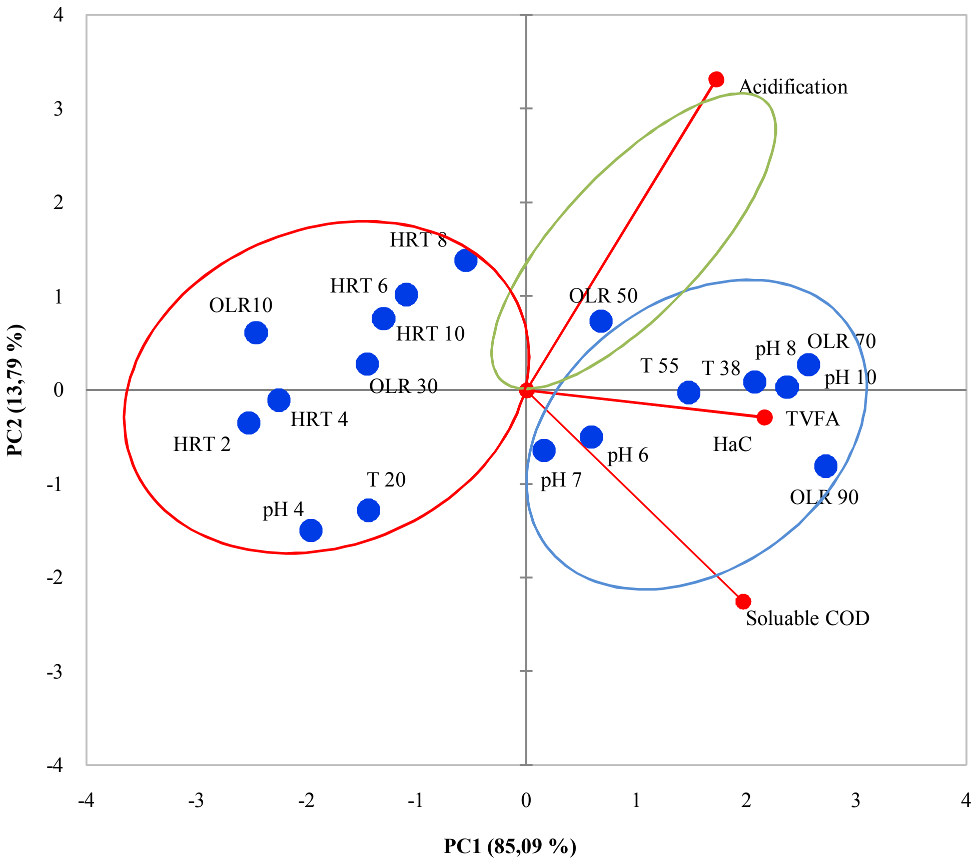


Figure 11. Relationships between effective parameters and processes

CONCLUSIONS

In this study, no pretreatment other than homogenization with mechanical disintegration was performed. It was determined that the effects of OLR, pH, Temperature parameters on acidification and acid production were higher in co-fermentation process (with Sweet Sorghum) compared to HRT parameter. Although the acidification percentage reaches upto 70% in the study carried out at pH 10, it is possible to say that the acidification is lower than pH 8, the amount of isobituric acid and propionic acid produced was increase and cause stress in the reactor. Maximum acid conversion (yield) is 193 gCOD/kgV at 70 gCOD/l-day OLR, 8 days, pH 8 and 38°C, meanwhile, the maximum solubility value is 308 gCOD/kg VS, at pH 10 was realized. In the operating conditions where it is in maximum acid conversion, acetic acid is 48%, butyric acid is 24%, and isobutyric was taken place in total acid as 13%.Acidification and COD conversion efficiency in fertilizer fermentation without different chemical and biological pretreatments are well below many studies in the literature.

ACKNOWLEDGEMENT
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ABBREVIATIONS
PCA: Principal component analysis
VFA: Volatile fatty acid (mg/l)
COD: Chemical oxygen demand (g/l)
sCOD: Soluable Chemical oxygen demand(g/l)
HRT: Hydraulic retention time (day^{-1})
OLR: Organic loading rate (g /l-day)
S_f: COD equivalent of VFA produced,(g/l)
S_i: COD value in influent (g/l)

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