Working Parameters Optimization of Hydrolysis-acidogenesis reactor in two stage anaerobic digestion of slaughterhouse Wastewater for Biogas Production

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Working Parameters Optimization of Hydrolysis-acidogenesis reactor in two stage anaerobic digestion of slaughterhouse Wastewater for Biogas Production

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Abstract

Background: Wastewater from agro-industries such as slaughterhouse is typical organic wastewater with high value of biochemical oxygen demand, chemical oxygen demand, biological organic nutrients (Nitrogen and phosphate) which are insoluble, slowly biodegradable solids, pathogenic and non-pathogenic bacteria and viruses, parasite eggs. Moreover it contains high protein and putrefies fast leading to environmental pollution problem. This indicates that slaughterhouses are among the most environmental polluting agro-industries. Anaerobic digestion is a sequence of metabolic steps involving consortiums of several microbial populations to form a complex metabolic interaction network resulting in the conversation of organic matter into methane (CH_4), carbon dioxide (CO_2) and other trace compounds. Separation of the phase permits the optimization of the organic loading rate and HRT based on the requirements of the microbial consortiums of each phase. The purpose of this study was to optimize the working conditions for the hydrolytic - acidogenic stage in two step/phase anaerobic digestion of slaughterhouse wastewater. The setup of the laboratory scale reactor was established at Center for Environmental Science, College of Natural Science with a total volume of 40 liter (36 liter working volume and 4 liter gas space). The working parameters for hydrolytic - acidogenic stage were optimized for six hydraulic retention time 1-6 days and equivalent organic loading rate of 5366.43 – 894.41 mg COD/L day to evaluate the effect of the working parameters on the performance of hydrolytic – acidogenic reactor.

Result: The finding revealed that hydraulic retention time of 3 day with organic loading rate of 1,788.81 mg COD/L day was a as an optimal working conditions for the parameters under study for the hydrolytic - acidogenic stage. The degree of hydrolysis and acidification were mainly influenced by lower hydraulic retention time (higher organic loading rate) and highest values recorded were 63.92 % at hydraulic retention time of 3 day and 53.26% at hydraulic retention time of 2 day respectively.

Conclusion: The finding of the present study indicated that at steady state the concentration of soluble chemical oxygen demand and total volatile fatty acids increase as hydraulic retention time decreased or organic loading rate increased from 1 day hydraulic retention time to 3 day hydraulic retention time and decreases as hydraulic retention time increase from 4 to 6 day. The lowest concentration of NH_4^+-N and highest degree of acidification was also achieved at hydraulic retention time of 3 day. Therefore, it can be concluded that hydraulic retention time of 3 day/organic loading rate of 1,788.81 mg COD/L .day was selected as an optimal working condition for the high performance and stability during the two stage anaerobic digestion of slaughterhouse wastewater for the hydrolytic-acidogenic stage under mesophilic temperature range selected (37.5°C).

Keywords: Slaughterhouse Wastewater, Hydrolytic – Acidogenic, Two Phase Anaerobic Digestion, Optimal Condition, Agro-processing wastewater

1. Background

In the recent centuries, the various ecosystems on which human life relies on were degraded due to the global industrialization, urbanization and population growth. Mismanagement of agro-industrial wastewater and over use of water creates maximum stress on fresh water bodies such as rivers, lakes (lotic and lentic), seas and oceans the decrease in the quality of aquatic ecosystem service was primarily due to the discharge of inadequately treated municipal and industrial wastewater.

Wastewater from agro-industries such as slaughterhouse is typical organic wastewater with high value of biochemical oxygen demand (BOD), chemical oxygen demand (COD), biological organic nutrients (Nitrogen and phosphate) which are insoluble, slowly biodegradable solids, pathogenic and non-pathogenic viruses and bacteria, and parasite eggs. Moreover it contains high protein and putrefies fast leading to environmental pollution problem. This indicates that slaughterhouses are among the most environmental polluting agro-industries.

Anaerobic digestion is a sequence of metabolic steps involving consortiums of several microbial populations to form a complex metabolic interaction network resulting in the conversation of organic matter into methane (CH_4), carbon dioxide (CO_2) and other trace compounds. During anaerobic digestion process complex organics such as: proteins, lipids and polysaccharides hydrolyzed to amino acids, fatty acids and sugars by enzymes. The above intermediates are then degraded further to volatile fatty acids (VFA) by acidogens. The acidogens are relatively grow faster less sensitive to the variation of pH then methanogens/acetogens which leads to the accumulation of volatile fatty acids, lowering of pH and suppression of methanogens. Scholars reported the instability/failure of the single-phase anaerobic reactor for different wastewaters mostly during high loading rate. The application of two-phase anaerobic reactor system was first and foremost conceptualized for the rationale of exploit the environmental conditions of different anaerobic process (hydrolysis and acidogenesis in the 1st phase and acetogenesis and methanogenesis in the 2nd phase) as specific micro-organism operate best at its optimal conditions.

To overcome the accumulation of VFA, fall of pH and separation of acidogenesis and methanogenesis bacteria so that they degrade the feedstock very efficiently, phase separation is very important. Separation of the phase permits the optimization of the OLR and HRT based on the requirements of the microbial consortiums of each phase. Therefore, two-phase anaerobic process can prevent the imbalance due to the groups of anaerobic bacteria occurring in single-phase reactors (Ghosh *et al*. 1987; Koutrouli et al. 2009). In hydrolysis phase optimization of working condition/parameters such as OLR, HRT, TVFA, SCOD and ammonia were very crucial. Formation of excess VFA, ammonia will result in system instability and even to failure (Cuetos et al. 2008).

Therefore, this research article was intended to designed for optimize the working condition of hydrolytic-acidogenic phase of the integrated two stage anaerobic digestion of slaughterhouse wastewater at mesophilic temperature range.

2. Materials and Methods

2.1. Source of feedstock

The experiment was carried out in laboratory, Center for Environmental Science (CES) at College of Natural Science of Addis Ababa University. The wastewater used as a feedstock for the study was from Organic export Abattoir slaughterhouse found in Modjo town, Ethiopia. Eight hundred to one thousand two hundred sheep and goats (each) per day were slaughtered at this slaughterhouse and the total of 400 L of water/sheep/goat was used. Almost equivalent amount of wastewater was discharged into the nearby Modjo River especially increasing the pollution load on Koka Lake the final destination of the Modjo River. The composite slaughterhouse wastewater was collected in an acidified 20 L polyethylene plastic 'jericuns' and transported to the Center for Environmental laboratory and stored at 4 °C until fed to the reactor.

2.2. Source of Inoculums

The stomach material (cud) from the same slaughterhouse was used as the source of the inoculums for the purpose of present study. A 1:1 ratio of the inoculums/cud as a source for the crucial microbes to feedstock (wastewater) was used to kick off the hydrolysis/acidogenesis system.

2.3. Experimental setup (digester design) of the laboratory scale digester

The setup used for the optimization of hydrolytic-acidogenic step was done using a 40L galvanized metal container (digester) with working volume of 36L and 4L gas space. The digesters were sealed with gasket maker to create anaerobic condition and tensioning bolts to support the sealing. The temperature of the digesters was maintained at 37 ℃ using hot water circulated from thermostat water bath (cu-420, China). Clean water pump (inC-CO, China) was used to circulate the hot water to maintain the digester temperature at 37 ℃.The pipes used for hot water circulation was composed of stainless steel pipes inside the digester and ¾ PPR pipe for the extension of the pipe outside the digester. Fig. 1 shows the photo of laboratory experiment setup.

Fi. 1: Photo of Laboratory Experiment setup

The digester has wastewater feeding and discharging, level regulation and sludge discharging ports with control valve at each port. Fig. 2. (Below) shows the detail laboratory scale experimental system setup.

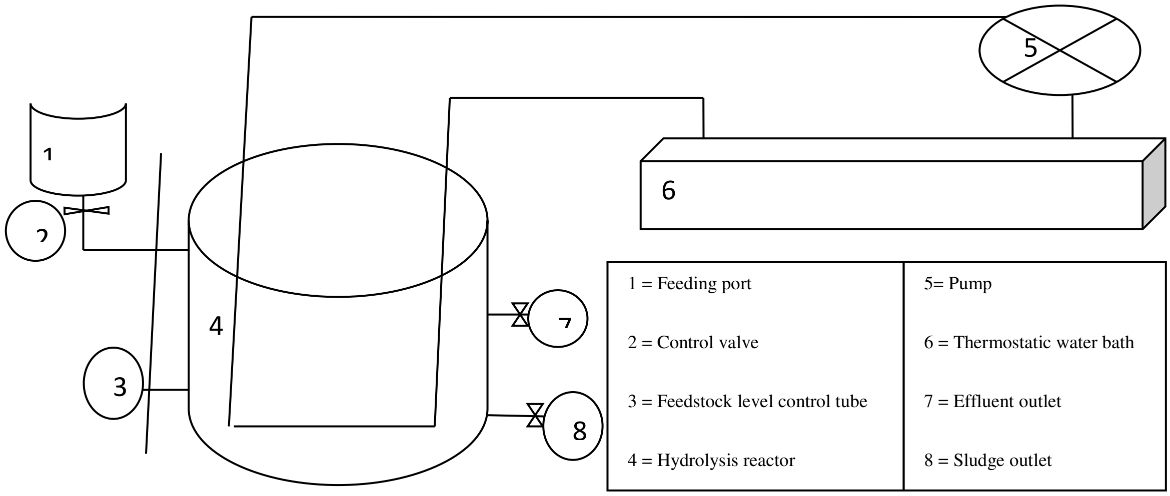

Fig. 2: Schematic diagram of the laboratory scale hydrolytic-acidogenic reactor experimental setup.

2.4. Operating procedure (conditions)

For the optimization of the working condition basically HRT and OLR of hydrolytic-acidogenic stage a 40 L total volume reactor was established at laboratory scale. In order to retain an anaerobic condition in the hydrolytic-acidogenic digester inert gas (nitrogen gas) was bubbled before starting the experiment to dissolve the oxygen in the digester. The detail operating condition of the hydrolytic-acidogenic digester was presented in Table 5. To kick up the system the reactor was fed with a 1:1 ratio of inoculums to slaughterhouse wastewater. The system was acclimatized by increasing the wastewater fed per day gradually till the working volume level achieved. The optimal conditions of the OLR and HRT for the hydrolysis stage anaerobic digester were determined by comparing the performance of the process at six different HRTs (1, 2, 3, 4, 5 and 6 days) at mesophilic temperature range 37.5℃ as indicated in Table 2. The digester efficiency parameters considered during the optimization were TCOD, SCOD, TVFA (the key parameters as it is the main acid stage product reflecting the organic matter that has been hydrolyzed) and NH_4^+-N (inhabitant of the reactor/system).

At each OLR, the values of the parameters understudy; TVFA, NH_4-N, TCOD and SCOD were evaluated at steady state condition. The steady state condition was assumed to be achieved when the concentration of the parameters under study was within 10% variation and fifteen (15) consecutive reading were taken for each parameter after realization of the steady state condition.

Table 1: Operating/working condition of Hydrolytic-acidogenic digester at different HRT/OLR

HRT (day)	OLR (mg COD/L.day)	Flow rate (L/day)
6	5366.43	6
5	2683.22	7.2
4	1788.81	9
3	1341.61	12
2	1073.27	18
1	894.41	36

2.5. Degree of acidification

Degree of acidification is a parameter used to measure the degree of success of acid fermentation which represents the amount of solubilized matter converted to VFAs. It was quantified using eq. 1 to evaluate the performance of the digester.

$$\boldsymbol{DA}\ (\%) = \frac{\boldsymbol{Sf}}{\boldsymbol{Si}}\mathbf{x100} \quad \textbf{Eq. 1}$$

Where, DA represent Degree of acidification, S*i*: initial Feedstock concentration expressed in mg/L of COD, S*f*: Net VFA produced (Final-initial) expressed as theoretical equivalent of COD concentration in mg/L. The COD equivalent of each VFA: Acetic acid (1.066), Butyric acid (1.816), Propionic (1.512), Valeric acid (2.036) and Caproic acid (2.204) (yilmaz and Demirer 2007).

2.6. Analytical Methods

Physico-chemical characteristics of the raw slaughterhouse wastewater, effluent from hydrolytic-acidogenic reactor were analyzed for the parameters mentioned below. Total solids and suspended solids (TS and TSS), Volatile and suspended solids (VS and VSS), Chemical Oxygen Demand (COD), Total Kjeldahl Nitrogen (TKN), Ammonium Nitrogen (NH_4^+-N), were characterized by standard method (APHA, 2012). Total Alkalinity and Total Volatile Fatty Acid (TVFA) were analyzed by titration method as described (APHA, 2012). The parameters such as, Oxidation Reduction Potential (ORP) and pH was analyzed using pH meter (JENWAY, UK). Resistivity, Salinity, Electrical Conductivity (EC), Total dissolved solids (TDS) were analyzed by multimeter (EUTECH CON2700, Spain).

2.7. Statistical analysis

The data generated from the analysis during the study were entered to the MS excel spreadsheet based on the objective set for further statistical analysis. The statistical analysis for mean, Standard deviation, correlation and One-way analysis of variance (ANOVA) performed at 95% confidence interval were also performed using excel statistical package to compare the performance of hydrolytic-acidogenic reactors' for 1, 2, 3, 4, 5 and 6 day HRTs and origin 8.0 software to draw graphs. All the samples analysis values for the parameters under study were taken at least triplicate to ensure reproducibility of the experiment.

3. Result and Discussions

3.1. Characteristics of Feedstock (Slaughterhouse wastewater)

The collected wastewater was stored at 4°C until feeding to the reactor/digester to reduce the microbial activity and maintain the characteristics of wastewater. The slaughtered animal showed variation in number due to the fluctuation of market demand, as a result the wastewater quantity and composition also varied. The main characteristics of the raw slaughterhouse wastewater utilized for this research is presented in Table 2. The main characteristics of the slaughterhouse wastewater is presented in Table 1. The wastewater had a mean ± SDV of 5366.43 ± 826.80, of which about 60 - 90.24 % was in soluble form and the other in particulate matter. The high values of COD and BOD_5 can be attributable to the fact that the slaughterhouse wastewater contains mainly organic molecules such as fat, glucose and proteins from blood and other body fluids (Abrha Mulu and Tenalem Ayenew 2015).

The pH of the wastewater ranges nearly neutral from 6.80-7.399. The temperature and ORP were ranges from 28.9 - 30.5°C and -62.5 to -101.1 mv respectively. The EC, TDS and salinity of the raw slaughterhouse wastewater used as feedstock during the study were ranged between 1348-1964 ppm, 1165 - 1684 ppm, 1210-1628 ppm and 290.9 - 425 Ω respectively which were very high. The high values of EC, TDS and Salinity indicated in this study were may be due to the mobile (dissolved) ions present in the slaughterhouse wastewater (Padilla et al. 2011). The mean TVFA, TCOD, SCOD and NH_4^+-N concentration were 816.6 ± 381.67, 5366.43 ± 826.80, 4842.21 ± 826.81 and 338.40 ± 58.13 (mg/L) respectively. This high quantity of organic matter require more quantity of oxygen to oxidized in to carbon dioxide and water and may contribute to the increase in the COD and BOD of the water body receiving the waste (Abdullahi A S et al. 2004). Scholars such as, Abraham and Tenalem (2015); Zemene Worku and Seyoum Leta (2017) reported the average COD concentration of slaughterhouse wastewater of 4752.66 ± 1156.27; 6942.59 ± 152.98 – 7079.69 ± 226.89 mg/L respectively.

Table 2: Physico-chemical characteristics of the slaughterhouse wastewater

S/N	Parameters	Raw Slaughterhouse wastewater
1	pH	7.055 ± 0.30
2	Salinity	1208.98 ± 428.48
3	Electrical Conductivity	1346 ± 462.88
4	Resistivity (Ω)	458.46 ± 155.75
5	TDS	1170.74 ± 399.84
6	ORP	-80.5 ± 18.13
7	TVFA	816.6 ± 381.67
8	TCOD	5366.43 ± 826.80
9	SCOD	4842.21 ± 826.81
10	NH_4^+-N	338.4 ± 58.13

3.2. Stability Evaluation of Hydrolysis reactor

The digestion of anaerobic process begins with the bacterial hydrolysis of the feedstock material in order to break down insoluble polymers such as carbohydrates, proteins, fats and make them available for the bacteria. Once absorbed, these insoluble organic polymers undergo bacterial degradation that results in the production of soluble sugars. Chen et al. (2008) stated that the anaerobic digestion is much susceptible than the aerobic process for the same degree of factor devotion from optimum condition during the waste treatment. Therefore, in this study the hydrolysis step stability of the reactor was evaluated based on the breakdown of large molecules to accumulation of intermediates; VFA, alkalinity SCOD and NH_4^+-N. Table 3 (below) shows the average steady state of the parameters for the stability indicators.

Table 3**: The average performance of Hydrolytic-acidogenic reactors at steady state for differ HRT/OLR**

Parameters	Raw Slaughterhouse wastewater	HRT 1 Effluent	HRT 2 Effluent	HRT 3 Effluent	HRT 4 Effluent	HRT 5 Effluent	HRT 6 Effluent
pH	7.055 ± 0.30	6.892 ± 0.730	6.759 ±0.149	6.489 ± 0.333	6.126 ± 0.480	6.809 ± 0.0143	6.733 ± 0.166
Salinity	1208.98 ± 428.48	1710.00±155.65	1538.33±175.04	1650.40±120.22	1785.27±183.54	1784.27±71.04	1784.67 ±122.26
EC	1346 ± 462.88	1835.87±142.18	1674.07±174.68	1809.73±122.07	1950.20±193.85	1964.80±80.26	1934.47±126.43
Resistivity (Ω)	458.46 ± 155.75	313.17±32.41	341.66±41.01	318.01±22.49	296.43±29.83	289.43± 12.49	292.23±16.00
TDS	1170.74 ± 399.84	1602.33±128.24	1469.80±168.75	1576.47±106.60	1702.00±165.20	1726.93±53.74	1697.27±109.48
ORP	80.5 ± 18.13	79.03±3.15	78.59±3.21	82.25±6.54	81.69±5.25	82.58±3.49	81.21±3.36
TVFA	816.6 ± 381.67	996.75± 138.60	1006.42± 298.35	1176.50± 81.66	1155.92± 163.20	1006.42± 298.35	1084.83± 139.37
TCOD	5366.43 ± 826.80	4793.92 ± 491.26	4915.25 ± 295.17	4944.75 ± 241.75	4872.00 ± 80.05	4872.00 ± 80.05	4302.33 ± 434.61
SCOD	4842.21 ± 826.81	2084.4 ± 710.00	3106.87 ± 720.65	3430.2 ± 800.44	2483.73 ± 467.72	2359.00 ± 395.79	2324.80 ± 249. 16

NH_4^+-N	338.4±58.13	346.42± 40.67	369.33± 51.75	228.08 ± 58.83	319.08 ± 40.21	281.67 ± 46.40	278.67 ± 47.25

3.2.1. The effect of OLR on Salinity, EC, TDS, Alkalinity and Resistivity

Alkalinity, Salinity and TDS are the buffering capacity enhancers in the anaerobic digestion system. The average values and the variation of the parameters at different HRT were indicated in Table 3 and Fig. 3, 4, 5 respectively. Salinity, TDS, EC, Alkalinity, ORP and Resistivity of the hydrolytic – acidogenic digester were ranges from 1785.27 ± 183.54 at HRT 4 day to 1538.33 ± 175.04 at digester 2, 1726.93± 53.74 HRT of 5 day to 1469.80 ± 168.75 at HRT of 2 day, 1964.80 ± 80.26 at HRT of 5 day to 1674.07 ± 174.68 at HRT of 2 day, alkalinity value, -82.58 ± 3.49 at HRT of 5 day to -78.59 ± 3.21 at HRT of 2 day and 341.66 ± 41.01 at HRT of 2 day to 289.43 ± 12.49 at HRT of 5 day respectively. The correlation statistical analysis of pH, ORP, EC, TDS, Salinity and Resistivity was also computed. Table 4 indicated the correlation matrix of some optimized parameters in the present study.

Table 4: Correlation matrix of optimized parameters

Parameters	pH	ORP	EC	TDS	Salinity	Resistivity
pH	1					
ORP	0.99	1				
EC	0.9	0.94	1			
TDS	0.86	0.91	1	1		
Salinity	0.9	0.93	0.99	0.98	1	
Resistivity	-0.87	-0.9	-1	-0.99	-0.98	1

Accordingly, pH, ORP, Salinity, EC and TDS have strong positive and negative correlation with each other and resistivity respectively (Table 4).

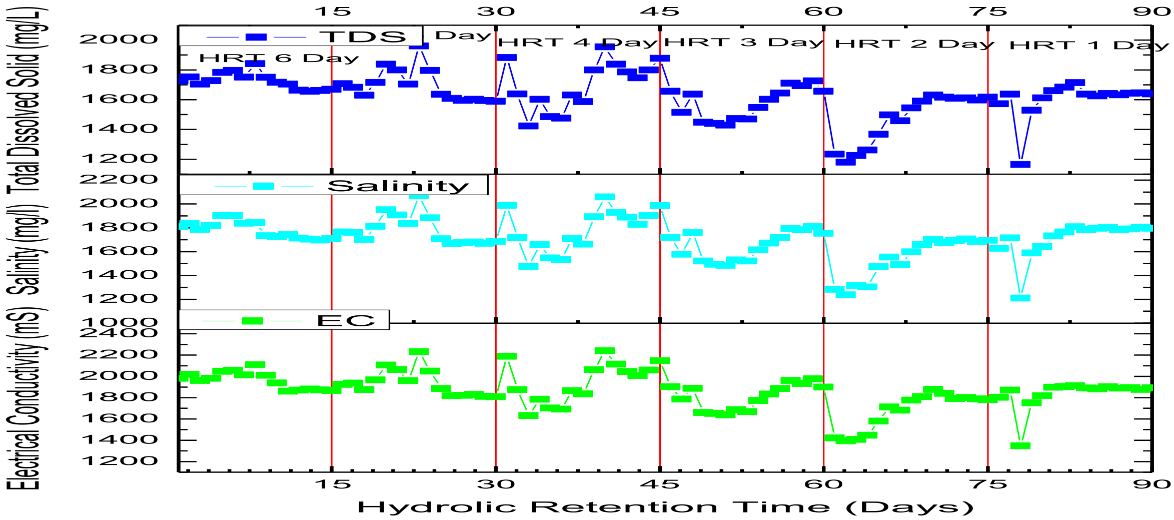

Fig. 3: Variation in TDS, Salinity and EC during the two stage anaerobic digestion of SHWW of hydrolytic-acidogenic reactor at different Hydraulic retention times

3.2.2. The effect of OLR/HRT on pH

The trends in pH variation of the hydrolytic-acidogenic reactor/digester during the study period at different OLR/HRT are shown in Fig. 4 and average values in Table 3. As indicated in the Fig. 4 the pH value and trend was influenced by the reactor operational conditions; OLR and HRT. The pH ranges were 7.054 – 6.813, 7.044 – 6.613, 7.03 – 6.174, 7.058 – 5.678, 7.044 – 6.672 and 7.019 – 6.594 at HRT of 6, 5, 4, 3, 2 and 1 days respectively. As indicated in the Fig. 4 the almost neutral pH values were recorded during the startup and gradually decrease until the system attain relative steady state at all the OLR and corresponding HRT. The decrease in pH during the start or acidification phase was likely due to the VFA intermediates, lactate and ethanol produced from the degradation of the organic matter in the slaughterhouse wastewater used as a feedstock (Angelidaki et al. 2002; Jiwei *et al*. 2014). Demirer and Alkaya (2011) also reported similar trends of pH during acidification of sugar beet processing wastes. Shifere Berhe and Seyoum Leta (2017) reported the pH values ranging from 7.98 – 4.90 during the optimization of hydrolytic-acidogenic digester operating condition for the anaerobic co-digestion of tannery and dairy wastewater at different HRT and OLR.

The pH range observed during the optimization process of acidogenic digester was 7.058 – 5.678 at OLR and equivalent HRT ranging from5366.43- 894.41 mg COD/L.day and one-six day respectively, which is in the range suitable for the growth of fermentative and acidogenic microorganisms (bacteria).

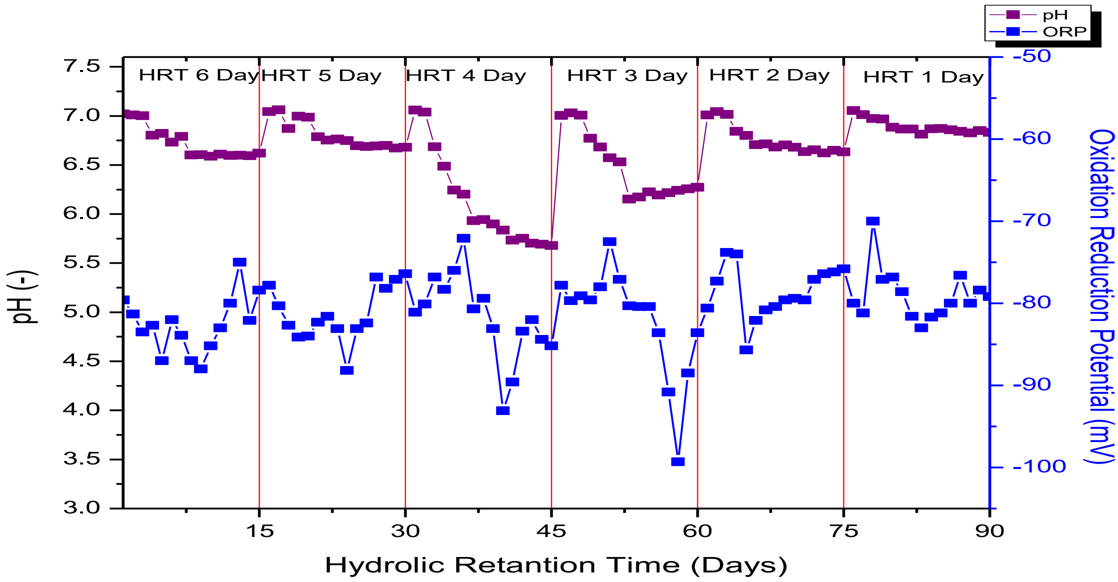

Fig. 4: Change in pH and ORP at hydrolytic-acidogenic step at different HRT

As it was seen from the Fig. 4 the pH at HRT of 1, 2, 5 and 6 day was above the optimum suitable range for hydrolytic-acidogenic consortium of bacteria. The pH at HRT of 3 and 4 day lay in the range of optimum pH for hydrolytic-acidogenic reactor of two stage anaerobic digester (Table 3).

3.2.3. The TVFA production at different HRT

In anaerobic digestion with separate hydrolysis and methanogenesis stage, VFA concentration in hydrolysis digester is the main indicators of system stability. The mean steady state period concentrations of TVFA in hydrolytic-acidogenic digester of each HRT are presented in Table 3. The average concentrations of TVFA produced during the optimization of the system were 996.75 ± 138.60, 1006.42 ± 298.35, 1176.50 ± 81.66, 1155.92 ± 163.20, 1006.42 ± 298.35 and 1084.83 ± 139.37 at HRT of 1, 2,3,4,5 and 6 days; OLR of 5366.43, 2683.22, 1788.81, 1341.61, 1073.27 and 894.41 mg/L of COD respectively. As can been seen from the Fig. 5 and Table 3 the OLR have effect on the TVFA concentration in the digester. The VFA concentration increased with the increase in OLR from 894.41- 5366.43 mg/l of COD; as the increasing the OLR would result in fast production of high intermediate product like TFVA by the hydrolytic-acidogenic bacteria. This is may be due to the large amount of biodegradable organic matter in the slaughterhouse wastewater. The TVFA concentration decrease from HRT of 4 to 6 day may be attributed to the washout of the hydrolytic-acidogenic consortia of bacteria (Jianguo et al. 2013). The VFA produced during the process is an indicator of the hydrolysis for the most insoluble organic matter in the digester. Scholars such as Shifare Berhe and

Seyoum Leta (2017) and Lim et al. (2008) also reported that the concentration of TVFA decrease as HRT increases and further increase of HRT will not further increase the production of TVFA. Moreover, Shifare Berhe and Seyoum Leta (2017) also reported the VFA ranging from 2,800 - 3,900 g$CaCO_3$/L at optimum condition during the two stage anaerobic co-digestion of tannery and dairy wastewater. High and low variation in TVFA concentration was observed at OLR (HRT) of 1,788.81 (3) and 5,366.43 (1) mg COD/L.day (day) respectively (Fig. 5; Table 3).

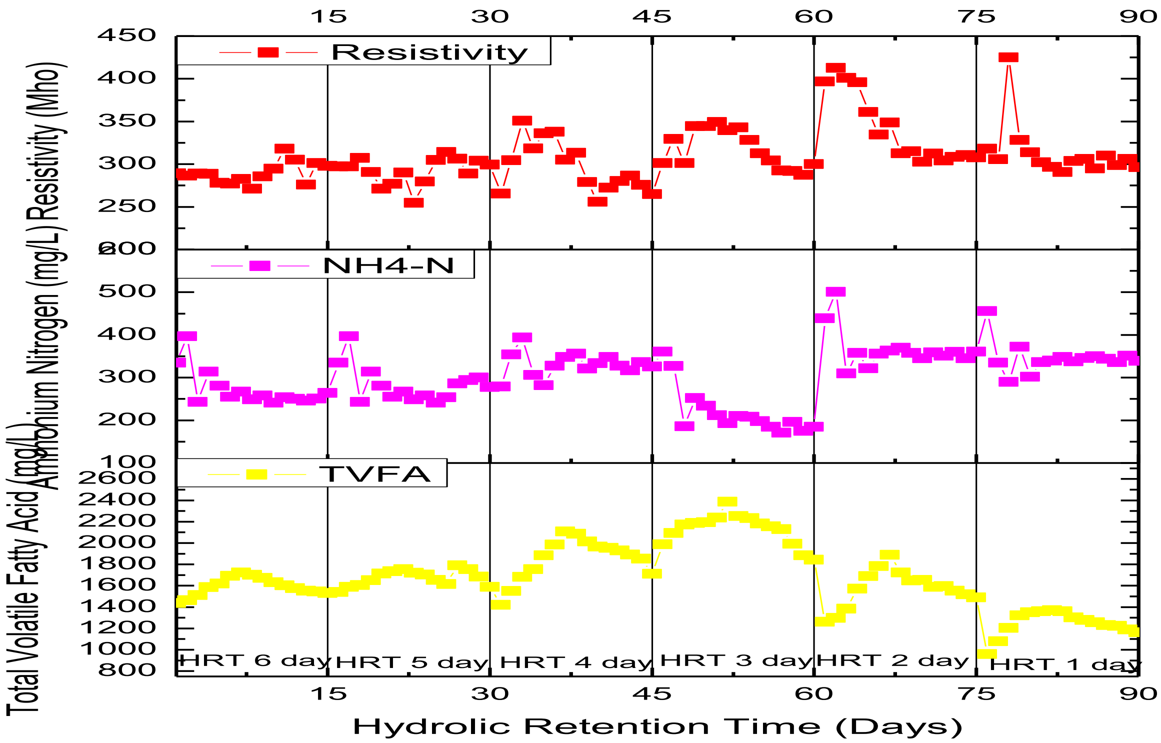

Fig.5: Variation of Resistivity, NH_4^+-N and TVFA at hydrolytic-acidogenic step with different HRT

The trends of the VFA produced at all HRT and corresponding OLRT during the experimental period were presented in Fig. 5. As it was indicated in the figure TVFA concentration during the reaction course shows the decreasing trend during the startup time and becomes nearly stable after 6th day of reaction time for each HRT under study. This may be due to the fact that microorganism's consortia usually take time to start their metabolic activity before becoming fully efficient.

The TCOD and SCOD production at different HRT

TCOD and SCOD were also among the parameters taken into consideration in present study in order to observe the performance of the hydrolytic-acidogenic digester. As presented in Table 3, the average

TCOD was 4793.92 ± 491.26, 4915.25 ± 295.17, 4944.75 ± 241.75, 4872.00 ± 80.05, 4872.00 ± 80.05, 4302.33 ± 434.61; at HRT of 1, 2,3,4,5 and 6 days; OLR of 5366.43, 2683.22, 1788.81, 1341.61, 1073.27 and 894.41 mg/L of COD respectively. As presented in Table 3, the average SCOD was4793.92 ± 491.26, 4915.25 ± 295.17, 4944.75 ± 241.75, 4872.00 ± 80.05, 4872.00 ± 80.05, 4302.33 ± 434.61; at HRT of 1, 2,3,4,5 and 6 days; OLR of 5366.43, 2683.22, 1788.81, 1341.61, 1073.27 and 894.41 mg/L of COD respectively. The trend of TCOD and SCOD concentration during the reaction time at each HRT and OLR were presented in Fig 6. The TCOD concentrations at the staring time for each HRT was fluctuating and come to stability after 7th day (Fig. 6). As depicted from Fig. 6, at each OLR/HRT the SCOD concentration shows a steady increase with reaction time. This is may be due to the fact that the microorganism consortia acclimatized and were acting at their optimal condition increasing the fermentation performance also increases fast solubilization observe at HRT/OLR of 3 day/1788.81 mg COD/L (Fig. 6). The highest TCOD (mg/L) and SCOD (mg/L) were achieved at HRT of 3 day and OLR of 1788.81 mg COD/L. Therefore, HRT of 3 day at OLR of 1788.81 mg COD/L was selected as the optimum HRT and OLR of hydrolytic-acidogenic digester as literatures suggest feedstock with high SCOD concentration yields high biogas Zhang et al. (2011) and used as a feedstock for methanogenesis digester for further study.

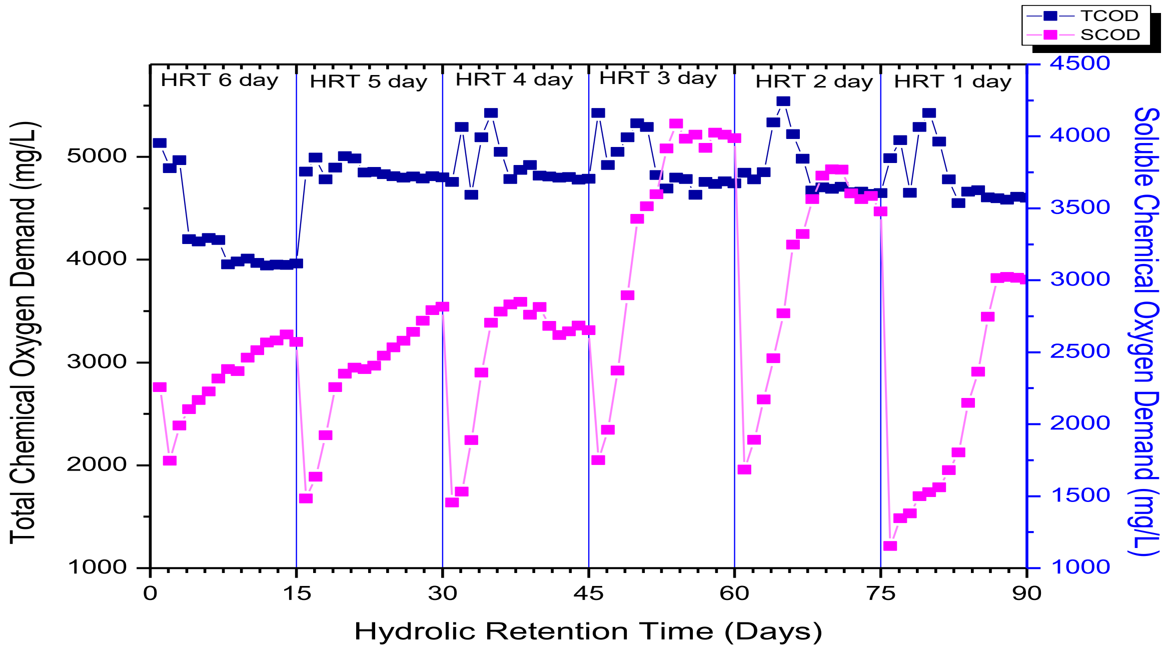

Fig.6: Variation trends of TCOD and SCOD during at hydrolytic-acidogenic step during two stage anaerobic digestion of SHWW at different HRT

3.2.4. Degree of Acidification

In this study the extent of acidification was assessed using the degree of acidification and their acidification performances were also compared and depicted in Fig. 7. Increases in OLR from 894.41 - 1342.61 mg/L of COD increase the DA from 17.17 - 57.26% and then increasing beyond this resulted in decrease DA (Fig. 7). The minimum and maximum acidification was achieved for the TCOD loading rate of 894.41 mg/L and 1342.61 mg/l respectively and influent SCOD of 2354.71 mg/L. In general, DA results (17.17 - 57.26%) obtained in this study is within the range of previously studied research. The values of DA reported in this study was in the range of the values reported (20 - 60%) by Demirel and Yenigu (2004) during the anaerobic digestion of dairy wastewater. Boualagui et al (2004) reported the DA of 38.9 - 44.4% in hydrolytic-acidogenic digester at HRT of 3 days on the study of two phase anaerobic digestion of fruit and vegetable waste mixture. The maximum DA (57.26%) value obtained is similar to the value reported by Shifare Berhe and Seyoum Leta, (2017) which is 55.5% at optimal condition in the study on two phase anaerobic co digestion of tannery and dairy wastewaters focusing on the effect of operational parameters on performance of hydrolytic-acidogenic step. The assumed optimum DA value required for anaerobic digester process stability lies between 40-50% (Alexiou and Anderson, 1997).

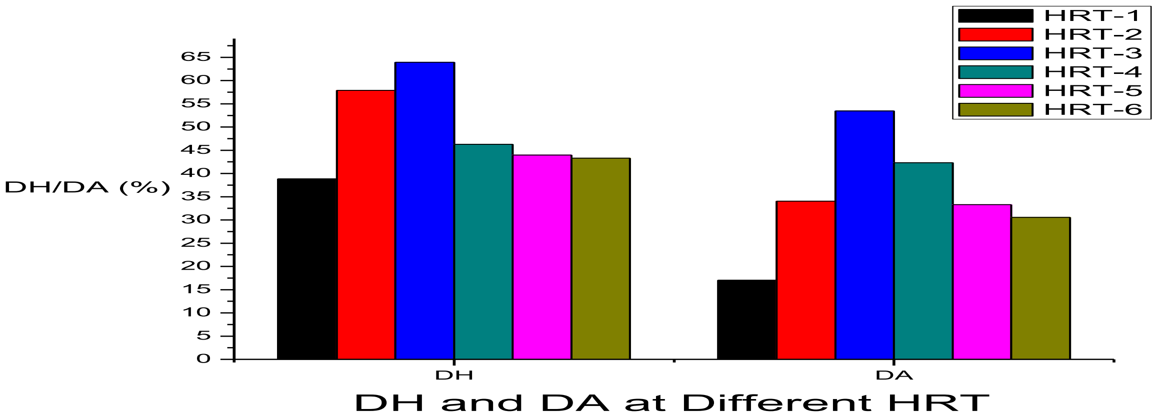

Fig. 7: DH and DA of the Hydrolytic-acidogenic digester at different HRT

3.2.5. Ammonium nitrogen production at different HRT

In reality, feedstock like slaughterhouse wastewater containing high nitrogen can frequently pose problems on the process stability of anaerobic digesters. The average NH_4^+-N concentration of the effluent of the hydrolytic-acidogenic digester during the optimization of the two stage anaerobic digestion of the slaughterhouse wastewater was presented in Table 3. The NH_4^+-N produced during the hydrolytic-acidogenic digestion of nitrogenous compounds mostly in the form of proteins, which were hydrolyzed into amino acids and further degraded into ammonia. The produced NH_4^+-N during hydrolysis has a significant role in buffering the digester, microbial growth and stabilizing of the hydrolysis process Garcia-Pena et al. (2011) and also a preferred nitrogen nutrient for methane forming bacteria but when present in high concentration it will cause reticence in anaerobic process (Nielsen and Ahring, 2007). The concentrations of NH_4^+-N produced were high at the startup of the reaction and gradually decrease and come to steady state almost after 4th day of the reaction time (Fig. 5) at each HRT. As indicated in the Table 3 the highest and lowest average NH_4^+-N concentration was observed at HRT of 2 day (369.33 ± 51.75 mg/L) and 3 day (228.08 ± 58.83 mg/L) respectively (Table 3) while the highest and lowest NH_4^+ -N concentration observed were at HRT of 2 and 3 day; on the 12th (171 mg/L) and 2nd (501 mg/L) days of reaction time during the course respectively (Fig. 5). Sossa et al. (2004) studied the ammonium inhibition on an anaerobic film enriched by methylaminotrohic methane producing Archaea and reported that 48.8 mg/L and 848.8 mg/L were the maximum and inhibitory ammonia concentration on the activity of methanogenic bacteria respectively. Different scholars reported different lowest NH_4^+-N inhibition levels. Braun (1981); Speece (1996); Chen et al. (2008); Angelidaki and Ahring (1994) reported that the NH_4^+-N concentration inhibition in anaerobic digester starts from 5000, 8500, 14000 and 400 mg/L respectively. The results of present study indicating that the concentration of the NH_4^+-N reported during the optimization process is not adversely disturb the performance and stability of the hydrolysis process.

Table 5: Summary of the mean values for the parameters indicate stability at optimum working condition of hydrolytic-acidogenic digester

S/n	Stability indicator parameters (working Conditions)	Concentration
1	pH	6.489 ± 0.33
2	SCOD(mg/L)	3,430.20 ± 800.44
3	NH_4^+-N (mg/L)	219.53 ± 55.21
4	TVFA (mg/L)	1,176.50 ± 81.66
5	DH (%)	63.92
6	DA (%)	57.26
7	HRT (day)	Three
8	OLR (mg COD/L. day)	1,788.81
9	Flow rate (L/day)	12

Table 5 shows the selected values for the parameter indicating the digester stability at the optimization of the hydrolytic-acidogenic stage of two stage anaerobic digestion of slaughterhouse wastewater. Therefore, the optimum values for the most of the stability indicating parameters were recorded at

HRT of 3 days/OLRT of 1, 788.81 mg COD/L (Table 5) and this HRT will be used for the further study of the methanogenic step.

4. Conclusion

In present study working parameters such as HRT and OLR were optimized in order to establish the suitable working condition for the hydrolytic-acidogenic stage of two stage anaerobic digestion of slaughterhouse wastewater at mesophilic temperature range (37.5°C). The DH and DA in the reactor were evaluated in terms of effluent SCOD, NH_4^+-N and TVFA concentration at steady state. The finding indicated that at steady state the concentration of SCOD and TVFA increase as HRT decreased or OLR increased from 1- 3 day HRT and decreases as HRT increase from 4 - 6 day. Furthermore, the lowest concentration of NH_4^+-N and highest DA was also achieved at HRT of 3 day. Therefore, it can be concluded that HRT of 3 day at OLR of 1,788.81 mg COD/L. day was selected as an optimal working condition for the high performance and stability during the two stage anaerobic digestion of slaughterhouse wastewater for the hydrolytic-acidogenic stage under mesophilic temperature range selected (37.5°C).

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Abbreviations

APHA: American Public Health Association; BOD: biological oxygen demand; COD: chemical oxygen demand; DA: degree of acidification; DH: degree of hydrolysis; DO: dissolved oxygen; EC: electrical conductivity; EPA: environmental protection authority; HRT: hydraulic retention time; NH4-N: Nitrogen ammonium; OLR: organic loading rate; ORP: oxidation reduction potential SCOD: soluble chemical oxygen demand; SHWW: slaughterhouse wastewater; TCOD: total chemical oxygen demand; TDS: total dissolved solid; TKN: total kjeldahl nitrogen; TSS: total suspended solids; TVFA: total volatile fatty acids; VFA: volatile fatty acids

Authors' contributions

All authors have made an obligatory intellectual involvement to this study. DTB designed the study, conducted the experiments, Collected, analyzed and interpreted the data and wrote the manuscript.

SLA participated on the study design, supervised the experiment, provided comments and suggestion for the whole work. MMK supervised the work, drafting and revising the primary manuscript, edited the manuscript, provided pertinent comments and suggestion on the manuscript. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The dataset and materials used for this manuscript is available and can be shared whenever necessary. Data was generated by the author from the field, substrate sample collection and laboratory analysis.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Photo of Laboratory Experiment setup

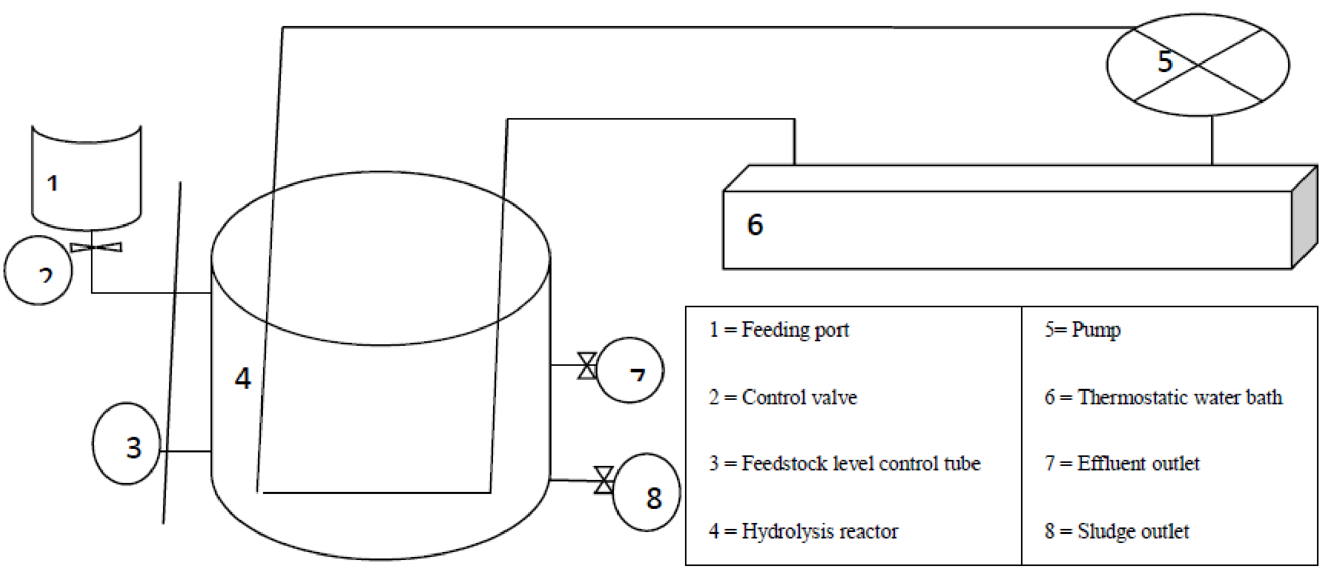

Figure 2

Schematic diagram of the laboratory scale hydrolytic-acidogenic reactor experimental setup.

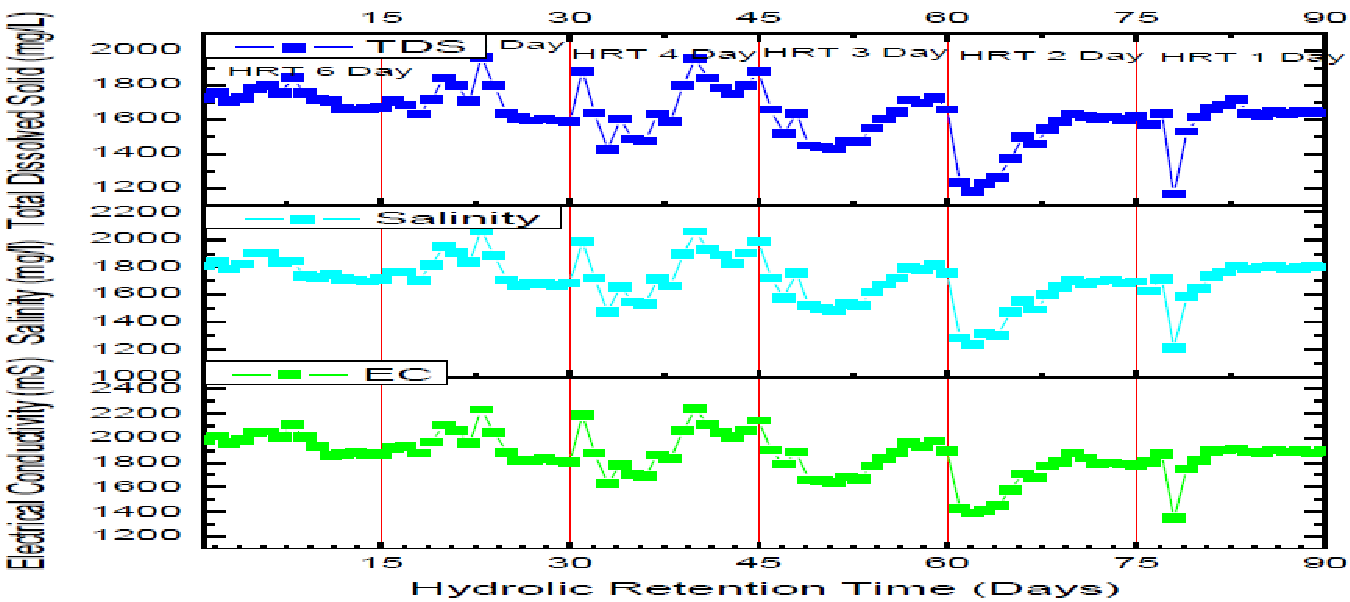

Figure 3

Variation in TDS, Salinity and EC during the two stage anaerobic digestion of SHWW of hydrolytic-acidogenic reactor at different Hydraulic retention times

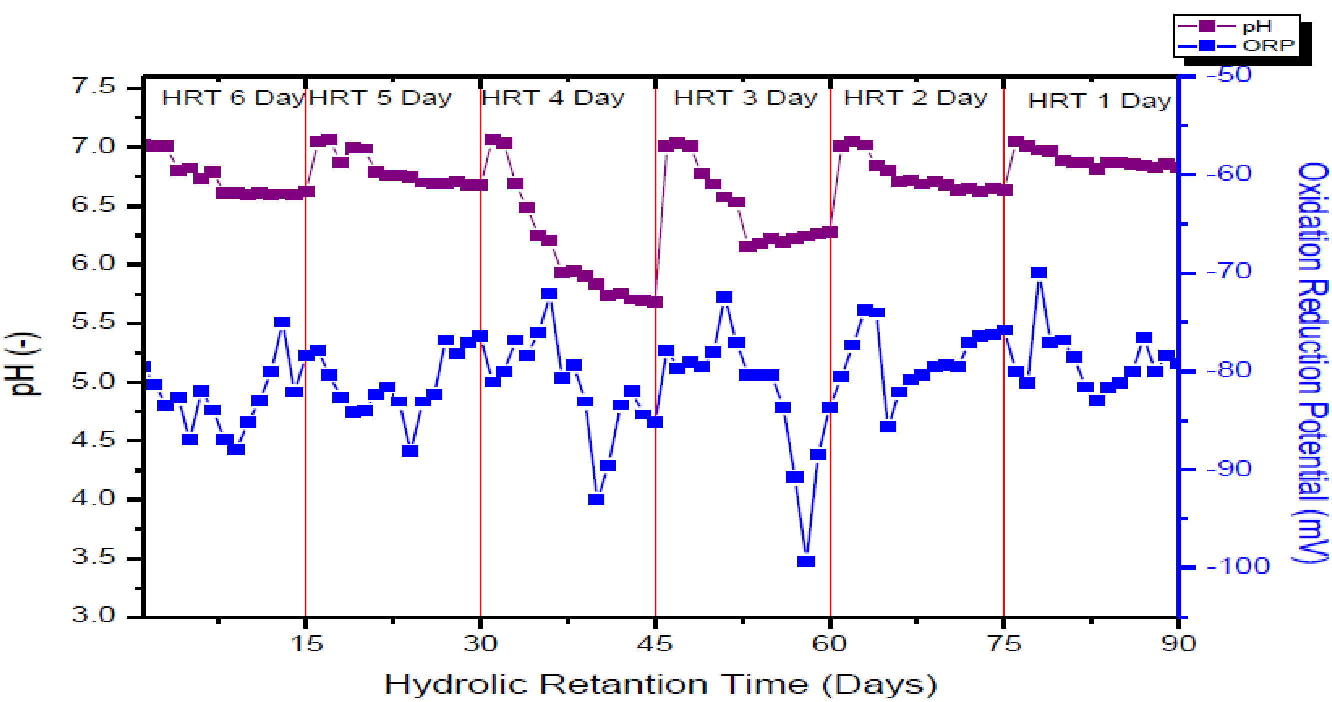

Figure 4

Change in pH and ORP at hydrolytic-acidogenic step at different HRT

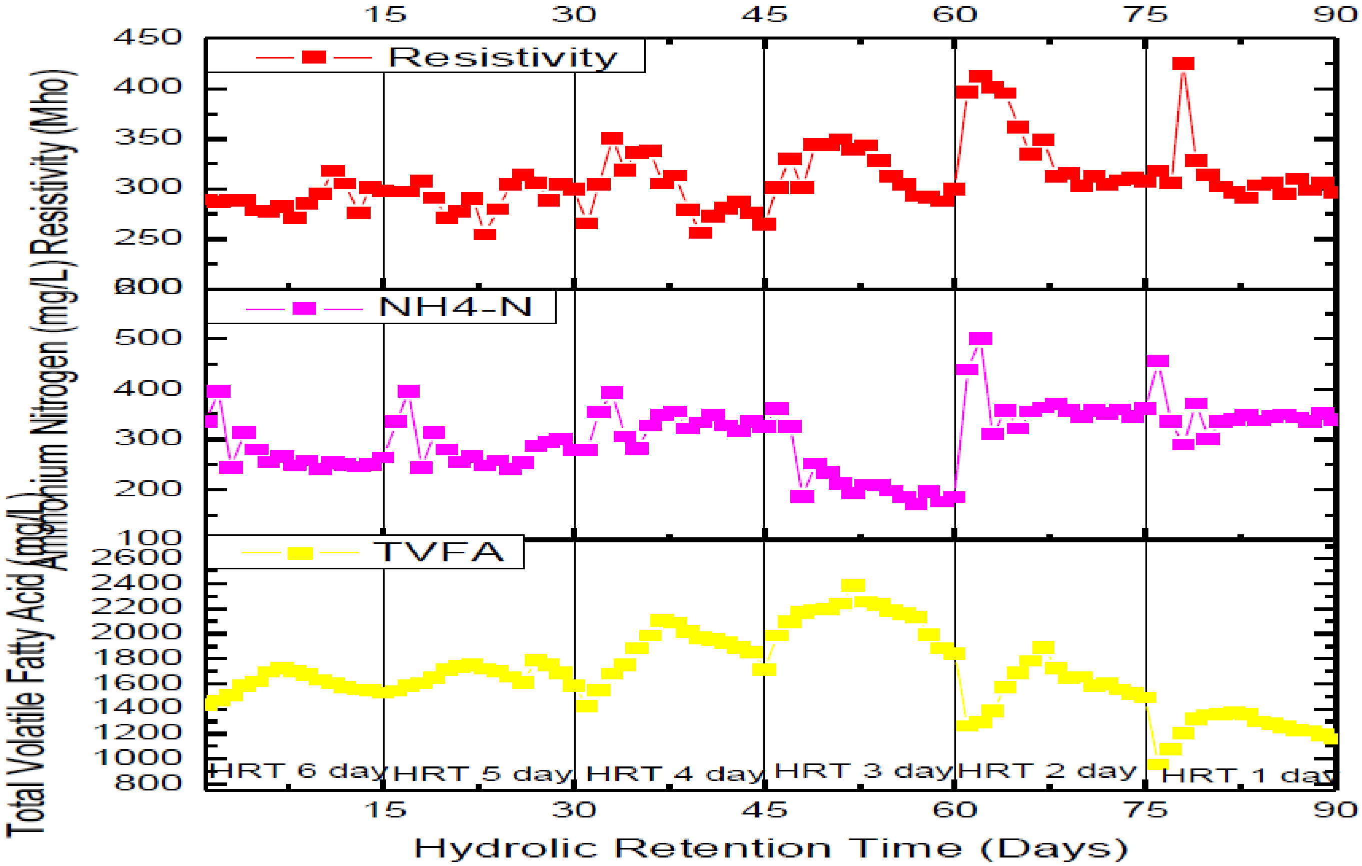

Figure 5

Variation of Resistivity, NH4+-N and TVFA at hydrolytic-acidogenic step with different HRT

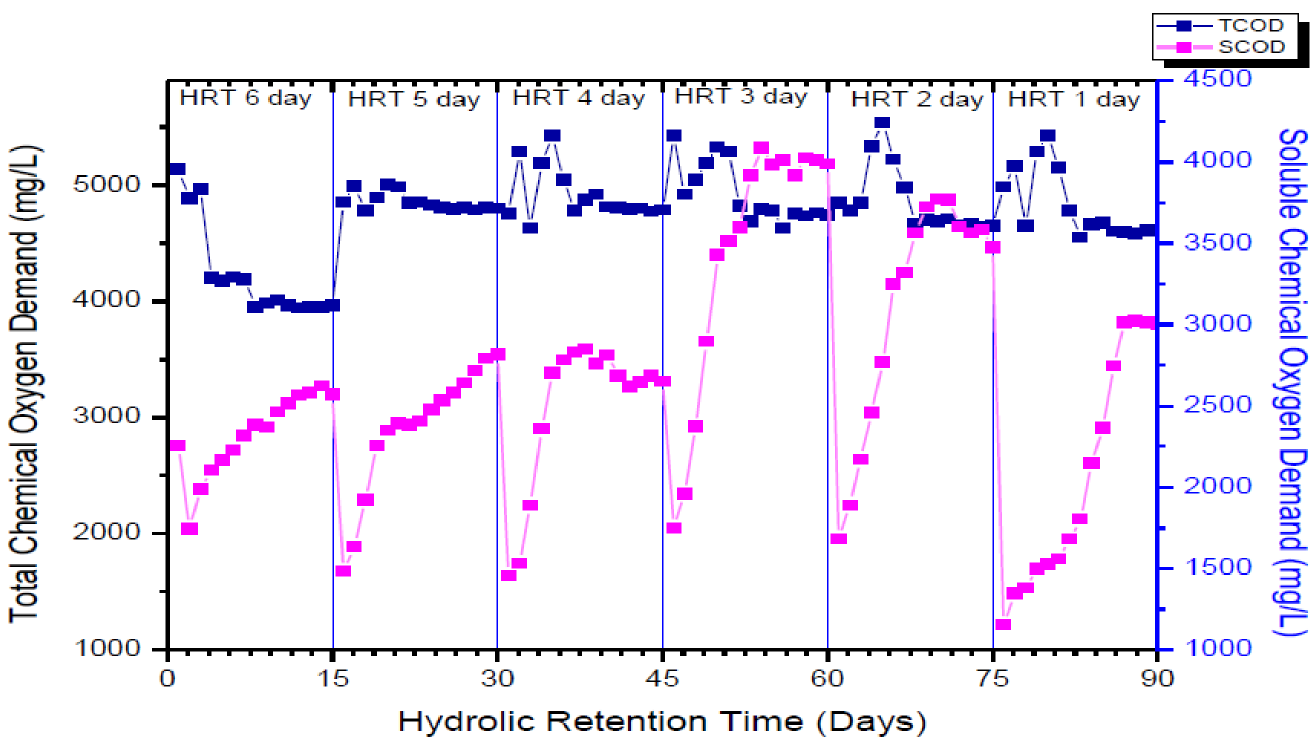

Figure 6

Variation trends of TCOD and SCOD during at hydrolytic-acidogenic step during two stage anaerobic digestion of SHWW at different HRT

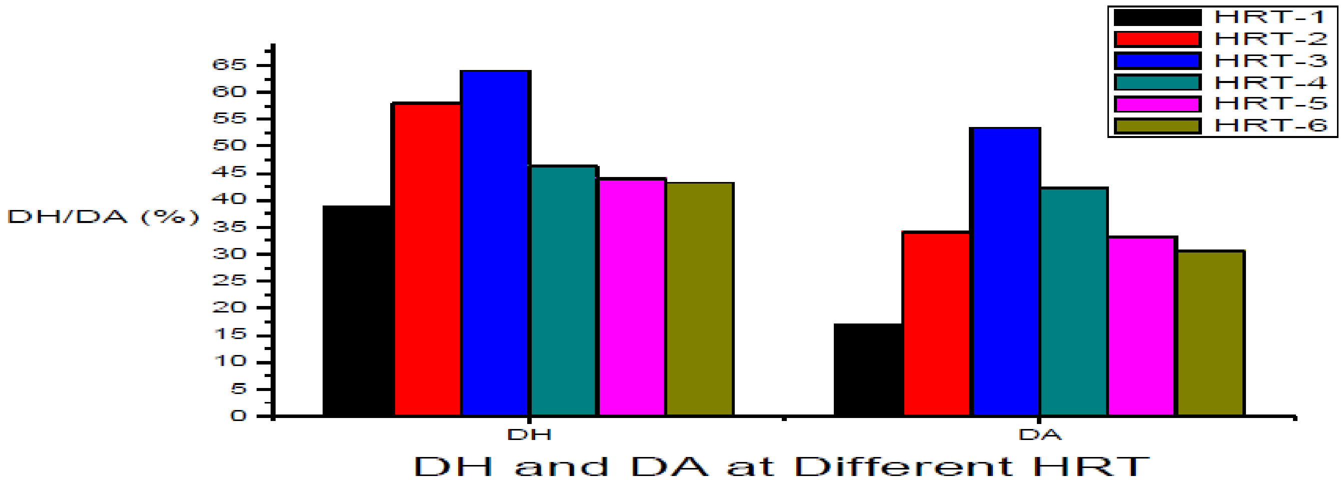

Figure 7

DH and DA of the Hydrolytic-acidogenic digester at different HRT