Microbial Dynamics in Laying Hen Waste and its Anaerobic Treatability at Mesophilic Temperature

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INTRODUCTION

- Chicken litter
  → mixture of feces/wasted feeds/bedding materials/feathers

- Chicken litter → $\geq 10x10^6$ tons every year in Turkey
  [usually spread on land as a low cost org. fert. due to ↑ nutrient cont.]

- Despite its high nutritional value, over-application ⇒ eutrophication, spread of pathogens, production of phytotoxic substances, air pollution & emission of greenhouse gases

- Chicken litter ⇒ also the source of human pathogens
  [*Salmonella, Campylobacter jejuni*, & *Listeria monocytogenes* – potentially cause contamination associated with foodborne outbreaks]

- Chicken litter ⇒ large/diverse population of microorg.

- Gram-positive bacteria (e.g., *Actinomycetes, Clostridia/Eubacteria* & *Bacilli/Lactobacilli*) account for ~ 90% of microbial diversity (reach ↑ 10^{10} CFU/gr litter).

- A variety of pathogens ⇒ detected in chicken litter-based org. fertilizers (e.g., *Actinobacillus, Bordetalla, Campylobacter, Clostridium, Corynebacterium, Escherichia coli, Globicatella, Listeria, Mycobacterium, Salmonella, Staphylococcus,* & *Streptococcus*).

- An investigation on pathogenic microorg. in poultry litter ⇒ also performed with selective medium [*Staphylococcus xylosus* predominant species].

- Additional approaches ⇒ such as physical, chemical & biological treatments should be considered for pathogen control in chicken litter.

- Anaerobic digestion ⇒ very efficient process for poultry litter (biogas with avr. $CH_4 \cong 60\%$).

- Following AD of poultry feces for 37 d ⇒ both coliform & *E. coli* counts ↓ drastically.

- Endogenous ammonia-nitrogen cont. rises considerably during AD.

- High levels of ammonium (>30 g/L) during AD of poultry litter ⇒ decrease in nb. of all physiological microbial groups as well as composition of methanogenic consortium changed (i.e. affected dominant methanogenic cultures_dominance of *Methanosarcinaceae* in manure digesters at high while *Methanosaetaceae* dominated in sewage sld digesters with low levels of NH_3).

- Acetate-utilizing methanogens offering thin filaments with great surface seemed to be more sensitive to NH_3. than hydrogenotrophic methanogens growing as rods (e.g., *Methanosarcinaceae* consisting of thick clumps).

- CM ⇒ often diluted prior to feeding to biosystem (TS%=3.00-8.25%).

- The highest biogas prod. rate ⇒ 554 mL/gVS_{feed} at OLR=2.17 g VS/L.d (i.e., corres. to 3.7% TS & 2.4% VS contents).

- Determination of structure of microbial communities in bioreactors ⇒ importance for achieving high efficien. during waste treatment.

- Development of culture independent molecular biological tech. of 16S rRNA gene analysis facilitated investigations of microbial communities of waste treatment systems, where microbial diversity is extremely high.

- Microbial sequencing ⇒ new tool in the field of molecular biology that has great potential for the development of environ. analysis.

- Microbial sequencing methods ⇒ achieve high sequencing depth which makes controlling the changes in the structure of a microbial community possible with a more in-depth understanding especially in bioreactors where biogas production occurs.

- Pyrosequencing ⇒ innovative NGS sys. with a promising position in environ. samples with remarkable genetic diversity.

- Illumina sequencing tech. ⇒ accepted as the most successful & widely adopted NGS technology worldwide.

OBJECTIVES

- to identify the microbial diversity in the raw manure as well as the dominant cultures (Illumina Miseq NGS).
- to investigate the anaerobic treatability of the diluted chicken manure & biogas potential in a lab-scale semi-continuous AD.

MATERIALS & METHODS

Characterization of the chicken waste

- Raw manure ⇒ fresh from a facility with a daily capacity of ~ 20000 eggs [275,000 livestocks_only laying-hen]

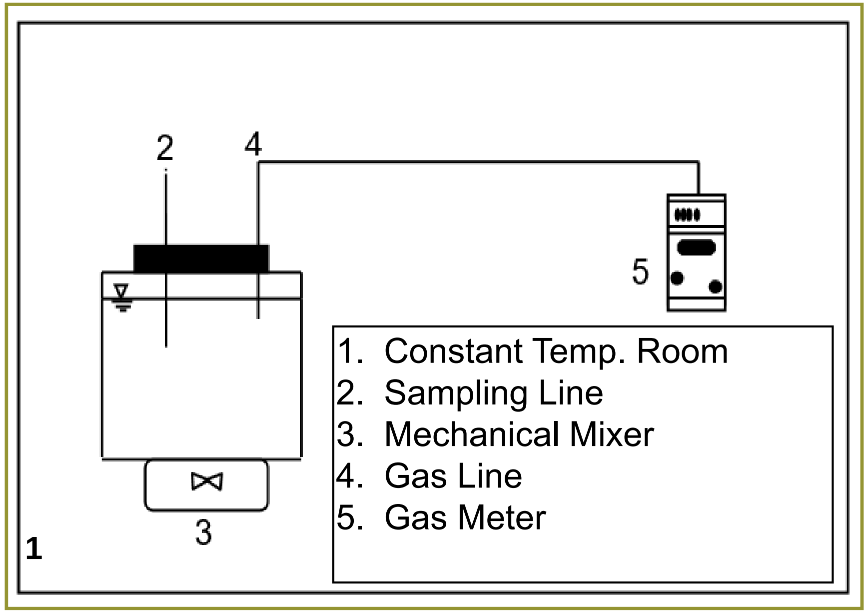
- CM ⇒ avr. TS ~ 28% (volatile cont. ~ 56%).

- Before feeding system ⇒ raw CM was diluted with tap water [TS ~ 5.5% according to previous optimization study using batch bioreactors]

Table 1. Characterization of the feed (diluted chicken waste)

Parameter	Unit	Value
TS	%	5.58
VS/TS	%	53
pH	-	8.00
Alkalinity	mg $CaCO_3$/L	15550
TAN	mg/L	2180
sCOD	mg/L	13325

Anaerobic reactor & inoculum sludge



1. Constant Temp. Room
2. Sampling Line
3. Mechanical Mixer
4. Gas Line
5. Gas Meter

Ritter Milligas
Counter_Gas-meter

Semi-cont. anaerobic reactor

[Opt. TS= 5.6%, $T_{operation}$=35°C (mesophilic)]

V_{tot} : 3000 mL
V_{eff} : 2500 mL
V_{seed} : 625 mL
V_{subs} : 1875 mL
SRT : 60 d

- ❖ Granular inoculum sludge ⇒ mesop. anaerobic Internal Circulation (IC) reactor (paper/cardboard ind.; TS=95 g/L; volatile content of 50%).
- ❖ Inoculation ⇒ 1:3 ratio (v/v)

Analytical procedure

Analysis

Parameters	Measurement Frequency
tCOD	2 / Week
sCOD	2 / Week
TAN	1 / Week
TS & VS	2 / Week
TSS & VSS	2 / Week
Alkalinity	2 / Week
pH	Daily
Gas Volume	Daily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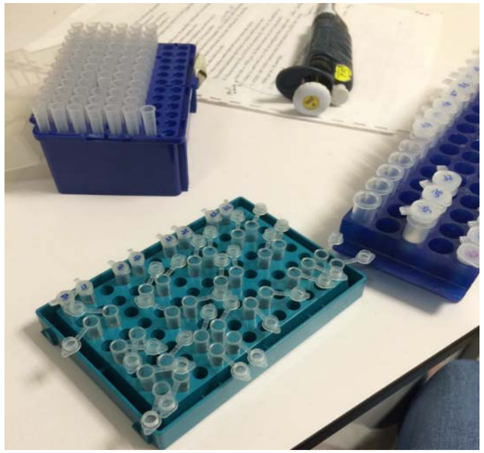
- Standard Methods

Molecular Analysis

✓ Microbial diversity in chicken feces

1. DNA Extraction



2. NGS

⇒ Illumina NGS

✓ Total DNAs ⇒ isolated from 1 mL sludge samples by PureLink Genomic DNA extraction kits (Invitrogen, U.K.).

✓ NanoDrop Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) ⇒ to determine the concentration.

✓ V4-V5 hypervariable region of 16S rRNA gene ⇒ reproduced with region-specific primers designed to contain Illumina adaptor & barcode sequences as follows;

518F-926R for bacteria & 518F-958R for archaea

Microbial diversity in the raw manure

RESULTS & DISCUSSION

Microbial diversity in raw manure

- ***Most abundant bacteria class***

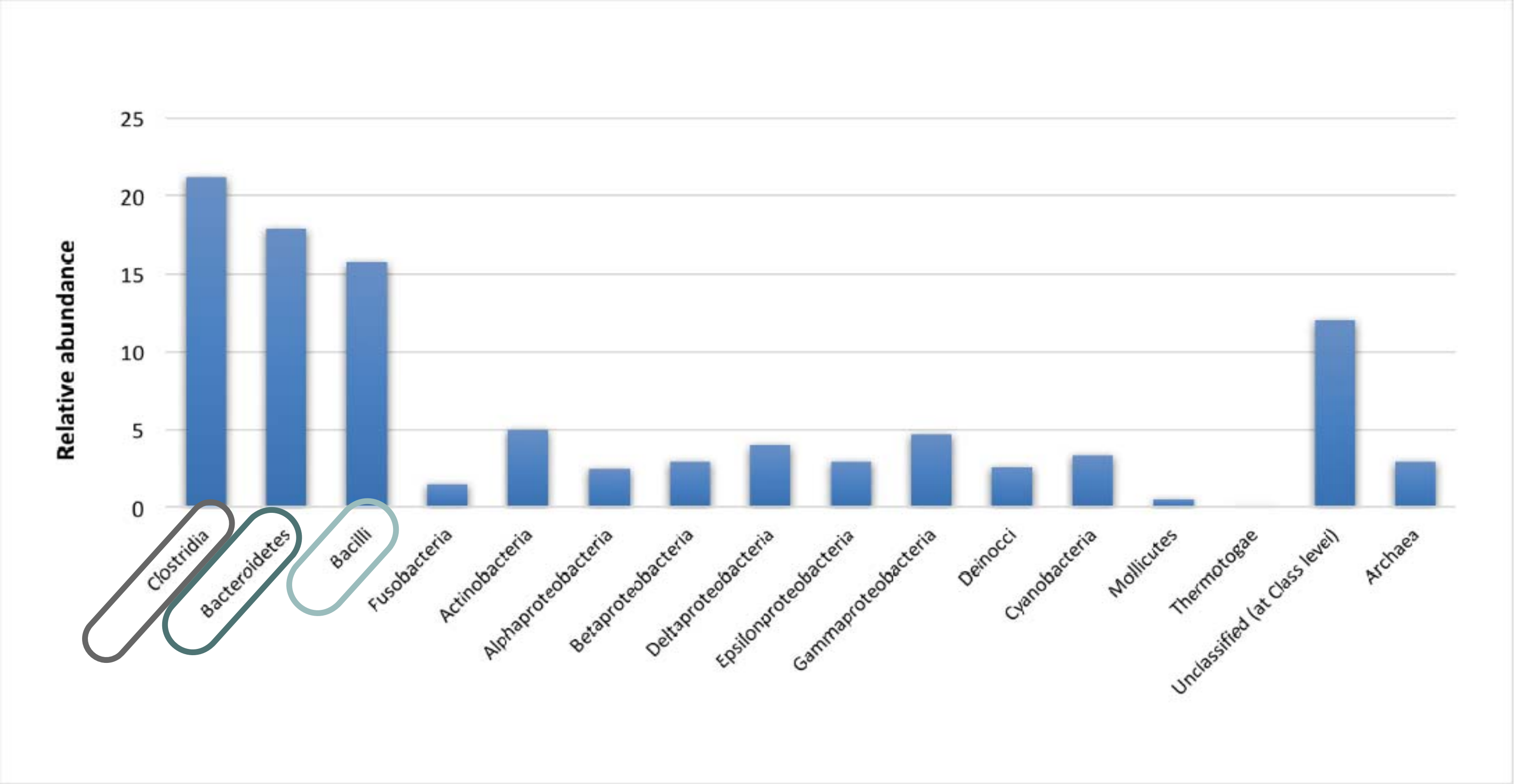
%21 *Clostridia*

%18 *Bacteroidetes*

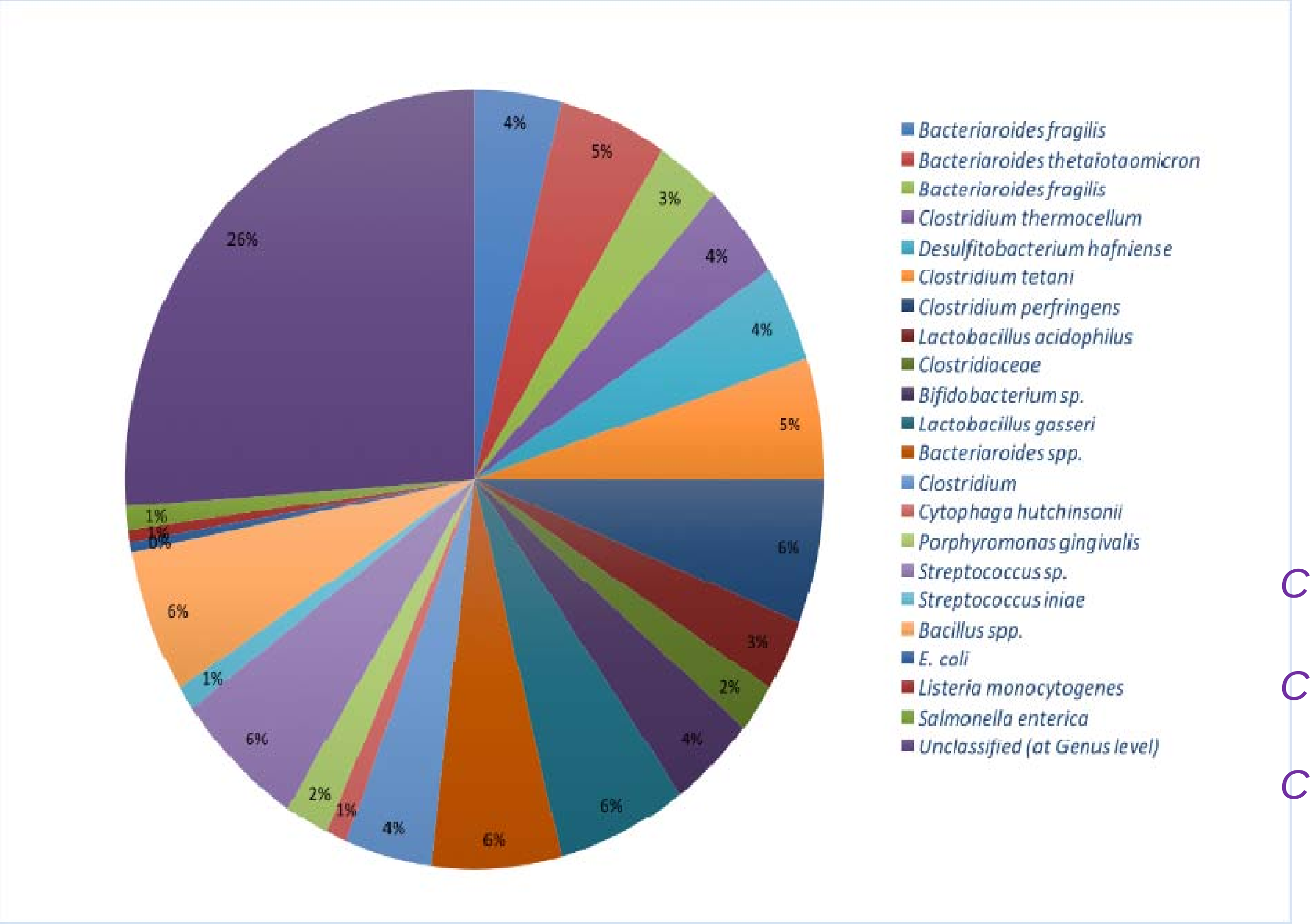
%16 *Bacilli*-like sequences

- Microbial community according to Illumina NGS

***Bacilli*, *Clostridia*, *Bacteroidetes*,**
***Fusobacteria*, *Actinobacteria*,**
***Alpha-proteobacteria*,**
***Beta-proteobacteria*,**
***Delta-proteobacteria*,**
***Epsilon-proteobacteria*,**
***Gamma-proteobacteria*,**
***Deinocci*, *Mollicutes*, *Thermotogae*,**
Cyanobacteria* & *Archaea



Microbial diversity in the raw manure



Clostridium tetani

Clostridium perfringens

Clostridium thermocellum

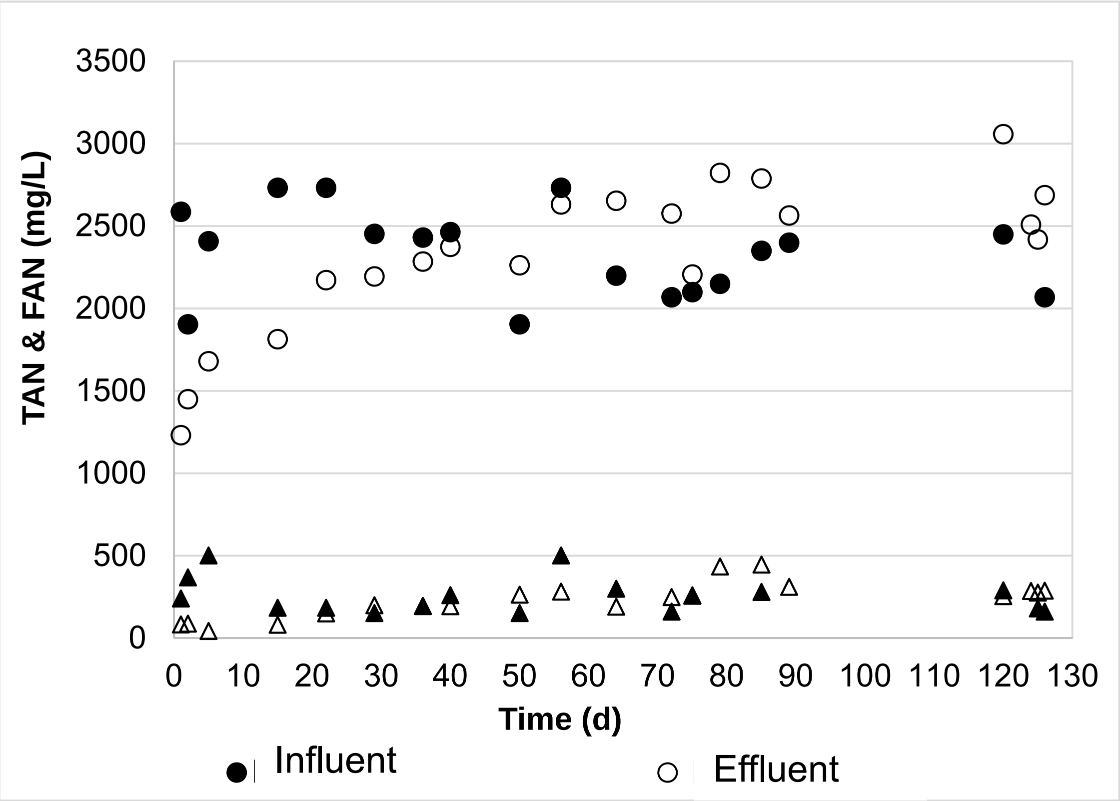
- NGS analysis in genus level ⇒ ~ 6% ***Streptococcus sp.*** (i.e., sphere-shaped gram-positive bacteria belonging to the phylum ***Firmicutes***)

A few of pathogenic bacterial species;

- *E. Coli*
- *Listeria monocytogenes*
- *Salmonella enterica*

Anaerobic treatability & biogas production

TAN

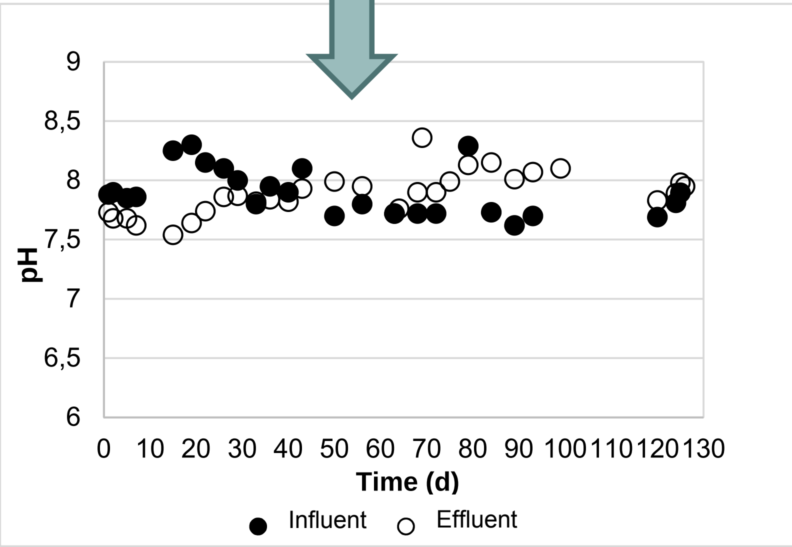


Avr. TAN influent ≈ 2340 mg/L

Avr. TAN effluent ≈ 2320 mg/L

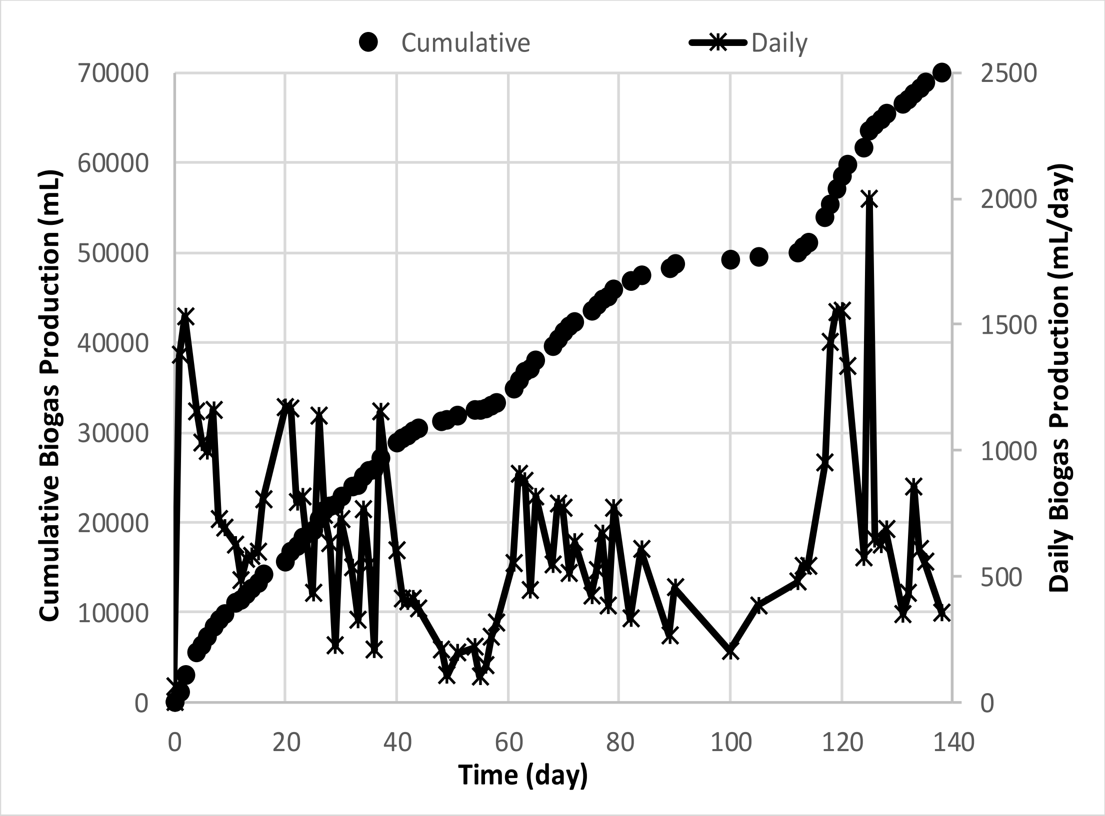
Avr. FAN influent ≈ 260 mg/L

Avr. FAN effluent ≈ 230 mg/L



- Alkalinity & pH in influent & effluent ⇒ 16190±2140 & 15900±5040 mg $CaCO_3$/L & 7.91±0.24 & 7.87±0.20, respectively

- Influent & effluent sCOD conc. ⇒ 12560±1660 & 6610±2715 mg/L, (Avr. Eff. ≅ 47%).
- Influent TS & VS conc. ⇒ 55950±9595 & 29050±4300 mg/L.
- Effluent TS & VS conc. ⇒ 33620±8590 & 14930±4045 mg/L.
- Avr. TS & VS removals ⇒ ~ 38% & 47%, respectively.



Biogas production from mesophilic AD of laying hen waste in this study

Biogas prod. during AD of poultry waste:
0.31 – 0.62 m^3/kg VS_{fed}

Biogas yield = **0.47 L/g VS_{fed}**

CONCLUSIONS

- Laying hen manure is an important renewable energy source & if managed/treated properly; significant biogas productions might be possible from the farms producing high amounts of chicken wastes.

- Eff. performance of semi-continuously fed anaerobic digester treating diluted laying hen manure regarding VS removal was ~ 47% on avr.

- Respective biogas yield was observed as ~ 0.47 L/gr VS_{fed} from bioreactor although TAN conc. was ~ 2300 mg/L on avr.

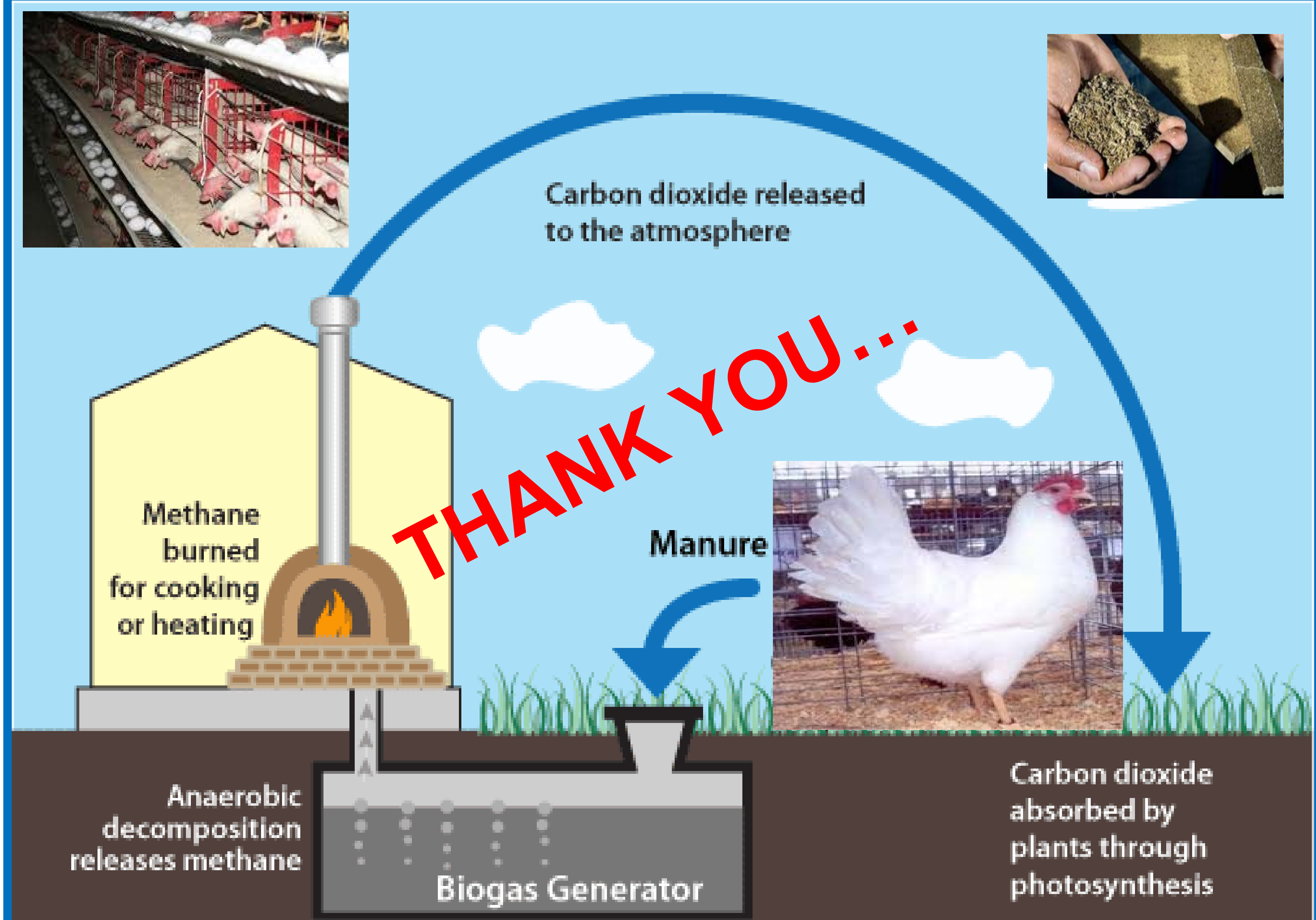
- FAN inhibition during the operating period of this study was not significant because the pH in the system did not increase $\geq$ 8.0.

CONCLUSIONS

- Microbial diversity in chicken feces revealed that *Bacilli, Clostridia, Bacteroidetes*, *Fusobacteria, Actinobacteria, Alpha-proteobacteria, Beta-proteobacteria, Delta-proteobacteria, Epsilon-proteobacteria, Gamma-proteobacteria, Deinocci, Mollicutes, Thermotogae, Cyanobacteria* & *Archaea* were present.

- The most abundant two bacteria classes were identified as ***Clostridia*** & ***Bacteroidetes*** whereas ***Bacilli***-like sequences were also characterized.

- A scarce sequence revealed similarity to ***Archaea*** (i.e., 3%) & pathogenic bacterial species were also identified in few numbers.



THANK YOU…

Department of Scientific Research Projects of ITU