EFFECT OF PERACETIC ACID ON ANAEROBIC TREATMENT OF POULTRY PROCESSING WASTEWATER

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SUMMARY

Peracetic acid (PAA) is a powerful disinfectant against a wide spectrum of microbes, used in healthcare, water treatment, and food industry. In poultry processing plants, PAA is used in chicken chiller tanks as well as for the disinfection of processing equipment as an antimicrobial agent. Although PAA achieves comparative disinfection efficacy with chlorine compounds and less disinfection by-products are formed, residual PAA in poultry processing wastewater may be carried to biological wastewater treatment systems with potential adverse effects. The overall goal of this research was to evaluate if anaerobic treatment of PAA-carrying poultry processing wastewater is feasible. The specific objectives of this research were to investigate: i) the effect of PAA on a mixed fermentative/methanogenic culture, acetoclastic methanogens, and hydrogenotrophic methanogens; and ii) the effect of PAA on the anaerobic treatment of poultry processing wastewater (dissolved air floatation effluent) and compare the effect of pre-decomposed and direct PAA addition to semi-continuously-fed, laboratory-scale anaerobic reactors, conditions reflecting normal operation and accidental spills, respectively, in poultry processing plants.

Pre-decomposed 40 and 100 mg/L PAA added to batch assays did not affect: 1) methane production, substrate COD conversion to CH_4, dextrin/peptone mixture ultimate biodegradability, and specific methanogenic activity (SMA) compared to PAA-free, controls under fermentative/methanogenic conditions; 2) methane production, substrate COD conversion to CH_4, and specific methanogenic activity (SMA) compared to PAA-free, control reactors under acetoclastic conditions; and 3) CH_4 produced/H_2 consumed

ratio compared to PAA-free, control reactors under hydrogenotrophic methanogenic conditions. On the other hand, the semi-continuously-fed anaerobic reactor (R2) showed that anaerobic treatment of dissolved air flotation (DAF) effluent carrying PAA is feasible, at least up to 80 mg/L PAA without observable biogas production difference compared to the PAA-free control reactor (R1).

Direct 40 mg/L and 100 mg/L PAA added to batch assays decreased the SMA but not the extent of methane production and substrate COD conversion to CH_4 compared to PAA-free, control reactors under fermentative/methanogenic and acetoclastic conditions, indicating that fermentative/methanogenic and acetoclastic methanogenesis were initially impacted but recovered over time. Under hydrogenotrophic methanogenic conditions, direct 40 mg/L PAA addition resulted in a similar CH_4 produced/H_2 consumed ratio compared to the PAA-free, control reactor. However, direct 100 mg/L PAA addition impacted hydrogenotrophic methanogens, resulting in a CH_4/H_2 ratio significantly lower compared to the PAA-free, control reactor.

Direct 40 mg/L PAA added to a semi-continuously-fed anaerobic reactor (R4) impacted both acidogenesis and methanogenesis, leading to lower biogas production compared to the PAA-free, control reactor (R1). After four feedings of 40 mg/L PAA directly (Phase 1: day 1 to 14), the total biogas of R4 decreased to 37% compared to the PAA-free control reactor (R1). After four feedings of 40 mg/L PAA directly for 14 days, it took 35 days for R4 to recover without feeding (Phase 2: day 14 to day 49). During the recovery period, acetoclastic methanogens recovered first then propionate-utilizers. After 35 days of recovery, when R4 was fed again with 40 mg/L PAA directly four times (Phase 3: day 49 to 63), the total biogas production declined to 59 % and then gradually increased to 67 %

compared to the PAA-free control reactor (R1). Compared to Phase 1, in which R4 had a normalized gas production of 37% compared to R1, R4 was more resistant to PAA during Phase 3 (second PAA feeding period).

Compared to R4, direct PAA addition with incrementally increasing PAA concentration from 5 to 40 mg/L (R3) resulted in an increasing resistance against PAA. In other words, the total biogas production of R3 was not impacted by direct PAA addition and VFAs did not accumulate. The resistance could be explained by introducing a low PAA concentration (5 mg/L) during Phase 1 that induced the anaerobic culture to synthesize antioxidative enzymes. Later on, antioxidative enzymes helped the fermentative/methanogenic culture to mitigate against higher PAA concentration (20 and 40 mg/L) addition.

The findings of the present study suggest that pre-decomposed PAA addition does not impact fermentative/methanogenic systems, especially acetoclastic methanogens, and hydrogenotrophic methanogens. On the other hand, direct 40 and 100 mg/L impacted the fermentative/methanogenic culture and acetoclastic methanogens, which recovered over time. Hydrogenotrophic methanogens were more resistant to direct PAA addition, given that hydrogenotrophic methanogens were not affected by direct 40 mg/L PAA addition. Thus, anaerobic processes can be used to treat poultry processing wastewater carrying PAA, at least up to 80 mg/L PAA. Even in the case of an accidental PAA spill, anaerobic treatment systems can recover over time providing that corrective measures are implemented (e.g., temporary wastewater diversion, pH adjustment).

CHAPTER 1

INTRODUCTION

Peracetic acid (PAA) is increasingly used in the food and water treatment industry over the last few decades because of its higher redox potential compared to hydrogen peroxide and comparative disinfection efficacy but less disinfection by-products (DBPs) compared to chlorine compounds (Luukkonen et al., 2016). In poultry processing plant, PAA is applied in chiller tanks, where chickens are transferred after processing, in order to inhibit microbial growth. In addition to chillers, PAA is also applied to disinfect other poultry processing equipment (e.g., electric stunning, scald, feather-picking fingers, and internal organ-clenchers).

The poultry processing wastewater is a combination of water used in chiller tanks as well as water used for the disinfection of processing equipment, along other waste streams from other processes. Poultry processing wastewater is commonly treated with a dissolved air floatation (DAF) system, followed by biological treatment, combined or individually. It was estimated that 25 % of U.S. poultry processing plants use biological treatment systems consisting of an anaerobic lagoon followed by an activated sludge system (Kiepper et al., 2003).

Although PAA is an effective disinfectant for food and water treatment, residual PAA in wastewater may be carried to biological wastewater treatment systems with potential adverse effects on the biological processes. A biofilter with 3 mg/L PAA had a lower total ammonia nitrogen (TAN) removal capacity but ammonia accumulation was not observed.

Nitrite partially accumulated with a delay of nitrite oxidation, indicating that nitrite oxidizing bacteria (NOB) were partially inactivated (Pederson et al., 2009).

The overall goal of this research was to evaluate the effect of PAA on the anaerobic treatment of poultry processing wastewater. The specific objectives of this research were to:

i) Investigate the effect of PAA on a mixed fermentative/methanogenic culture, acetoclastic methanogens, and hydrogenotrophic methanogens.

ii) Investigate the effect of PAA on the anaerobic treatment of poultry processing wastewater (DAF effluent) and compare the effect of pre-decomposed and direct PAA addition to semi-continuously-fed, laboratory-scale anaerobic reactors, conditions reflecting normal operation and accidental spills, respectively, in poultry processing plants.

CHAPTER 2

BACKGROUND

2.1 Peracetic Acid

Peracetic acid (PAA) has gained a high popularity in the food and water treatment industry over the last few decades because of its many qualities as an ideal disinfectant. These qualities include: effective disinfectant towards microorganisms but not to higher forms of life; comparative disinfection efficacy, but less disinfection by-products (DBPs) compared to chlorine compounds; simple to apply; long shelf life; and reasonable cost (Luukkonen et al., 2016). The food industry, for example poultry processing, applies PAA as a disinfectant in chicken chiller tanks as well as to processing equipment. Table 2.1, summarizes the structure, physical, and chemical properties of PAA (Luukkonen et al., 2016; Zhang et al., 2018). Commercially available PAA solutions are quaternary equilibrium mixtures of acetic acid, hydrogen peroxide, peracetic acid, and water. Table 2.2 shows typical components concentration in commercial PAA solutions used in poultry processing plants.

Table 2.1. Physical and chemical properties of peracetic acid (PAA).

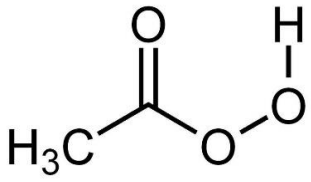
Structure	Parameter	Value
	Physical State	Liquid
	Appearance	Clear colorless
	Odor	Strong pungent
	Melting point (°C, at 1 atm)	-0.2
	Boiling point (°C, at 1 atm)	110
	Water solubility (g/L @ 25°C	1000
	Vapor pressure (kPa @20°C)	2.6
	Molecular weight (g/mol)	76.051
	Specific gravity	1.130
	Standard redox potential (V vs. SHE)	1.748
	pK_a	8.2

Table 2.2. Typical PAA solution components (wt %) used in poultry processing.

Component	Source A	Source B
PAA	20-23	14.7-15.7
Acetic acid	33-39	40-50
H_2O_2	8.5-10.5	5-6

PAA achieves its disinfection capacity by formation of reactive oxygen species (ROS), such as hydroxyl radical (HO·), superoxide anion ($\cdot O_2^-$), and peracetyl radical ($CH_3COO\cdot$). The following equations summarize the formation of ROS from PAA (Bach et al., 1996; Rokhina et al., 2010).

$$CH_3COOOH \rightarrow CH_3COO\cdot + HO$$
$$CH_3COOOH + HO\cdot \rightarrow CH_3CO\cdot + O_2 + H_2O$$
$$CH_3COOOH + HO\cdot \rightarrow CH_3COOO\cdot + H_2O$$
$$CH_3COO\cdot \rightarrow \cdot CH_3 + CO_2$$
$$2CH_3COO\cdot \rightarrow 2\cdot CH_3\cdot + 2CO + O_2$$
$$\cdot CH_3 + O_2 \rightarrow CH_3OO\cdot$$
$$CH_3COO\cdot + HO\cdot \rightarrow CH_3COOOH$$

The ROS can damage biomolecules according to the following mechanisms (Kitis et al., 2004): (1) Reaction with thiol (-SH), disulfide (S-S) and double bonds in proteins, enzymes, and other biomolecules. (2) Inactivation of catalase enzyme, which scavenges hydroxyl radicals during oxidation. (3) Disruption of the chemiosmotic function of the lipoprotein cytoplasmic membrane. (4) Protein denaturation. (5) Reaction with DNA bases.

The disinfection efficacy of PAA depends on temperature and pH (Kitis et al., 2004). PAA can be used over a wide temperature range; however, similar to other disinfectants, the PAA disinfection efficacy increases with increasing water temperature. PAA has a greater activity at lower pH, as the undissociated acid form (the PAA pK_a is 8.2) is considered as its more biocidal form. The difference in disinfection efficacy is low between pH 5 and 8, but a decrease in efficacy was demonstrated at pH 9 (Kitis et al., 2004).

The decomposition rate of PAA depends on pH, concentration, temperature, amount of organic material, transition metal ions, salinity, and water hardness (Luukkonen et al., 2016). In a concentrated solution, PAA can be decomposed according to three mechanisms (Zhao et al., 2008):

1. Hydrolysis under alkaline conditions (pH > 8.2):

$$CH_3COOOH + H_2O \rightarrow CH_3COOH + H_2O_2$$

2. Spontaneous decomposition under neutral and acidic conditions:

$$CH_3COOOH \rightarrow CH_3COOH + {}^1/_2\, O_2$$

3. Transition metal-catalyzed decomposition:

$$CH_3COOOH \xrightarrow{M^+} CH_3COOH + {}^1/_2\, O_2 + other\ products$$

The presence of organic and inorganic species affects the PAA decomposition rate in wastewater (Domínguez Henao et al., 2018a). Typical inorganic species (e.g. NH_4^+, NO_2^-, NO_3^-, Fe^{2+}, PO_4^{3-}) in wastewater affect the PAA decomposition rate. The PAA decomposition rate is not affected by inorganic nitrogen species, but it is affected by ferrous ion and orthophosphate. Ferrous ion facilitates the decomposition of PAA; on the contrary, orthophosphate acts as a chelating agent of transition metals and mitigates the effect of transition metals. In addition to inorganic species, among organic compounds (e.g., carbohydrates: glucose, cellulose; lipids: butyric acid, oleic acid; proteins: peptone and casein), only proteins result in a high initial PAA demand/decomposition (Domínguez Henao et al., 2018a). That is, proteins consume a significant amount of PAA instantly. Among all amino acids, sulfur-containing amino acids, such as cysteine and methionine, and imidazole-containing amino acid - histidine are the most reactive upon PAA treatment (Du et al., 2018).

Comparison between PAA and other disinfection agents, such as hypochlorite and ozone has been reported (Mezzanotte et al., 2007). To achieve a comparative logarithmic inactivation (4-5 $\log_{10}$) of *E. coli*., fecal or total coliforms, ozone needs the least dosage and contact time (3.6 mg/L, 12.8 min), followed by hypochlorite (7.5 mg/L, 18 min), whereas PAA is the least efficient (15 mg/L, 36 min). Although PAA has been reported to have a lower disinfection efficacy than hypochlorite and ozone, one of the advantages of PAA is its less possibility to form disinfection by-products (DBPs), which is favored in both the food and water treatment industry.

PAA has been reported to form DBPs, such as aldehydes and halogenated DBPs, but not epoxides and N-nitrosamines (Luukkonen et al., 2016). Amino acids, phenols, and

other aromatic substances present in raw water may be oxidized into aldehydes under PAA treatment. However, the concentration of aldehydes after PAA treatment has been reported to be around the limit of detection or much less than WHO's guidelines (Nurizzo et al., 2005). The halogenated DBPs are also limited or undetectable under typical PAA treatment (PAA concentration 1-10 mg/L, contact time 30-90 min). Nonetheless, at high concentrations of PAA and halides, halide ions are oxidized into hypohalous (HO-X) acids, which can further react with dissolved organic matter to form halogenated DBPs (Domínguez Henao et al., 2018b).

2.2 Poultry Wastewater

Poultry processing is now a highly automated industry, in which chickens are passed through electric stunning and then slaughtered. Then, the chickens are scalded in order to facilitate feathers removal. In the next step, the chickens are passed through the rubber-picking finger to remove feathers. After picking, the chickens' internal organs are removed by automated clencher. Throughout the above processing steps, cross-contamination with pathogenic microbes could occur. Thus, chickens are transferred to chiller tanks which contain cold water (pre-chiller maintained at 18.3℃, the main chiller maintained at 4.4℃; Kiepper, 2003) and antimicrobial agents to inhibit microbial growth. The poultry processing wastewater is a combination of water used in chiller tanks as well as water used for the disinfection of processing equipment, along with other waste streams from other processes.

Generally, the soluble and particulate organic matter of poultry processing wastewater is treated prior to discharge in order to comply with established environmental regulations. The most popular physical/chemical wastewater treatment in poultry processing plants is dissolved air floatation (DAF). DAF is a process that introduces flocculants and fine air bubbles at the bottom of the wastewater tank in order to improve water-solids separation. Air bubbles attached to aggregates enhance the buoyancy of the solids matrix, which cause it to rise to the surface of the water where it is collected as DAF skimmings. The DAF underflow wastewater (or effluent), depending on the plant location, is then further treated biologically at the plant site or discharged to municipal sewers. Table 2.3 summarizes the characteristics of poultry processing DAF effluent previously reported (de Nardi et al., 2008; Del Nery et al., 2016), as well as measured in the present study.

Table 2.3. Characteristics of poultry processing DAF effluent.

Parameter	De Nardi et al., 2007	Del Nery et al., 2016	This study
pH	5.8-7.2	7.0-7.6	6.37
tCOD (mg/L)	1764 ± 409[b]	2485 ± 385	785 ± 10[a]
sCOD (mg/L)	-	-	740 ± 28[a]
BOD (mg/L)	1094 ± 191[b]	1379 ± 264	-
TSS (mg/L)	488 ± 155[c]	-	-
VSS (mg/L)	449 ± 147[c]	-	-
TS (g/L)	-	-	0.99 ± 0.05[a]
VS (g/L)	-	-	0.51 ± 0.03[a]
Oil and grease (mg/L)	92 ± 17[d]	85 ± 16	60 ± 43 [a]
Crude protein (mg/L)	-	-	587 ± 7[a]

[a] Mean ± standard deviation (*n*=3)
[b] Mean ± standard deviation (*n*=20)
[c] Mean ± standard deviation (*n*=18)
[d] Mean ± standard deviation (*n*=19)

In addition to physical/chemical treatments, such as DAF, many poultry processing plants also apply biological wastewater treatment to further remove organics and pathogens from DAF effluent. A poultry processing plant used a combination of a DAF system, an upflow anaerobic sludge blanket (UASB) reactor, an aerated-facultative pond (AFP) and a chemical-DAF system, with the following removal extent: COD, 97.9 ± 1.0%; BOD, 98.6 ± 1.0%; and oil and grease, 91.1 ± 5.2% (Del Nery et al., 2016).

2.3 Anaerobic Digestion

Biological treatment is widely used to treat poultry processing wastewater. It was estimated that 25 % of U.S. poultry processing plants use biological treatment systems consisting of an anaerobic lagoon followed by an activated sludge system (Kiepper et al., 2003).

Anaerobic digestion is a process that can transform organic matter via a series of hydrolysis and fermentation reactions into CH_4 and CO_2. Anaerobic digestion is widely used as a biological treatment for the stabilization of municipal wastewater sludges and the degradation of solid wastes (Rittmann and McCarty, 2001). In addition to sludge reduction and stabilization, anaerobic digestion is also able to produce energy in the form of CH_4, which leads to currently increasing interest in energy neutral wastewater treatment plant (WWTP) operation (McCarty et al., 2011).

Anaerobic digestion is a complicated biochemical process which converts complex organic matter to CH_4 and CO_2. The whole process can be divided into four sub-processes: (1) hydrolysis, (2) acidogenesis, (3) acetogenesis, and (4) methanogenesis (Rittmann and McCarty, 2001). Anaerobic digestion starts with hydrolysis of organic particulate and colloidal, polymeric material – such as carbohydrates, protein, and lipids – into simple carbohydrates, amino acids and long-chain fatty acids by hydrolytic and fermentative bacteria. The hydrolysis products then undergo acidogenesis, by which simple carbohydrates and organic acids are fermented or anaerobically oxidized into volatile fatty acids (VFAs; mainly, acetate, propionate, butyrate) and H_2. The VFAs, other than acetate, as well as other low molecular weight organic compounds (e.g., lactate, methanol, and ethanol) are then partially oxidized to acetic acid, H_2, and CO_2 by acetogenic bacteria. At

the end, methanogenesis is completed by transforming acetate and H_2/CO_2 into CH_4 by acetoclastic methanogens and hydrogenotrophic methanogens, respectively (Pavlostathis, 2011).

Temperature and pH control are crucial for the successful operation of anaerobic digestion systems. The optimal pH for anaerobic digestion ranges from 6.6 to 7.6. pH slightly below 6.6 may result, as there are organic acids (i.e., VFAs) produced as intermediates during anaerobic digestion. The optimal temperature for methanogens ranges from 35 to 40°C (mesophilic) and from 55 to 65°C (thermophilic), respectively. The overall process rate at thermophilic temperature is usually 50 % to 100 % higher than at mesophilic temperature (Rittmann and McCarty, 2001). Therefore, a higher operating temperature can reduce the reactor volume to treat the same amount of organic feed, but has a higher energy consumption to maintain higher reactor temperature.

Compared with aerobic treatment, the anaerobic treatment process has several advantages. (1) Low production of waste biological solids and low nutrient requirements as a result of the low microbial yield and growth rate; (2) Production of CH_4, a readily available and renewable energy with an energetic value of 36.8 kJ/L at STP (0^oC, 1 atm); (3) Higher organic loading rate per unit reactor volume, but poor removal efficiency with dilute wastewater (Rittmann and McCarty, 2001).

CHAPTER 3

ANALYTICAL METHODS AND GENERAL PROCEDURES

3.1 pH

All pH measurements were performed using the potentiometric method with an ATI Orion Model 370 digital pH meter (Orion Research Inc., Boston, MA) and a gel-filled combination pH electrode (VWR International, West Chester, PA). The meter was calibrated weekly with pH 4.0, 7.0, and 10.0 standard buffer solutions (Fisher Scientific, Pittsburg, PA).

3.2 Chemical Oxygen Demand (COD)

COD was measured using the closed reflux, colorimetric method as described in *Standard Methods* (Eaton et al., 2005). An aliquot of 2 mL of sample was added to the vial containing 3 mL pre-made digestion solution (HACH Company, Loveland, CO). After tumbling the vial for 4-8 times, the content in the vials was digested at 150°C for 2 hours and then cooled down to room temperature. The absorbance was measured at 620 nm with a spectrophotometer (DR3900, HACH Company, Loveland, CO). Samples were centrifuged and filtered through a 0.45 μm polypropylene membrane filter if the soluble COD was measured, otherwise well-mixed samples were used after appropriate dilution for total COD measurements. All samples were prepared and measured in either triplicate (total COD) or duplicate (soluble COD).

3.3 Total and Volatile Solids (TS and VS)

Total solids content of samples was determined according to procedures outlined in *Standard Methods* (Eaton et al., 2005). Samples were weighed in pre-ignited (550°C) and cooled ceramic crucibles using a PG503-S balance (METTLER TOLEDO, Columbus, OH). The samples were then dried at 105°C for 24 hours in a Fisher Isotemp Model 750G oven. After drying, the crucibles were transferred to a desiccator until cooled, and then the dry weight was measured. If VS were to be determined, the crucibles were transferred to a Fisher Isotemp Model 550-126 muffle furnace and ignited at 550°C for 20 minutes. After ignition, the samples were cooled in a desiccator and the remaining solids weight was measured. TS was calculated as the difference between the weight of the crucible after the sample was dried at 105°C and the tare weight of the crucible divided by the sample volume. VS was calculated as the difference between the weight of the crucible after the sample was dried at 105°C and the weight of the crucible after the sample was combusted at 550°C divided by the sample volume.

3.4 Total Gas Production

Total gas produced by anaerobic reactors was measured by displacement of an acidified brine solution (10% NaCl w/v and 2% H_2SO4 v/v) in graduated burettes equilibrated to atmospheric pressure. Total gas produced in batch assays using serum bottles was measured using a pressure transducer (resolution ±1.974 atm, accuracy to 0.002 atm; Sper Scientific, Scottsdale, AZ). Unless otherwise stated, gas data reported throughout this work are at 22^{o}C and 1 atm.

3.5 Gas Composition

The CH_4 and CO_2 compositions were measured using a gas chromatography (GC) unit (Agilent Technologies, Model 6890N; Agilent Technologies, Inc., Palo Alto, CA) equipped with two columns and two thermal conductivity detectors. CH_4 was separated with a 15 m HP-Molesieve fused silica, 0.53 mm i.d. column (Agilent Technologies, Inc.) (front column). CO_2 was separated with a 25 m Chrompac PoraPLOT Q fused silica, 0.53 mm i.d. column (Varian, Inc., Palo Alto, CA) (back column). Helium was used as the carrier gas for both front and back column at a constant flow rate of 6 mL/min for all gases. The 10:1 split injector was maintained at 150°C, the oven was set at 40°C and the detector temperature was set at 150°C. All gas analyses were performed by injecting a 200 μL gas sample. The H_2 composition was measured using a gas chromatography (GC) unit (Agilent Technologies, Model 6850N; Agilent Technologies, Inc., Palo Alto, CA), and separated with a 15 m HP-Molesieve fused silica, 0.53 mm i.d. column (Agilent Technologies, Inc.). Nitrogen was used as the carrier gas at a constant flow rate of 6 mL/min, the splitless injector was maintained at 90°C, the oven was set at 50°C and the thermal conductivity detector temperature was set at 150°C. All gas analyses were performed by injecting a 200 μL gas sample. The results of gas composition analysis using gas standards are summarized in Table 3.1.

Table 3.1. Results of gas composition analysis

Gas	Retention time (min)	Slope[a]	R^2
CH_4	1.524	15974	1.00
CO_2	2.533	7369	0.99
H_2	0.813	10591	0.99

[a]Slope = peak area/gas concentration (%)

3.6 Volatile Fatty Acids (VFAs)

VFAs (C_1 to C_7; i.e., formic, acetic, propionic, iso-butyric, n-butyric, iso-valeric, n-valeric, iso-caproic, n-caproic, and heptanoic acids) were quantified using a high-performance liquid chromatography (HPLC) unit equipped with a UV-vis detector (Agilent 1100, Santa Clara, CA). A HPX-87H column (BioRad, Hercules, CA) was used with an eluent of 5 mM H_2SO_4 (v/v) at a flow rate of 0.6 mL/min. The injection volume was 20 µL, and the column was maintained at 65℃. The wavelength of 210 nm was used for all VFAs detection. Samples used for the measurement of VFAs were prepared by centrifugation at 10,000 rpm for 20 minutes and filtration through 0.2 µm Nuclepore track-etched polycarbonate membranes (Whatman Inc., Florham Park, NJ) prior to injection. The 10 mM VFAs standard solution was obtained from Supelco (Product No. CRM46975; Bellefonte, PA), all VFAs had quantification ranges from 0.25 to 10 mM. The results of VFAs analysis are summarized in Table 3.2.

Table 3.2. Results of VFAs analysis

VFA	Retention time (min)	Slope[a]	R^2	Molecular Weight (mg/mmol)	COD conversion factor (mg COD/mg VFA)
Formate	13.085	89.14	1.00	46.03	0.35
Acetate	14.267	68.28	1.00	60.05	1.07
Propionate	16.722	83.42	1.00	74.08	1.51
i-Butyrate	18.807	118.93	1.00	88.11	1.82
n-Butyrate	20.285	95.84	0.99	88.11	1.82
i-Valerate	23.154	115.42	1.00	102.13	2.04
n-Valerate	27.468	96.03	0.99	102.13	2.04
i-Caproate	33.963	104.67	1.00	116.16	2.20
n-Caproate	39.543	99.81	0.99	116.16	2.20
Heptanoate	61.499	94.86	0.99	130.18	2.34

[a]Slope = peak area/VFA concentration (mM)

The calculation of VFAs in terms of COD is as follows:

$$VFA\ COD\ \left(\frac{mg}{L}\right) = \frac{peak\ area}{slope} \times molecular\ weight\ \times CODconversion\ factor$$

3.7 Ammonia

Ammonia concentration was measured using the distillation method described in *Standard Methods* (Eaton et al., 2005). The samples were centrifuged at 10,000 rpm for 20 minutes and filtered through a 0.2 μm Nuclepore track-etched polycarbonate membranes (Whatman Inc., Florham Park, NJ). The ammonia distillation was performed using a Labconco distillation apparatus (Labconco., Kansas City, MO) and the distillate was received in a mixed indicator solution of 20 g H_3BO_3/L, methyl red 13.3 mg/L and methylene blue 6.67 mg/L. The distillate was then titrated with 0.2 N H_2SO_4 and the ammonia was quantified.

3.8 Phosphate

Phosphate concentration was measured using a Dionex DX-100 ion chromatography unit (Dionex Corporation, Sunnyvale, CA) equipped with a suppressed conductivity detector, a Dionex IonPac AG14A (4x50 mm) precolumn, and a Dionex IonPac AS14A (4x250 mm) analytical column. The unit was operated in autosuppression mode with 1 mM $NaHCO_3$/8 mM Na_2CO_3 eluent at a flow rate of 1mL/min. All samples were filtered through 0.2 μm Nuclepore track-etched polycarbonate membranes (Whatman Inc., Florham Park, NJ) prior to injection. The phosphate retention time was 11.290 minutes. The calibration slope was 0.0535 (peak area versus mg P/L; R^2 = 0.990), and the quantification range was from 1 to 25 mg P/L.

3.9 Peracetic Acid (PAA)

Commercial peracetic acid solutions are mixtures of peracetic acid, hydrogen peroxide, and acetic acid. To quantify the two peroxides, one can differentiate peracetic acid and hydrogen peroxide by their different redox properties: a two-step quantitative redox reaction of PAA oxidizes methyl *p*-tolyl sulfide (MTS) into methyl *p*-tolyl sulfoxide (MTSO) and hydrogen peroxide oxidizes triphenylphosphine (TPP) into triphenylphosphine oxide (TPPO). These reagents and their products can be separated by HPLC equipped with a reverse-phase column using water/acetonitrile gradient. Based on polarity, from polar to non-polar, MTSO elutes first, followed by TPPO, MTS, and at last TPP (Pinkernell et al., 1997).

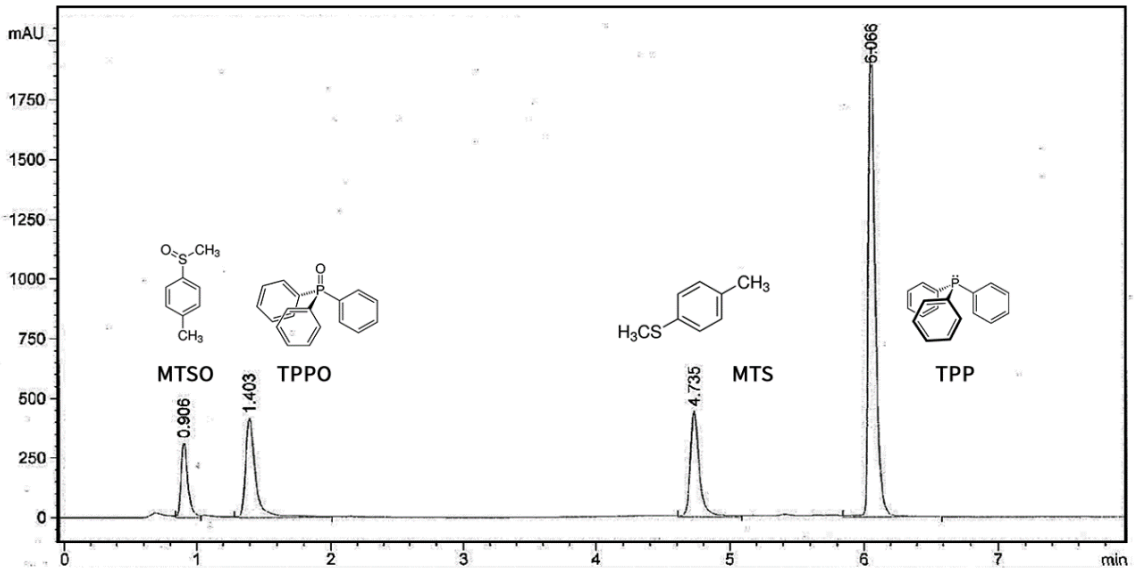


Figure 3.1. Retention time of MTS, MTSO, TPP, and TPPO using a Luna C8(2) column.

The two-step redox reaction starts with the oxidation of MTS into MTSO by PAA: 100 µL of a 20 mM solution of MTS in acetonitrile and 300 µL of deionized water were added to 100 µL of sample. After a reaction time of 10 min, 400 µL of acetonitrile and 100 µL of a 10 mM solution of TPP in acetonitrile were added to start the second redox reaction. The resulting solution was stored in the dark for 30 minutes then was injected into an HPLC unit equipped with a UV-vis detector (Agilent 1100, Santa Clara, CA), and a Luna C8(2) column (150x3.0 mm; particle size of 5 µm and pore size of 100 Å; Phenomenex, Torrance, CA). The eluent consisted of (A) 40% acetonitrile in deionized water (v/v) and (B) 100% acetonitrile at a flow rate of 1 mL/min. The elution gradient was as follows: from 0 to 3 min, the acetonitrile concentration of eluent gradually increased from 40 % to 100 % in deionized water (v/v); from 3 to 8 min, the acetonitrile concentration of eluent gradually decreased from 100 % to 40 % in deionized water (v/v). The injection volume was 5 µL, and the column was maintained at 35℃. The wavelength of 225 nm was used for MTS, MTSO, TPP, and TPPO detection. The elution order and retention time are shown in Figure 3.1 (MTSO at 0.906 min, TPPO at 1.403 min, MTS at 4.735 min, and TPP at 6.066 min).

The optimal quantitative ranges for MTSO and TPPO are both from 0.01 to 1 mM, Therefore, if needed, samples were diluted to the range of 0.076 to 76.05 mg PAA/L and 0.34 to 34.01 mg H_2O_2/L. The calibration curves are shown in Figure 3.2.

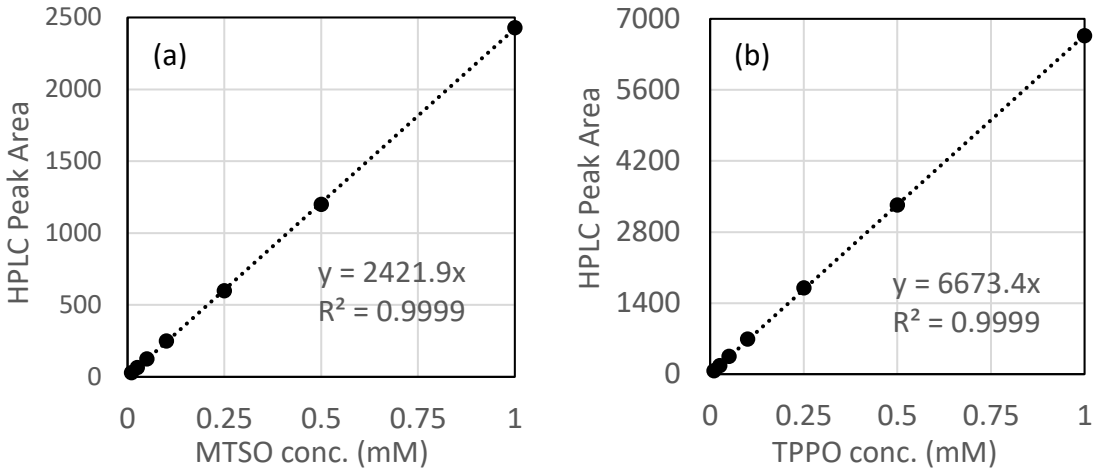


Figure 3.2. Calibration curve for (a) MTSO and (b) TPPO (Optimal quantitative range for both MTSO and TPPO is 0.01 to 1 mM).

To determine the concentration of PAA and hydrogen peroxide in the PAA working solution or a sample, a blank with deionized water followed the procedures described above was also prepared. The background MTSO and TPPO peak areas were usually 0.00 and 482, respectively. The calculation of the concentration of PAA and hydrogen peroxide in the working PAA solution was as follows.

$$\text{PAA (mM)} = \frac{MTSO\ area_{wk\ sln.} - MTSO\ area_{bk}}{slope_{MTSO}}$$

$$H_2O_2\ \text{(mM)} = \frac{TPPO\ area_{wk\ sln.} - TPPO\ area_{bk}}{slope_{TPPO}}$$

3.10 Peracetic Acid Decomposition

To investigate the effect of pre-decomposed PAA addition and direct PAA addition to anaerobic reactors, a series of PAA decomposition assays were conducted in an anaerobic culture medium (see Table 4.1) and dissolved air floatation (DAF) effluent, obtained from a poultry processing plant (see Chapter 2, section 2.2). The kinetics of PAA decomposition in both the anaerobic culture medium and the DAF effluent were assessed.

3.10.1 Chemicals

Peracetic acid solution was obtained from Sigma Aldrich (Product No. 101878085; St. Louis, MO). The density of the PAA solution was 1.15 g/mL, and the composition (by weight) was 39.3% peracetic acid, 42.3% acetic acid, and 6% hydrogen peroxide. Therefore, the initial concentrations were: 451.95 g PAA/L, 486.45 g acetate/L, and 69.00 g H_2O_2/L.

3.10.2 PAA Decomposition Assays

All PAA decomposition assays used a ratio of 39:1 (v:v) of the anaerobic culture medium or the DAF effluent to the PAA solution. The targeted initial PAA concentration for the anaerobic culture medium was 500 mg PAA/L, and for the DAF effluent was 40 and 80 mg PAA/L. At each sampling interval, the pH and PAA concentration were measured. PAA concentrations were determined as described in Section 3.9. Samples were centrifuged at 10,000 rpm for 20 minutes and filtered through 0.2 µm Nuclepore track-etched polycarbonate membranes (Whatman Inc., Florham Park, NJ) prior to injection into the HPLC. Each assay contained a background control, that is, the anaerobic culture

medium or the DAF effluent without any PAA addition. Quantification of PAA followed the procedure described in Section 3.9.

3.10.3 Results and Discussion

Both the anaerobic culture medium (Figure 3.3) and the DAF effluent (Figure 3.4) showed an initial high PAA demand, followed by slower decomposition rates.

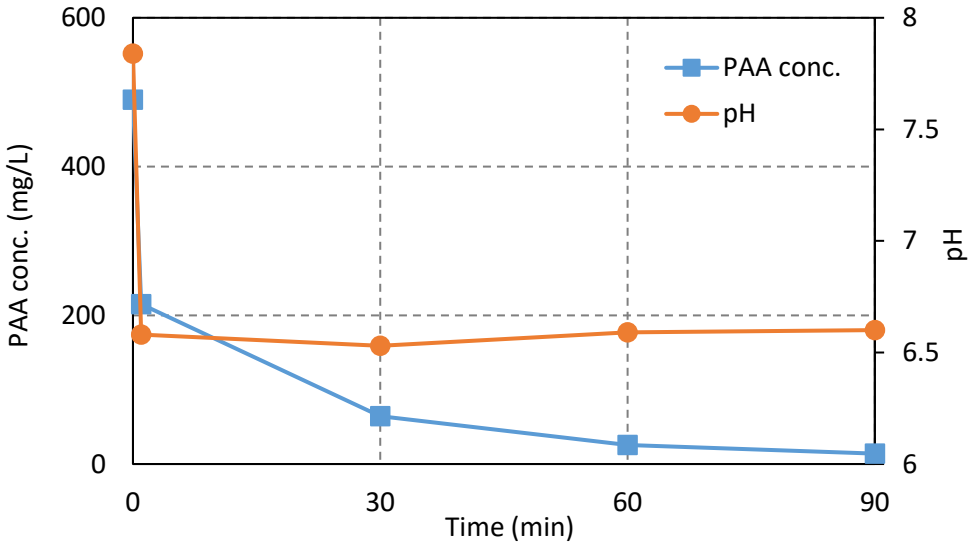


Figure 3.3. PAA decomposition in the anaerobic culture medium and pH change over time. $[PAA]_{initial}$ = 490 mg/L.

For the anaerobic culture media, the initial PAA concentration was 490 mg /L, and the initial pH was 7.84. After PAA addition, the pH dropped to 6.58 and was around 6.60 over 90 minutes. The initial PAA demand of the anaerobic culture media was 56.11 % of the initial PAA. After 1 hour, PAA decomposed by 94.81 %, and its concentration was under the limit of detection (LOD; ca., 0.76 mg/L) after 90 minutes.

Unlike the anaerobic culture media, the DAF effluent was not well-buffered. After adding PAA into the DAF effluent, the pH dropped to between 4.07 and 4.32. Under neutral and acidic conditions, the PAA decomposition rate decreased as the pH decreased, as previously reported (Zhao et al., 2008). Thus, NaOH was added to increase the pH of DAF effluent after PAA addition to 5 and 6.4 to accelerate the PAA decomposition rate. The PAA concentration and pH were measured at 1, 5, 15, 30, and 60 minutes after PAA addition, and NaOH added 1 minute after PAA addition. Table 3.3 summarizes the conditions and results of PAA decomposition in DAF effluent.

Table 3.3. Results of batch PAA decomposition in DAF effluent (multiple tests).

Initial PAA conc. (mg/L)	Initial acetate conc. (mM)	NaOH addition	pH at 60 min[a]	PAA decomposition (%) at 15 min[a]
40. 4	17.0	--	3.97	45.6
49.3	16.3	4.5 mL of 0.1 N NaOH	5.00	91.4
41.2	16.5	1.07 mL of 1 N NaOH	6.42	95.5
79.6	33.8	3 mL of 1 N NaOH.	6.79	99.2

[a] Time after PAA addition

The PAA decomposition in the DAF effluent, with an initial PAA concentration of 40 mg PAA/L, without any pH adjustment resulted in 45.6% PAA decomposition in 15 minutes after PAA addition. In contrast, at a similar initial PAA concentration and pH adjusted to 5.00 and 6.42, PAA decomposed by 91.4 and 95.5% in 15 minutes after PAA addition, respectively. As the initial PAA concentration increased to 80 mg/L and the pH was adjusted to 6.79, the extent of PAA decomposition was higher reaching 99.2 % in 15 minutes after PAA addition. Thus, the PAA decomposition rate increased as the pH increased (Figure 3.4).

The amount of NaOH needed to adjust the pH to the targeted pH value depends on the condition of the PAA working solution and buffer capacity of the DAF effluent. The PAA in the working solution was slowly decomposed into acetic acid, which has a pKa of 4.76. To maintain the same initial PAA concentration as the working PAA solution kept decomposing (assays were conducted on different dates), the amount of PAA working solution added into the DAF effluent increased. That is, the initial acetic acid added into the DAF effluent increased and the DAF effluent had a stronger buffer capacity around pH of 4.76. Therefore, the amount of NaOH needed to adjust the pH to the targeted pH values increased.

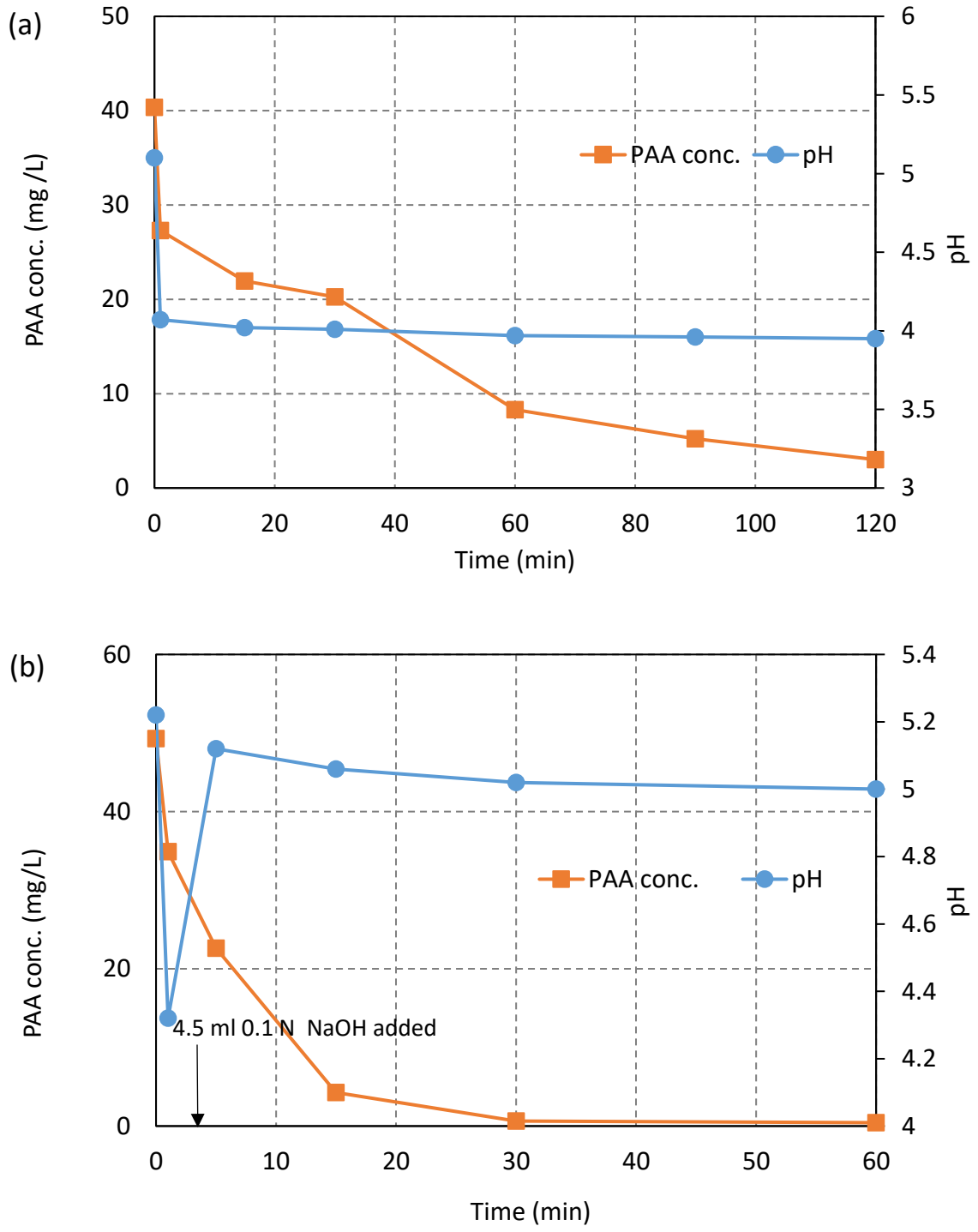


Figure 3.4. PAA decomposition in DAF effluent and pH change over time. (a) $[PAA]_{initial}$ = 40 mg/L and pH maintained at 4; (b) $[PAA]_{initial}$ = 49 mg/L, with addition of 4.5 mL of 0.1 N NaOH and pH maintained at 5.

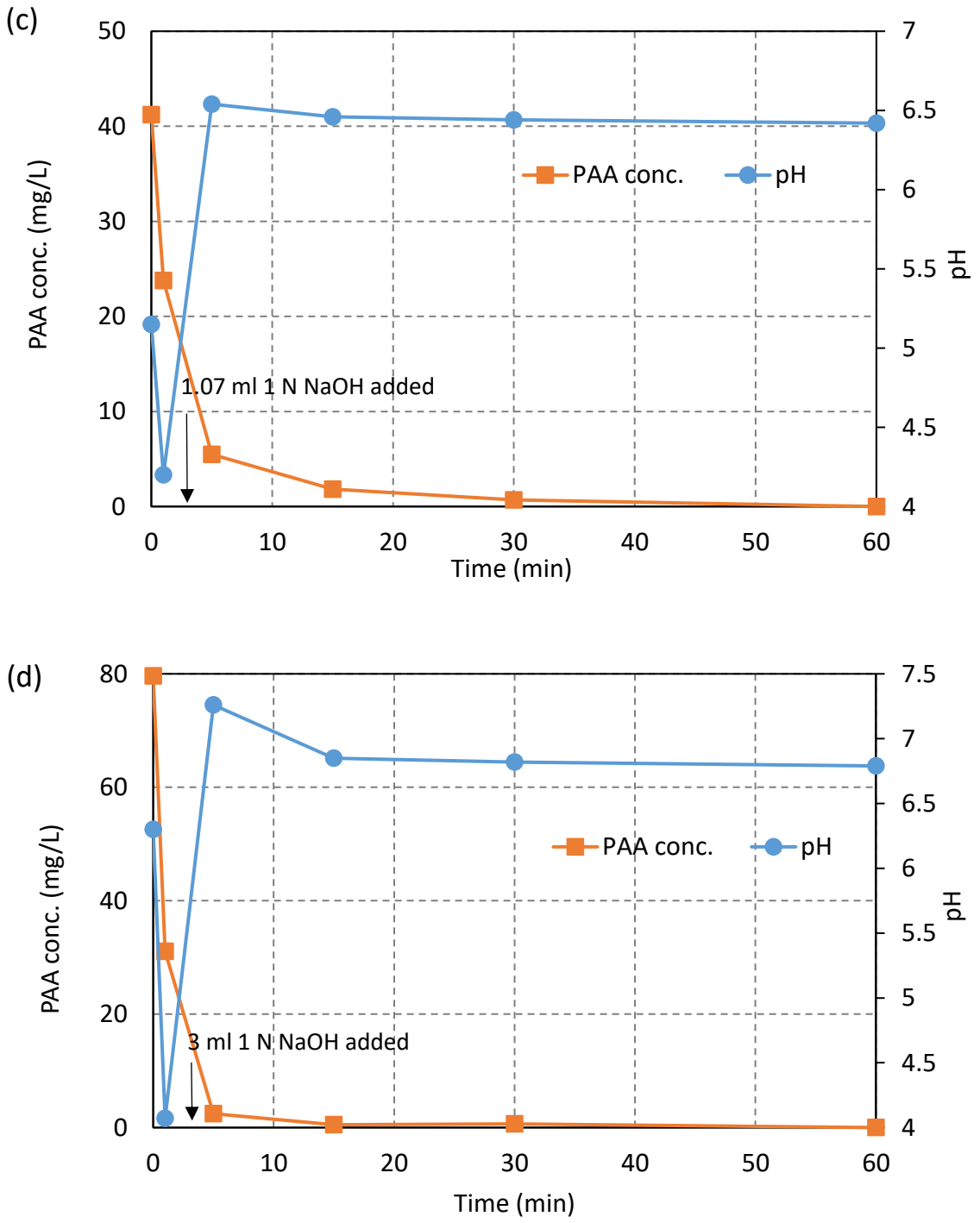


Figure 3.4. (continued) PAA decomposition in DAF effluent and pH change over time. (c) $[PAA]_{initial}$ = 41 mg/L, with addition of 1.07 mL of 1 N NaOH and pH maintained at 6.4 (d) $[PAA]_{initial}$ = 80 mg/L, with addition of 3 mL of 1 N NaOH and pH maintained at 6.8.

In summary, when the pH of DAF effluent was adjusted above 5, 40 and 80 mg/L PAA decomposed by ca. 90 % in 15 minutes after PAA addition (Figure 3.5, i.e., overlay of Figure 3.4 (a)-(d)). Therefore, in the follow-up batch assays conducted with pre-decomposed PAA, PAA was added first to the anaerobic culture medium or the DAF effluent, followed by culture seed addition after at least 15 minutes has elapsed since the PAA addition. In other words, the PAA concentration had decreased below the limit of detection (ca. 0.76 mg/L) prior to adding the culture seed.

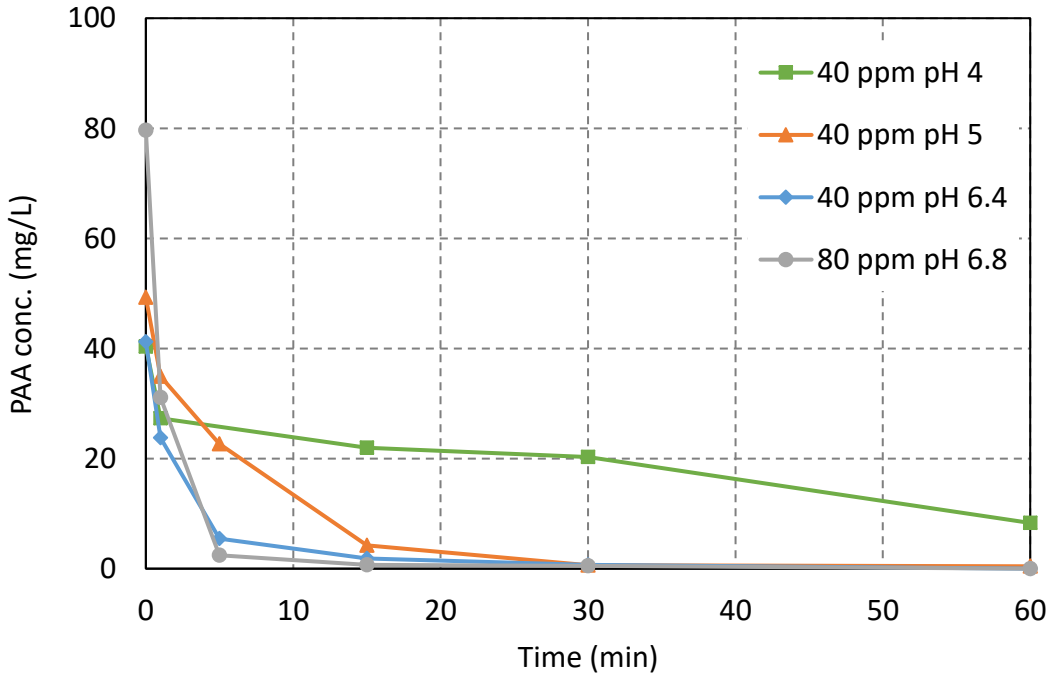


Figure 3.5. Extent of PAA decomposition in DAF effluent amended with different initial PAA concentrations and resulting pH values.

CHAPTER 4

EFFECT OF PERACETIC ACID ON A MIXED FERMENTATIVE/METHANOGENIC CULTURE

4.1 Introduction

Peracetic acid (PAA) is a strong disinfectant against a wide spectrum of microbes, used in healthcare, water treatment, and food industry. Residual PAA in wastewater may be carried to biological wastewater treatment systems with potential adverse effects. However, it is not well known how PAA will affect the anaerobic treatment processes.

Anaerobic digestion is a multi-stage process of series and parallel reactions transforming organic matter into methane and carbon dioxide. The reactions are typically grouped into four subprocesses: (1) hydrolysis, (2) acidogenesis, (3) acetogenesis, and (4) methanogenesis. Among all microbes responsible for the above-mentioned reactions, archaeal methanogens are generally the most vulnerable. Therefore, in this study, the PAA effect on methanogens was assessed based on different types of substrates. The main substrates used by methanogens, acetate and H_2/CO_2, are converted into CH_4 by acetoclastic methanogens and hydrogenotrophic methanogens, respectively (Pavlostathis, 2011).

The objectives of this phase of the study were to i) investigate the effect of PAA on a mixed fermentative/methanogenic culture, acetoclastic methanogens, and hydrogenotrophic methanogens; and ii) compare the effect of pre-decomposed and direct

PAA addition, conditions reflecting normal operation and accidental spills, respectively, while applying PAA for disinfection in poultry processing plants.

4.2 Mixed Fermentative/Methanogenic Culture

4.2.1 Culture Setup and Feeding

The stock mixed fermentative/methanogenic culture used in this study was initiated with inoculum obtained from a mesophilic, municipal anaerobic digester, fed with a mixture of dextrin and peptone and maintained at 35°C for several years (Dykstra and Pavlostathis, 2017). The inoculum was transferred into a 5 L reactor with a total liquid volume of 4 L. The reactor's headspace was connected to a graduated cylinder containing an acid brine solution (10% NaCl w/v and 2% H_2SO_4 v/v) for gas collection and measurement by liquid displacement. The reactor was continuously mixed using a magnetic bar and stir plate and maintained at room temperature (22±2^{o}C) with a hydraulic/solids retention time (HRT/SRT) of 21 days. The reactor was batch-fed every 3 and 4 days with a mixture of dextrin/peptone (D/P) (stock of 96 g dextrin/L, 48 g peptone/L; initial D/P concentration upon feeding, 1440 and 1908 mg COD/L, respectively; mean organic loading, 478 mg COD/L-day). In addition to the D/P mixture, the anaerobic culture medium (Table 4.1-Table 4.3) was added to maintain a total liquid phase volume of 4 L.

Table 4.1. Composition of the anaerobic culture medium.

Component	Concentration
$K_2 HPO_4$	0.9 g/L
$KH_2 PO_4$	0.5 g/L
$CaCl_2 \cdot 2H_2O$	0.1 g/L
$MgCl_2 \cdot 6H_2O$	0.2 g/L
$FeCl_2 \cdot 4H_2O$	0.1 g/L
NH_4Cl	0.5 g/L
$Na_2S \cdot 9H_2O$	0.5 g/L
$NaHCO_3$	3.5 g/L
1 g/L Resazurin Stock	2.0 mL/L
Vitamin Stock[a]	1.0 mL/L
Trace Metal Stock[b]	1.0 mL/L

[a] Vitamin stock solution composition described in Table 4.2
[b] Trace metal stock solution composition described in Table 4.3

Table 4.2. Composition of vitamin stock solution.

Vitamin	Concentration (g/L)
Biotin	0.2
Folic Acid	0.2
Pyridoxine hydrochloride	1.0
Riboflavin	0.5
Thiamine	0.5
Nicotinic Acid	0.5
Pantothenic Acid	0.5
Vitamin B12	0.01
p-Aminobenzoic Acid	0.5
Thioctic Acid	0.5

Table 4.3. Composition of trace metals stock solution.

Chemical	Concentration (g/L)
$ZnCl_2$	0.5
$MnCl_2 \cdot 4H_2O$	0.3
H_3BO_3	3.0
$CoCl_2 \cdot 6H_2O$	2.0
$CuCl_2 \cdot 2H_2O$	0.1
$NiSO_4 \cdot 6H_2O$	0.2
$Na_2MoO_4 \cdot 2H_2O$	0.3

4.2.2 Anaerobic Digester Performance

The stock anaerobic digester had a steady total biogas production and gas composition throughout the study, and it was suitable to serve as an anaerobic seed for other assays conducted in this study. The total gas production was 2821 ± 80 ml and 3766 ± 94 ml (n = 10) for 3-day and 4-day feeding cycle (Figure 4.1), respectively. The normalized gas composition was calculated as follows:

$$CH_{4,normlized}\ (\%) = \frac{CH_4(\%)}{CH_4\ (\%) + CO_2(\%)}; \qquad CO_{2,normlized}\ (\%) = \frac{CO_2(\%)}{CH_4\ (\%) + CO_2(\%)}$$

The normalized CH_4 and CO_2 content of the biogas was 65.1 ± 0.8 % and 35.0 ± 0.8 % (n = 19), respectively (Figure 4.2 a). The soluble COD before each feeding was initially ca. 600 mg COD/L and gradually decreased to 300 mg COD/L (Figure 4.2 b).

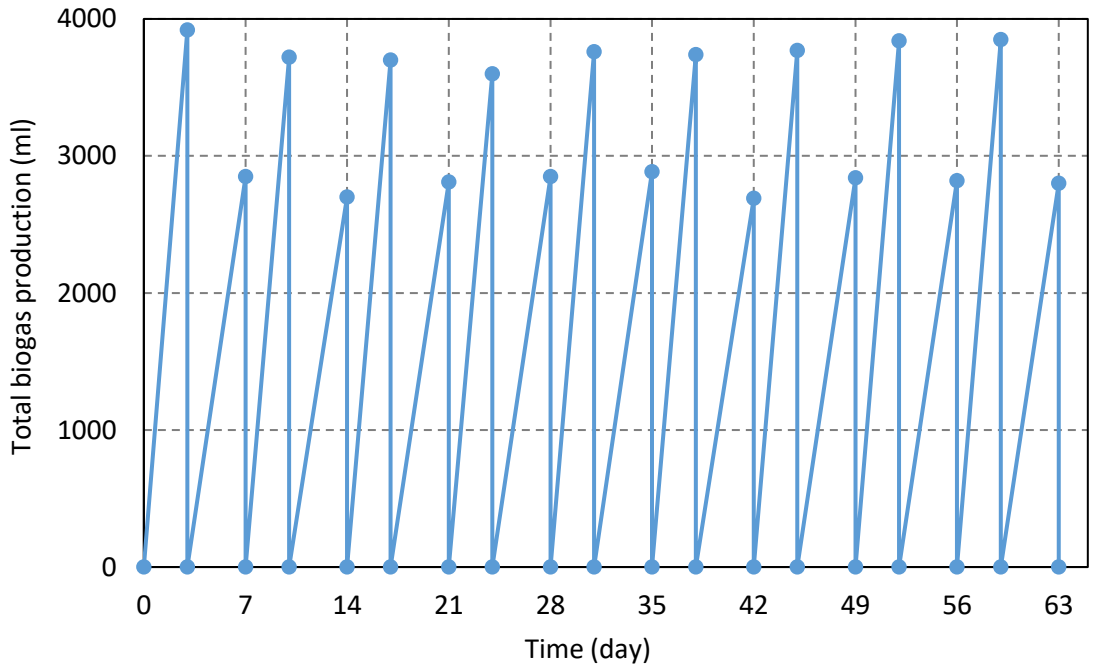


Figure 4.1. Total biogas production over 3-day and 4-day feeding cycles over the initial 63 days of anaerobic digester operation (22°C, 1 atm).

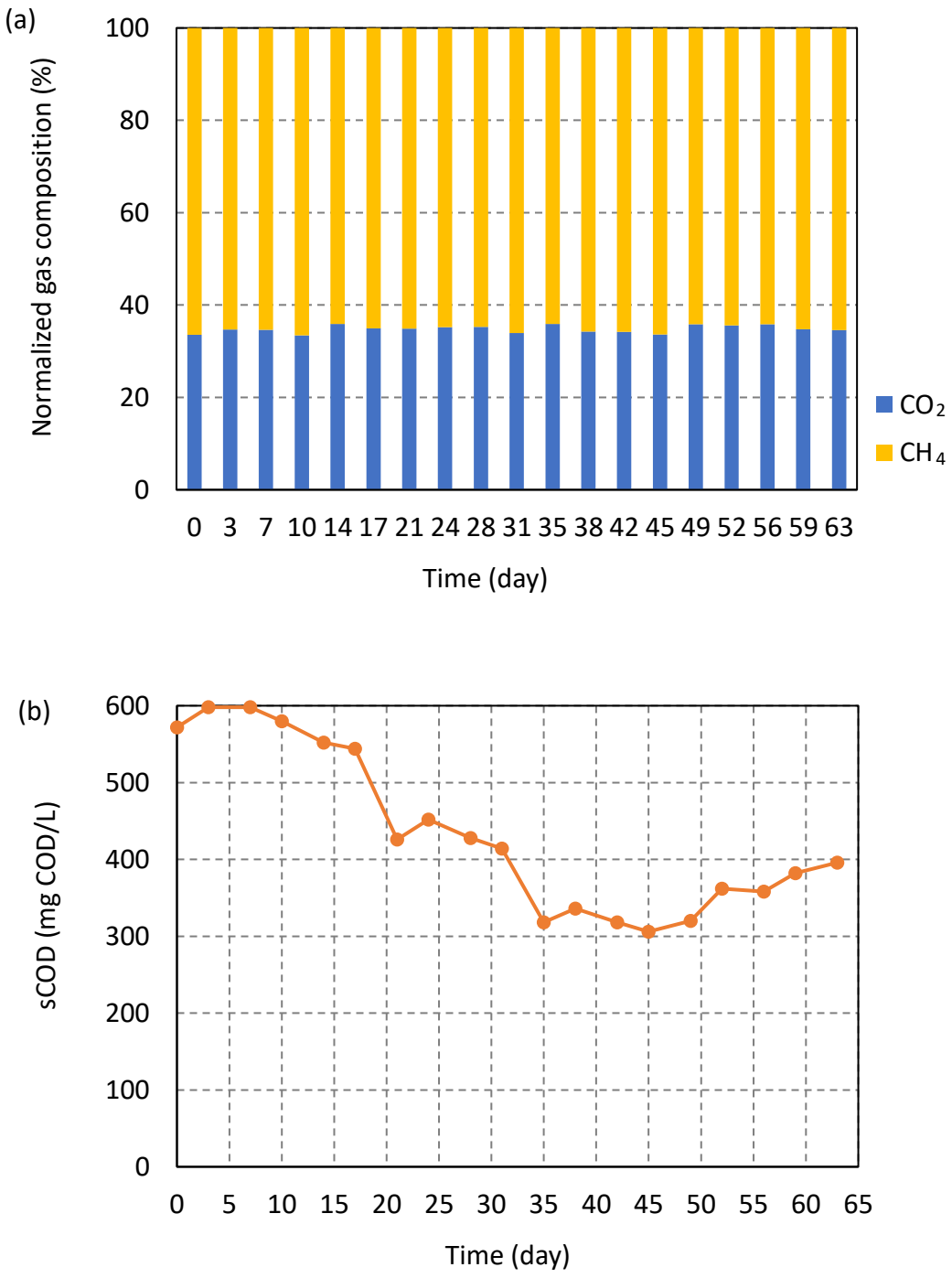


Figure 4.2. Normalized gas composition (a) and soluble COD (b) of the anaerobic digester over the initial 63 days of operation.

4.3 Effect of Peracetic Acid on the Mixed Fermentative/Methanogenic Culture

4.3.1 Materials and Methods

4.3.1.1 Effect of PAA on the Mixed Fermentative/Methanogenic Culture fed with Dextrin/Peptone

The objective of this phase of the study was to investigate the PAA effect on the mixed fermentative/methanogenic culture and the effect of pre-decomposed and direct PAA addition, conditions reflecting normal operation and accidental spills in poultry processing plants, respectively. A batch assay was conducted with six culture series: seed-blank, reference (PAA-free), direct 40 mg/L PAA addition, direct 100 mg/L PAA addition, pre-decomposed 40 mg/L PAA, and pre-decomposed 100 mg/L PAA (details shown in Table 4.4). Six 600-mL glass reactors, with rubber stoppers on their top and outlets in the bottom for liquid sample collection, were flushed with helium gas for 15 minutes at 10 psig. Anaerobic culture medium (100 mL) was first transferred anaerobically into the reactors the night before the assay was conducted. For the 40 mg/L and 100 mg/L pre-decomposed series, 2 and 5 mL of 10 g PAA/ L working solution were added 12 hours before the assay was conducted, respectively. On day 0, 10 mL of dextrin/peptone (96 g dextrin/L, 48 g peptone/L) were added to all series except the seed-blank. Then, DI water was added according to Table 4.4. Last, needles (Precision Glide^{TM} Needle; 1.6mm × 40mm, Becton, Dickinson and Company, Franklin Lakes, NJ) were inserted to the rubber stoppers to all series to vent out excess gas, followed by the addition of 385 mL of the anaerobic seed (see Section 4.2). The seed was waste collected weekly from the stock anaerobic digester, kept at 22°C in the laboratory for two weeks without feeding. Last, for direct 40 mg/L and 100

mg/L PAA addition series, 2 and 5 mL of 10 g PAA/ L was added, respectively. Before the beginning of the incubation, 100 mL of sample was removed from each series to measure initial pH, tCOD, sCOD, TS, VS, and ammonia. To avoid introducing air into the reactors while taking out sample, the reactors headspaces were attached to a water displacement column containing helium. After taking out 100 mL of sample, excess gas pressure was released from the reactors to equilibrate their headspace to atmospheric pressure. During the incubation, total gas production and gas composition were measured at appropriate time intervals. At the end of the incubation, the reactors were opened and the pH, tCOD, sCOD, TS, VS, ammonia, and VFAs were measured.

The hydrogen peroxide concentration of the PAA working solution was typically one tenth (by weight) of PAA concentration; therefore, in this study, hydrogen peroxide was considered negligible to affect microbial activity.

Table 4.4. Component volume (mL) in the batch assay set up to evaluate the PAA effect on the mixed fermentative/methanogenic culture fed with dextrin/peptone.

Component	Seed-blank	Reference	Direct		Pre-decomposed	
			40 mg PAA/L	100 mg PAA/L	40 mg PAA/L	100 mg PAA/L
Anaerobic culture seed	385	385	385	385	385	385
96 g dextrin/L, 48 g peptone/L	0	10	10	10	10	10
Anaerobic culture media	100	100	100	100	100	100
10 g PAA /L	0	0	2	5	2	5
DI water	15	5	3	0	3	0
Total volume	500	500	500	500	500	500

The D/P mixture, PAA decomposed to acetate and acetate in the PAA working solution could serve as substrates for microbial growth. The COD of PAA decomposed to acetate and acetate in the PAA working solution combined is referred to as PAA working solution COD in the following sections. Table 4.5, summarizes the substrate COD in each series set up to evaluate the PAA effect on the mixed fermentative/methanogenic culture fed with dextrin/peptone.

Table 4.5. Component of substrate COD concentration (mg COD/L) in the batch assay set up to evaluate the PAA effect on the mixed fermentative/methanogenic culture fed with dextrin/peptone.

Component	Seed-blank	Reference	Direct		Pre-decomposed	
			40 mg PAA/L	100 mg PAA/L	40 mg PAA/L	100 mg PAA/L
D/P mixture	0	2655	2655	2655	2655	2655
PAA decomposed to acetate	0	0	34	84	34	84
Acetate in PAA working solution	0	0	116	290	116	290
Total substrate COD	0	2655	2805	3029	2805	3029

4.3.1.2 Effect of PAA on Acetoclastic Methanogens

The objective of this phase of the study was to investigate the PAA effect on acetoclastic methanogens and the effect of pre-decomposed and direct PAA addition. A batch assay similar to the one described in Section 4.3.1.1, was conducted. The only difference was replacing dextrin/peptone with the same COD-equivalent sodium acetate (2.07 M in stock). Details are summarized in Table 4.6, and substrate COD is summarized in Table 4.7. The setup, operations and measurements were the same as those described in Section 4.3.1.1.

Table 4.6. Component volume (mL) in the batch assay set up to evaluate the PAA effect on acetoclastic methanogens.

Component	Seed-blank	Reference	Direct		Pre-decomposed	
			40 mg PAA/L	100 mg PAA/L	40 mg PAA/L	100 mg PAA/L
Anaerobic culture seed	385	385	385	385	385	385
2.07 M Sodium acetate	0	10	10	10	10	10
Anaerobic culture media	100	100	100	100	100	100
10 g PAA /L	0	0	2	5	2	5
DI water	15	5	3	0	3	0
Total volume	500	500	500	500	500	500

Table 4.7. Component of substrate COD concentration (mg COD/L) in the batch assay set up to evaluate the PAA effect on acetoclastic methanogens.

Component	Seed-blank	Reference	Direct		Pre-decomposed	
			40 mg PAA/L	100 mg PAA/L	40 mg PAA/L	100 mg PAA/L
Sodium acetate	0	2650	2650	2650	2650	2650
PAA decomposed to acetate	0	0	33	83	33	83
Acetate in PAA working solution	0	0	49	122	49	122
Total substrate COD	0	2650	2732	2855	2732	2855

4.3.1.3 Effect of PAA on Hydrogenotrophic Methanogens

The objective of this phase of the study was to investigate the PAA effect on hydrogenotrophic methanogens and the effect of pre-decomposed and direct PAA addition. A batch assay was conducted with six culture series: seed-blank, reference (PAA-free), direct 40 mg/L PAA addition, direct 100 mg/L PAA addition, pre-decomposed 40 mg/L PAA, and pre-decomposed 100 mg/L PAA (details shown in Table 4.8). Six 160-mL serum bottles were sealed with rubber stoppers and aluminum caps, then flushed with helium gas for 15 minutes at 10 psig. Two Precision Glide^{TM} Needles (1.6mm × 40mm) penetrated through the rubber stopper, one for adding liquid, the other one for venting out gas. The night before day 0, 20 mL of the anaerobic culture medium was added to each bottle. For PAA pre-decomposed series, 1 and 2.5 mL of 4 g PAA/L PAA working solution was added, respectively. On day 0, 77 mL of the anaerobic seed was added to each bottle. For the direct PAA addition series, 1 and 2.5 mL of 4 g PAA/L PAA working solution was added, respectively. Table 4.9, summarizes the PAA and acetate COD in each series and projected CH_4 production based on acetate in the PAA working solution, plus acetate produced from PAA decomposition. All serum bottles were then flushed with H_2/CO_2 (80:20; v/v) at 5 psig for 5 minutes, then pressurized to 21.80 psig. The gas pressure and gas composition were measured immediately after pressurization and then at predetermined times. Total gas production was measured using a pressure transducer (resolution ±1.974 atm, accuracy to 0.002 atm). When one of the series' pressure dropped below 1.01 bar, all series were re-pressurized to 21.80 psig (1.503 bar). To improve the mass transfer of hydrogen gas, all serum bottles were placed on an Innova 2100 platform orbital shaker (New Brunswick

Scientific, Edison Township, NJ) and mixed at 150 rpm. At the end of the assay, pH, tCOD, sCOD, and VFAs were measured.

Table 4.8. Component volume (mL) in the batch assay set up to evaluate the PAA effect on hydrogenotrophic methanogens.

Component	Seed-blank	Reference	Direct		Pre-decomposed	
			40 mg PAA/L	100 mg PAA/L	40 mg PAA/L	100 mg PAA/L
Anaerobic culture seed	77	77	77	77	77	77
Anaerobic culture media	20	20	20	20	20	20
4 g PAA /L	0	0	1	2.5	1	2.5
DI water	3	3	2	0.5	2	0.5
Total volume	100	100	100	100	100	100

Table 4.9. Component of substrate COD concentration (mg COD/L) and projected CH_4 production in the batch assay set up to evaluate the PAA effect on hydrogenotrophic methanogens. [a]

Component	Seed-blank	Reference	Direct		Pre-decomposed	
			40 mg PAA/L	100 mg PAA/L	40 mg PAA/L	100 mg PAA/L
PAA decomposed to acetate	0	0	32	84	34	82
Acetate in PAA working solution	0	0	51	135	53	130
Projected CH_4 COD	0	0	83	219	87	212

[a] Calculated COD values of PAA solution measured at different days.

4.3.2 Results and Discussion

4.3.2.1 Effect of PAA on the Mixed Fermentative/Methanogenic Culture fed with Dextrin/Peptone

The initial and end of incubation characteristics of the six series are summarized in Table 4.10. Incubation lasted for 60 days. The pH in all series was above 7, which is optimal for methanogens (Rittmann and McCarty, 2001). Both TS and VS decreased throughout the incubation period, except for the TS of the seed-blank series. Ammonia increased after the incubation in all groups, except in the seed-blank series.

Table 4.10. Initial and final characteristics of six series in the batch assay testing the effect of PAA on the mixed fermentative/methanogenic culture fed with dextrin/peptone (without seed blank correction).

Series		pH	tCOD mg COD/L	sCOD mg COD/L	TS g/L	VS g/L	NH_3-N mg/L
Seed-blank	Initial	7.55	2106	212	6.03±0.07[a]	1.57±0.06[a]	169
	Final	7.47	1536	232	6.21±0.10	1.42±0.12	146
Reference	Initial	7.25	5024	1892	7.72±0.13	3.34±0.06	158
	Final	7.07	2233	238	6.77±0.09	1.92±0.11	230
Direct 40 mg/L PAA	Initial	7.02	5208	2146	7.93±0.07	3.36±0.07	160
	Final	7.03	2273	288	6.87±0.04	1.96±0.04	244
Direct 100 mg/L PAA	Initial	7.06	5262	2460	7.94±0.08	3.18±0.03	171
	Final	7.04	2555	284	6.98±0.02	2.15±0.03	210
Pre-decomposed 40 mg/L PAA	Initial	7.13	4994	1670	7.76+0.35	3.19±0.18	165
	Final	7.05	2316	242	6.95±0.05	2.02±0.03	224
Pre-decomposed 100 mg/L PAA	Initial	7.06	5458	2226	7.86±0.15	3.33±0.04	162
	Final	7.04	2349	218	6.78±0.14	2.00±0.00	228

[a] Mean ± standard deviation (n = 3)

The anaerobic culture medium provided great capacity to decompose PAA. Figure 4.3 shows the color of the anaerobic culture medium, which was originally black due to sulfide precipitates. The series with pre-decomposed 40 mg/L PAA became pink-purple, and the series with the pre-decomposed 100 mg/L PAA became milky white after the PAA addition. In the PAA-amended series, all black precipitates disappeared after PAA addition. PAA oxidized the sulfide precipitates and the dihydroresorufin (a product of resazurin) in the anaerobic culture medium. Resazurin (dark blue), which serves as a redox indicator in the anaerobic culture medium, was irreversibly reduced to resorufin (pink), and further underwent reversible reduction to dihydroresorufin (colorless) (Twigg et al., 1945). For the PAA-free reference series, the anaerobic culture medium was composed of dihydroresorufin (colorless) and sulfides (black precipitates); in the pre-decomposed 40 mg/L PAA series, PAA oxidized sulfides and dihydroresorufin into resorufin (pink); in the pre-decomposed 100 mg/L PAA series, PAA oxidized sulfides and dihydroresorufin into oxidized-resorufin (white) (its specific structure has not been reported).

Figure 4.3. Color of the anaerobic culture medium of the reference (PAA-free), pre-decomposed 40 mg/L PAA, and pre-decomposed 100 mg/L PAA series (from left to right).

The cumulative total biogas, carbon dioxide, and methane produced over the incubation period are shown in Figure 4.4. The pre-decomposed 40 mg/L PAA series experienced a spill during the setup and ca. 30 mL of liquid phase was lost; thus, all gas volume and COD mass data related to this series were multiplied by 1.1. All series had greater CO_2 production than CH_4 during the first 10 days of incubation, except for the direct 100 mg/L PAA series, for which it took 18 days for CH_4 to surpass CO_2, indicating that fermentation started first, and methanogenesis proceeded later on. All series, except the seed blank, had a similar CO_2 production rate during the first 10 days of incubation. On the contrary, the direct 100 mg/L PAA series had a delay in CH_4 production, indicating that the 100 mg/L PAA added directly impacted methanogenesis but not fermentation.

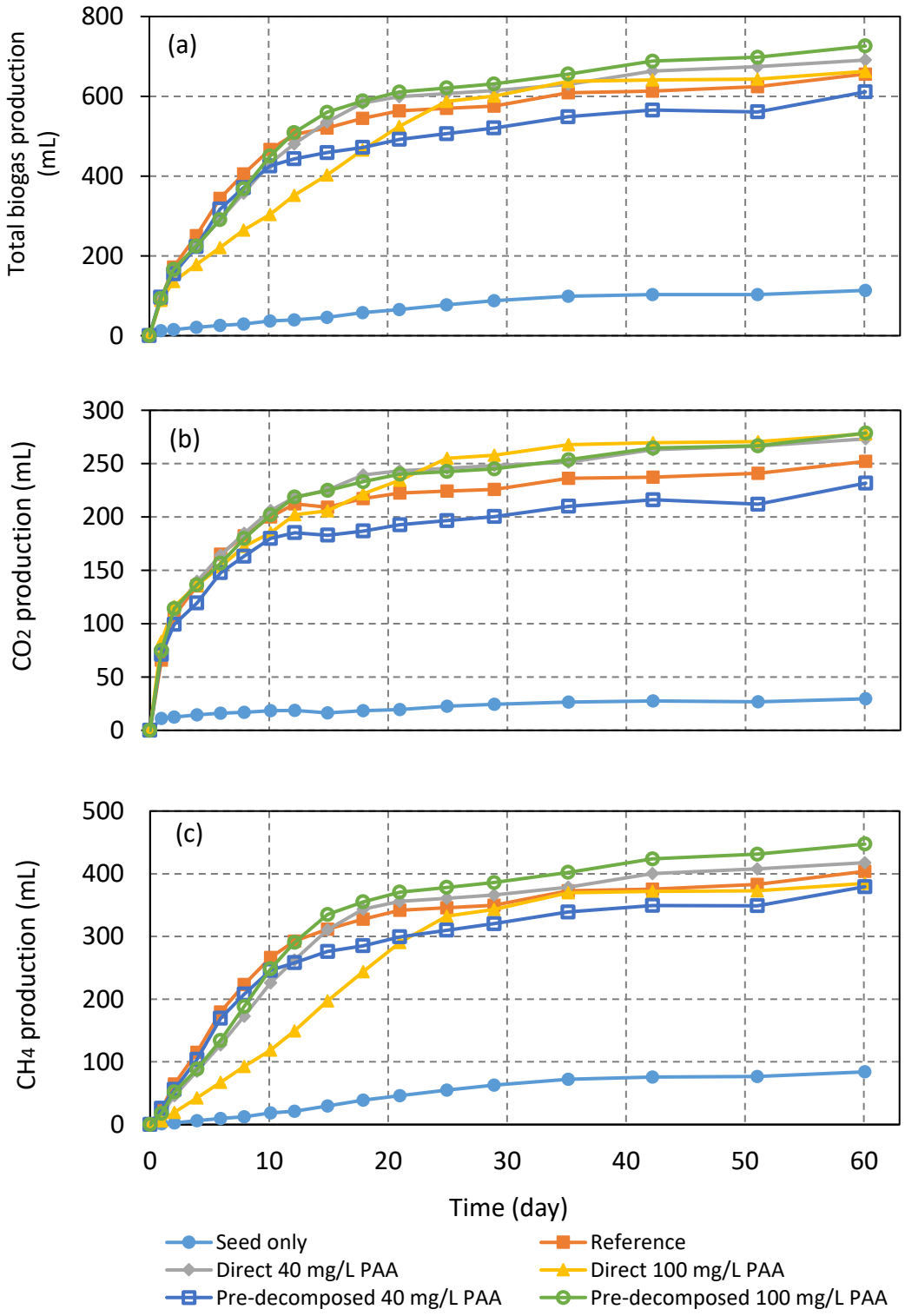


Figure 4.4. PAA effect on the mixed fermentative/methanogenic culture fed with dextrin/peptone: (a) total biogas, (b) carbon dioxide, and (c) methane production (22°C, 1 atm).

The seed blank-corrected performance of all series is summarized in Table 4.11. The CO_2 and CH_4 content were 42.0 ± 1.9 % and 58.0 ± 1.9 % (n = 5), respectively. The small standard deviation indicates that all series had similar gas composition. COD balance and total COD destruction were calculated as follows:

$$COD\ balance(\%) = \frac{tCOD_{Initial} - tCOD_{Final} - COD_{CH_4}}{tCOD_{Initial}} \times 100$$

$$Total\ COD\ destruction(\%) = \frac{tCOD_{Initial} - tCOD_{Final}}{tCOD_{Initial}} \times 100$$

Table 4.11. Parameters measured at the end of the assay set up to evaluate the PAA effect on the mixed fermentative/methanogenic culture fed with dextrin/peptone (after seed blank correction).

Parameter	Reference	Direct		Pre-decomposed	
		40 mg PAA/L	100 mg PAA/L	40 mg PAA/L	100 mg PAA/L
CO_2 (mL)[a]	222.6	243.7	248.4	202.2	249.2
CH_4 (mL)[a]	319.8	333.7	300.7	295.4	363.3
Total biogas (mL)[a]	542.4	577.4	549.0	497.6	612.5
CO_2 (%)	41.03	42.21	45.24	40.64	40.68
CH_4 (%)	58.97	57.79	54.76	59.36	59.32
COD balance (%)	3.6	5.1	4.7	5.4	4.1
CH_4_COD/Initial COD (%)	72.5	71.1	63.0	67.6	71.7
Total COD destruction (%)	76.1	76.2	67.7	73.0	75.7
Normalized Total COD destruction (%)[b]	100.0	100.2	89.0	95.9	99.5

[a] All gas data at 22°C and 1 atm; [b] Normalized to the reference series.

All series had COD balance between 3.6 and 5.4 %, showing the measurements were very reliable. The reference series achieved a total COD destruction of 76.1 %. Direct 40 mg/L PAA and pre-decomposed 100 mg/L PAA had similar total COD destruction (100.2 % and 99.5 % normalized to the reference). Indirect 40 mg/L PAA had a lower total COD destruction (95.9% normalized to the reference), which might be the result of losing ca. 30 mL of liquid phase during setup. Direct 100 mg/L PAA showed 89.0 % (normalized to the reference) of total COD destruction, indicating that COD destruction was impacted in this series. The total degradable COD was the sum of COD converted to CH_4 and biomass COD. In these series, the D/P mixture, PAA decomposed to acetate, and acetate in the PAA working solution were substrates used for microbial metabolism. To calculate the biodegradability of D/P mixture, PAA working solution acetate was considered negligible to serve as carbon source for microbial growth, i.e., all carbon for microbial growth came from D/P mixture. The net VS increase in the series was converted to COD equivalents by using a COD:VS ratio value of 1.42 g COD/g VS (Rittmann and McCarty, 2001) to represent the COD used for biomass growth. Therefore, the biodegradability of the D/P mixture was calculated as follows:

$$\text{Biodegradability}_{D/P} = \frac{D/P\ to\ CH_4COD + biomass\ COD}{\text{Initial D/P tCOD}}$$

All series had biodegradability of the D/P mixture between 95.7 and 97.4 % (details shown in Table 4.12). The series with direct 100 mg/L PAA addition had the least methane production per COD destroyed and the highest biomass COD, indicating that 100 mg/L of PAA addition impacted methanogens and the methane production was inhibited, while the D/P mixture was utilized as the carbon source for microbial growth.

Table 4.12. Substrate COD composition and biodegradability of the D/P mixture in the bioassay set up to evaluate the PAA effect on the mixed fermentative/methanogenic culture (liquid volume = 400 mL).

Parameter	Reference	Direct		Pre-decomposed	
		40 mg PAA/L	100 mg PAA/L	40 mg PAA/L	100 mg PAA/L
PAA [a] (mg COD)	0	14	34	14	34
Acetate [b] (mg COD)	0	46	116	46	116
PAA working solution (mg COD)	0	60	145	60	145
Initial tCOD (mg COD)	1167	1241	1262	1155	1341
Initial D/P tCOD [c] (mg COD)	1167	1181	1113	1095	1191
CH_4 equivalent COD (mg COD)	846	883	795	781	961
D/P to CH_4 [d] equivalent COD (mg COD)	846	823	646	722	811
Net VS increased (mg VS)	201	217	295	244	232
Biomass COD (mg COD)	286	308	419	346	331
D/P biodegradability (%)	97.0	95.8	95.7	97.4	95.9

[a] PAA decomposed to acetate
[b] Acetate in PAA working solution
[c] Initial tCOD = Initial D/P tCOD + PAA working solution COD
[d] CH_4 COD = D/P to CH_4 COD + PAA working solution COD

Figure 4.5, shows the seed blank-corrected methane COD equivalent production normalized to initial total substrate COD. The normalized methane production rate over the first 10 days of incubation decreased in the following order: Reference > pre-decomposed 40 mg/L PAA > pre-decomposed 100 mg/L > direct 40 mg/L PAA > direct 100 mg/L PAA, showing that direct PAA addition impacted methanogenesis, which recovered over time.

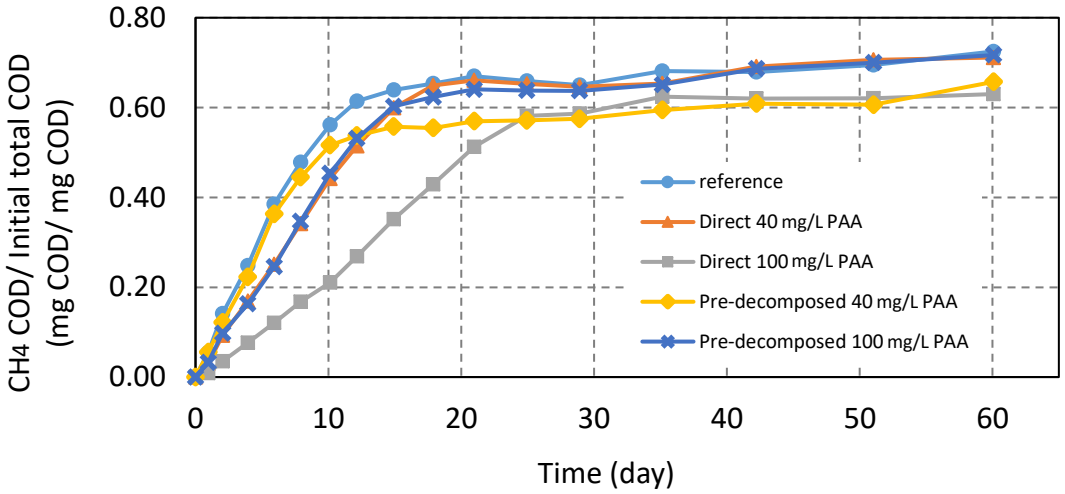


Figure 4.5. Methane COD equivalent production normalized to initial substrate COD (after seed blank correction) during the batch assay set up to evaluate the PAA effect on the mixed fermentative/methanogenic culture fed with dextrin/peptone.

The specific methanogenic activity (SMA) was calculated as the initial, linear methane production rate divided by the biomass VS concentration (Sørensen et al., 1993). Table 4.13, summarizes the SMA values for all series. SMA decreased in the following order: Reference > pre-decomposed 40 mg/L PAA > pre-decomposed 100 mg/L > direct 40 mg/L PAA > direct 100 mg/L PAA, indicating that direct PAA addition impacted the methanogenesis more than the pre-decomposed PAA addition.

Table 4.13. Specific methanogenic activity (SMA) of the mixed fermentative/methanogenic culture fed with dextrin/peptone as a function of PAA concentration and method of addition.

	SMA $\frac{mmole\ CH_4}{g\ VS - day}$	R^2	Normalized SMA[a]
Reference	1.451	0.996	1.00
Direct 40 mg/L PAA	1.094	0.999	0.75
Direct 100 mg/L PAA	0.627	0.996	0.43
Pre-decomposed 40 mg/L PAA	1.342	0.996	0.92
Pre-decomposed 100 mg/L PAA	1.201	0.997	0.83

[a] Normalized to the reference series.

In summary, both direct PAA addition and pre-decomposed PAA addition did not affect significantly the carbon dioxide production rate and extent (i.e., fermentation). On the contrary, any PAA addition affected the initial methane production rate. Direct PAA addition impacted methanogenesis more than pre-decomposed PAA addition and inhibition increased as the PAA concentration increased. Both addition methods did not affect the biodegradability of the D/P mixture. However, 100 mg/L PAA added directly to the culture resulted in lower rate and extent of methane production and higher COD equivalent biomass increased (i.e., higher biomass growth), indicating that methanogens were impacted and the D/P mixture served as carbon source for microbial growth of fermentative bacteria more than in other experimental series.

4.3.2.2 Effect of PAA on Acetoclastic Methanogenesis

The initial and end of incubation characteristics of six series are summarized in Table 4.14. Incubation lasted for 40 days. The pH in all series was maintained above 7. Both TS and VS decreased throughout the incubation period. Ammonia increased in all series after the incubation. The total biogas production, CO_2, and CH_4 production are shown in Figure 4.6. All series had greater CO_2 production than CH_4 during the first 3 days of incubation. Compared to the fermentative/methanogenic culture assay conducted with D/P as the main substrate (section 4.3.2.1), methane production in the acetoclastic methanogenic assay took place sooner, as acetate was the direct substrate without requiring substrate fermentation. Both direct 40 mg/L PAA and direct 100 mg/L PAA series had a delay in CH_4 production.

Table 4.14 Initial and final characteristics in the six series of batch assay testing the effect of PAA on the acetoclastic methanogens.

Series		pH	tCOD mg COD/L	sCOD mg COD/L	TS g/L	VS g/L	NH_3-N mg/L
Seed-blank	Initial	7.44	2340	292	6.37±0.11[a]	1.61±0.08[a]	162
	Final	7.36	1609	232	5.86±0.25	1.39±0.05	210
Reference	Initial	7.35	4970	2828	9.65±0.08	2.99±0.06	168
	Final	7.61	1779	300	8.05±0.06	1.32±0.06	202
Direct 40 mg/L PAA	Initial	7.32	5100	2884	9.75±0.11	3.01±0.13	129
	Final	7.60	1801	330	8.11±0.06	1.42±0.05	202
Direct 100 mg/L PAA	Initial	7.21	5225	2972	9.64±0.04	2.95±0.06	144
	Final	7.56	1905	308	8.32±0.08	1.56±0.06	190
Pre-decomposed 40 mg/L PAA	Initial	7.36	5030	2864	9.57+0.13	2.98±0.08	112
	Final	7.58	1797	252	8.23±0.05	1.53±0.00	199
Pre-decomposed 100 mg/L PAA	Initial	7.28	5000	2842	9.82±0.04	3.16±0.06	162
	Final	7.58	1792	320	8.29±0.09	1.49±0.06	199

[a] Mean ± standard deviation (n = 3)

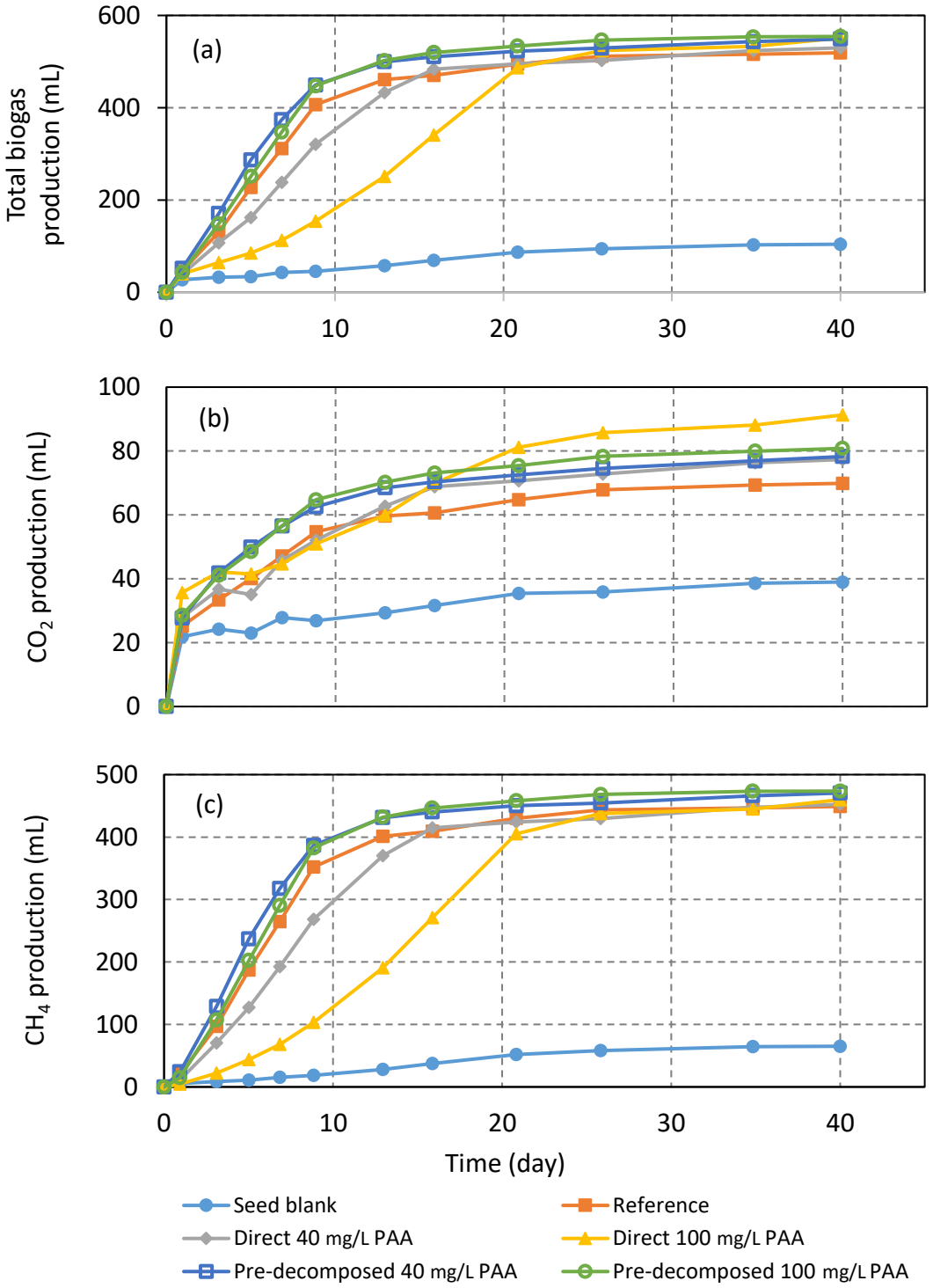


Figure 4.6. PAA effect on the acetoclastic methanogenesis: (a) total biogas, (b) carbon dioxide, and (c) methane production (22ºC, 1 atm).

The seed blank-corrected performance of all series is summarized in Table 4.15. The CO_2 and CH_4 content were 9.3 ± 1.5 % and 90.7 ± 1.5 % (n = 5), respectively. Based on the stoichiometry of acetate fermentation to CO_2 and CH_4 ($CH_3COOH \rightarrow CO_2 + CH_4$), the expected gas content of CO_2 and CH_4 content should be 50 % and 50 %. However, CO_2 has a high solubility in water (Henry's constant of $10^{-1.47}$; Sander, 2014), resulting in lower CO_2 content in the gas phase. The molar ratio of CO_2 in the liquid and gas phase was calculated as follows (parameters are summarized in Table 4.16) (Ramaswami et al., 2005):

$$K_{H,CO_2} = \frac{\{H_2CO_3^*\}}{p_{co_2}} = \frac{n_{co_2,liquid} \times V_{liquid}}{\frac{n_{co_2,gas}}{V_{gas}} \times RT}$$

$$\frac{n_{co_2,liquid}}{n_{co_2,gas}} = K_{H,CO_2} \times RT \times \frac{1}{V_{liquid} \times V_{gas}} = 10^{-1.47} \times 0.082 \times \frac{295}{0.2 \times 0.4} \cong 10.25$$

Only 8.9 % of CO_2 [= 100/(10.25 + 1)] produced is expected in the gas phase, which is consistent with the measurement in this study (9.3%) and the model developed by Sung et al. (2012) (ca. 8%).

Table 4.15. Parameters measured at the end of the assay set up to evaluate the PAA effect on the acetoclastic methanogenesis (after seed blank correction).

Parameter	Reference	Direct		Pre-decomposed	
		40 mg PAA/L	100 mg PAA/L	40 mg PAA/L	100 mg PAA/L
CO_2 (mL)[a]	30.9	38.4	52.3	39.3	41.8
CH_4 (mL)[a]	384.0	387.2	394.7	405.4	408.5
Total biogas (mL)[a]	414.9	425.6	447.0	444.7	450.3
CO_2 (%)	7.45	9.01	11.70	8.84	9.29
CH_4 (%)	92.55	90.99	88.30	91.16	90.71
COD balance (%)	-3.0	0.3	-0.8	-6.7	-8.4
CH_4_COD/ Initial COD (%)	96.6	92.8	90.5	99.7	101.6
Total COD destruction (%)	93.6	93.0	89.7	93.0	93.1
Normalized Total COD destruction (%)[b]	100	99.4	95.9	99.4	99.5

[a] All gas data at 22°C and 1 atm; [b] Normalized to the reference series.

Table 4.16. Parameters for calculation of CO_2 partitioning between gas and liquid phase.

Symbol	Description	Value	Unit
$K_{H,CO2}$	Henry's constant of CO_2	$10^{-1.47}$	M/atm
$\{H_2CO_3^*\}$	Total dissolved carbonate species		M
p_{CO2}	Partial pressure of CO_2		atm
$n_{CO2,\ liquid}$	Mole of CO_2 in liquid phase		mol
$n_{CO2,\ gas}$	Mole of CO_2 in gas phase		mol
V_{liquid}	Volume of liquid phase	0.4	L
V_{gas}	Volume of gas phase	0.2	L
R	Ideal gas constant	0.082	$\frac{L \cdot atm}{K \cdot mol}$
T	Absolute temperature	295	K

All series had COD balance between -8.4 and 0.3 %; negative values might have resulted from overestimation of CH_4 production. All series had total COD destruction between 89.7 and 93.6 %, indicating that ca. 90 % (on COD basis) of acetate transformed to CH_4. As a comparison, in the fermentative/methanogenic culture assay in which D/P was the only substrate, only ca. 75 % (on COD basis) of D/P mixture was transformed to CH_4. The difference could be explained by the microbial yield coefficient on carbohydrates (0.20 g VSS/g COD), proteins (0.056 g VSS/g COD), and acetate (0.038 g VSS/g COD) (Rittmann and McCarty, 2001). The D/P mixture ratio used in the fermentative/methanogenic assay was 2:1 (on COD basis) with a calculated mean microbial yield coefficient of 0.15 g VSS/g D/P mixture, which accounts for the observed difference in total COD destruction and the fraction of substrate COD converted to methane

Figure 4.7, shows the seed blank-corrected methane production (methane COD equivalent) normalized to initial substrate tCOD. For the initial first 10 days the methane production rate decreased in the following order: pre-decomposed 40 mg/L PAA > pre-decomposed 100 mg/L PAA > Reference > direct 40 mg/L PAA > direct 100 mg/L PAA. At the end of the incubation, the methane COD equivalent normalized to initial substrate tCOD of all series ranged from 90.5 to 101.6 %, showing that direct PAA addition impacted the methanogenesis, which recovered over time.

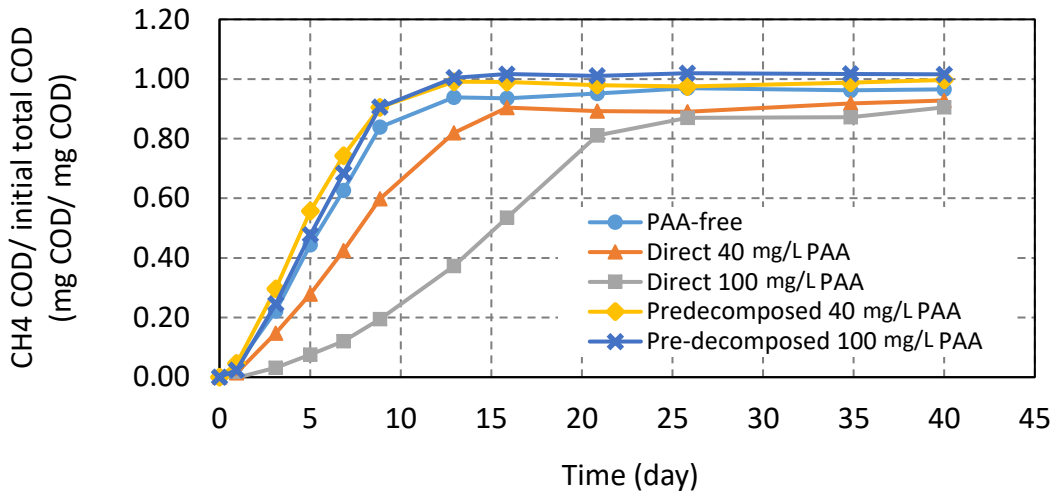


Figure 4.7. Methane COD equivalent production normalized to initial substrate tCOD for the assay set up to evaluate the PAA effect on the acetoclastic methanogenesis (after seed blank correction).

Table 4.17 summarizes the specific methanogenic activity (SMA). SMA decreased in the following order: pre-decomposed 40 mg/L PAA > pre-decomposed 100 mg/L PAA > Reference > direct 40 mg/L PAA > direct 100 mg/L PAA. The reference series had a SMA value of 2.63 mmole CH_4/g VS-day, which is comparable to previously reported SMA values for acetoclastic methanogens (1.46 to 2.33 mmole CH_4/g VS-day; Sørensen et al., 1993). The SMA value in the acetoclastic assay for the reference series (2.63 mmole CH_4/g VS-day) is greater than the SMA value in the fermentative/methanogenic assay in which D/P was used as the substrate (1.45 mmole CH4/g VS-day), as acetate was initially provided at a higher concentration than the acetate produced by D/P fermentation in the mixed fermentative/methanogenic culture. Both pre-decomposed PAA addition series had higher SMA values, which might be attributed to the higher initial acetate concentration resulting from PAA decomposition to acetate and acetate originally in the PAA working solution. Direct 40 and 100 mg/L PAA addition had normalized SMA values of 0.72 and 0.40, respectively (Table 4.17), which are similar to those achieved in the

fermentative/methanogenic assay, indicating direct PAA addition impacted methanogenesis.

Table 4.17. Specific methanogenic activity (SMA) of the mixed fermentative/methanogenic culture as a function of PAA concentration and method of addition[a].

	SMA $\frac{mmole\ CH_4}{g\ VS-day}$	R^2	Normalized SMA[b]
Reference	2.634	0.993	1.00
Direct 40 mg/L PAA	1.902	0.992	0.72
Direct 100 mg/L PAA	1.042	0.979	0.40
Pre-decomposed 40 mg/L PAA	2.993	0.993	1.14
Pre-decomposed 100 mg/L PAA	2.909	0.993	1.10

[a] Acetate was the main substrate; [b] Normalized to the reference series.

In summary, only direct PAA addition impacted acetoclastic methanogenesis. The specific methanogenic activity decreased by 28 % in the direct 40 mg/L PAA series and by 60 % in the direct 100 mg/L PAA series. Although the direct PAA addition affected the specific methanogenic activity, total COD destruction showed little difference (Table 4.15), indicating that direct PAA addition impacted acetoclastic methanogenesis initially, but recovered over time.

4.3.2.3 Effect of PAA on the Hydrogenotrophic Methanogenesis

Figure 4.8 shows the seed blank-corrected methane production over time. All series were not shaken over the first 4 days of incubation. Therefore, the methane production rate over the first 4 days was limited by the mass transfer of H_2 from the gas-phase to the liquid-phase. On day 4, all series were placed on a platform orbital shaker and continuously mixed, which enhanced the H_2 mass transfer and methane production rate. The low methane production in the direct 100 mg/L PAA series shows that hydrogenotrophic methanogenesis was severely impacted.

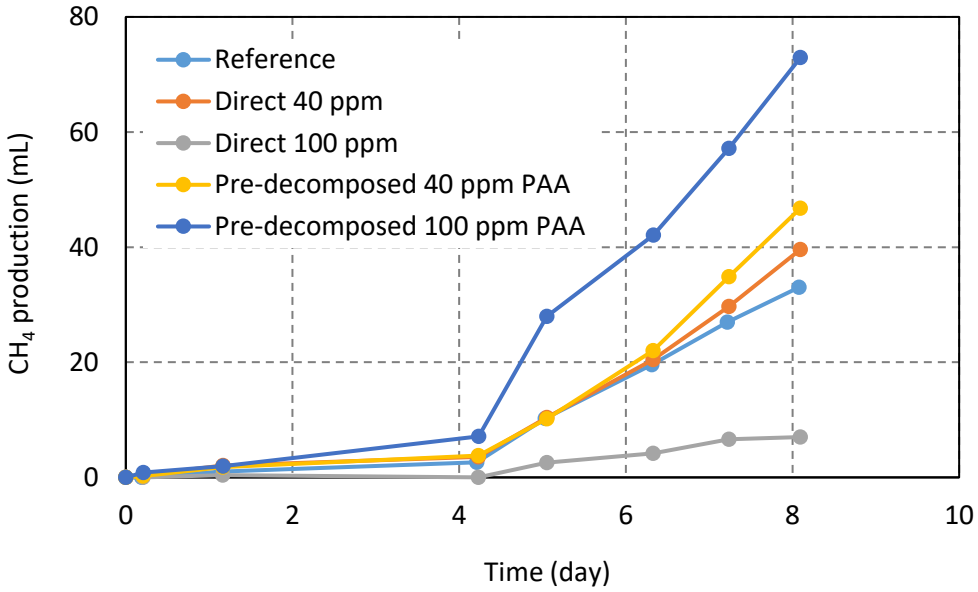


Figure 4.8. PAA effect on the hydrogenotrophic methanogenesis. All series pressurized initially and then re-pressurized on day 4.2, 5.1, 6.3, and 7.2.

The performance (seed blank-corrected) of the five series at the end of the 8-days incubation is summarized in Table 4.18. The difference in methane production among all series is attributed to the fact that each series had a different H_2 consumption rate although all series were re-pressurized at the same time to the same pressure (21.8 psig) without gas release. While re-pressurizing, series with a higher residual gas pressure received less H_2/CO_2 mixture over the entire incubation period. At the end of the incubation, the calculated total volume of H_2/CO_2 mixture added decreased in the following order: pre-decomposed 100 mg/L PAA > pre-decomposed 40 mg/L PAA > direct 40 mg/L PAA > Reference > direct 100 mg/L PAA.

Because each series received a different volume of H_2/CO_2 mixture, the methane production was also different. It is more indicative to compare each series by normalizing the cumulative CH_4 production to cumulative H_2 consumption. Based on stoichiometry, the volume ratio of methane produced to H_2 added should be 0.25 ($4\,H_2 + CO_2 \rightarrow CH_4 + 2H_2O$). A ratio greater than 0.25 suggests that additional substrates contributed to methane formation (e.g., acetate in PAA working solution, acetate formed as a result of PAA decomposition, fermentation/methanogenesis of cell lysis products). On the other hand, a ratio lower than 0.25 means that hydrogenotrophic methanogenesis was impacted. The CH_4/H_2 ratio in the direct 100 mg/L PAA addition series was 0.149, which is much lower than 0.25, showing that hydrogenotrophic methanogenesis was impacted. The remaining series had CH_4/H_2 ratio values between 0.333 and 0.445, meaning there were other substrate sources which contributed to methane formation. One of the sources of methane formation was acetate produced from the decomposition of PAA as well as acetate in the PAA working solution. By subtracting the theoretical methane produced related to the PAA

working solution and normalizing to cumulative H_2 consumption, all series, except the direct 100 mg/L PAA addition, had a CH_4/H_2 ratio between 0.333 and 0.396. On the other hand, the direct 100 mg/L PAA series had a negative value because it had lower cumulative CH_4 production than the projected methane related to the PAA working solution. The final acetate concentration of the direct 100 mg/L PAA series was 200 mg COD/L, which is comparable to the initial acetate concentration of 219 mg COD/L (Table 4.9), indicating that both acetoclastic and hydrogenotrophic methanogenesis was impacted.

Compared to the acetoclastic methanogens, hydrogenotrophic methanogens were not affected by direct 40 mg/L PAA addition, indicating they are more resistant to direct PAA addition than the acetoclastic methanogens. Previous research showed that *Methanosarcina thermautotrophicus*, which is a model hydrogenotrophic methanogen, had upregulation of an operon-like gene cluster (encoded redox enzymes, such as, rubredoxins, rubrerythrins, thioredoxins, and thioredoxin reductase) under H_2O_2 oxidative treatment (Kato et al., 2008).

In summary, only direct 100 ppm PAA addition impacted hydrogenotrophic methanogenesis. Direct 40 ppm PAA, as well as 40 and 100 ppm pre-decomposed PAA had a similar CH_4/H_2 ratio between 0.346 and 0.396 compared to the theoretical CH_4/H_2 ratio of 0.25 by hydrogenotrophic methanogenesis alone. Higher CH_4/H_2 ratio values indicate that substrates other than H_2 and CO_2 contributed to methane production.

Table 4.18. End of incubation results of the batch assay evaluating the PAA effect on the hydrogenotrophic methanogenesis.

Parameter	Reference	Direct		Pre-decomposed	
		40 mg PAA/L	100 mg PAA/L	40 mg PAA/L	100 mg PAA/L
Final pH	7.09	7.25	6.91	7.14	7.19
H_2 consumed (mL)	98.8	102.6	47.2	124.3	164.0
CH_4 formed (mL)	36.8	39.0	7.01	46.3	72.9
CH_4 produced from PAA solution (mL)	0	3.11	8.29	3.29	8.00
Initial tCOD (mg COD/L)	1979	2076	2222	2136	2268
Final tCOD (mg COD/L)	1893	1940	2144	2011	1999
Final sCOD (mg COD/L)	108	122	208	118	84
Final VFAs (mg COD/L)	ND	ND	265.2	ND	ND
H_2 COD consumed (mg COD/L)	653	678	312	822	1084
CH_4 COD formed (mg COD/L)	921	1060	291	1343	1536
COD balance (%)	-6.9	-8.9	3.9	-13.4	-5.5
$\frac{CH_4\ formed}{H_2\ consumed}$ [a]	0.333	0.380	0.149	0.373	0.445
$\frac{CH_4\ formed - PAA\_CH_4}{H_2\ consumed}$	0.333	0.350	NA	0.346	0.396

[a] After seed blank-correction; ND, not detected; NA, non-applicable.

4.4 Summary

The anaerobic culture medium had a great capacity to decompose PAA and mitigate the potential adverse effect on the mixed fermentative/methanogenic culture, acetoclastic methanogens, and hydrogenotrophic methanogens. Therefore, pre-decomposed PAA addition showed little difference with PAA-free reference in terms of specific methanogenic activity, total COD destruction, ultimate D/P biodegradability, and CH_4/H_2 ratio.

Direct PAA addition did not affect the initial CO_2 production rate and extent in all batch assays, indicating fermentation was not impacted. In the case of direct 40 mg/L PAA addition, the specific methanogenic activity decreased by 25 % and 28% in the assay which used D/P as the substrate and acetoclastic methanogenic assay, respectively. Hydrogenotrophic methanogenesis, on the contrary, was not impacted by direct 40 mg/L PAA addition and had similar CH_4/H_2 ratio with the PAA-free reference series. In the case of direct 100 mg/L addition, the specific methanogenic activity decreased by 57 % and 60 % in the fermentative/methanogenic assay with D/P substrate and the acetoclastic methanogenic assay, respectively. Hydrogenotrophic methanogens were impacted by direct 100 mg/L PAA addition and had a very low CH_4 production.

Although the kinetics of methanogenesis were impacted by direct PAA addition, total COD destruction and D/P biodegradability were similar to those achieved by the PAA-free reference series, demonstrating that methanogenesis recovered over time. Direct 40 mg/L PAA addition impacted acetoclastic methanogens but not hydrogenotrophic methanogens, indicating acetoclastic methanogens are more vulnerable to direct PAA addition.

CHAPTER 5

EFFECT OF PERACETIC ACID ON THE PERFORMANCE OF ANAEROBIC REACTORS FED WITH POULTRY PROCESSING WASTEWATER

5.1 Introduction

Poultry processing wastewater consists of processing equipment disinfection wastewater (e.g., electronic stunning, slaughter, scalding, and removal of internal organs) as well as chicken chiller's water, which contain antimicrobial agents to inhibit microbial growth (Kiepper et al., 2003). Peracetic acid (PAA) is increasingly used in poultry processing plants as an antimicrobial agent due to its high redox potential and less possibility to form disinfection by-products (DPBs) (Domínguez Henao et al., 2018b). However, residual PAA in poultry processing wastewater may be carried to wastewater treatment systems, including anaerobic systems such as lagoons, with potential adverse effects.

The optimum temperature ranges for anaerobic wastewater treatment are 35 to 40°C (mesophilic) and 55 to 65°C (thermophilic), respectively (Rittmann and McCarty, 2001). However, in poultry processing plants, anaerobic lagoons are not heated to maintain their temperature at optimal temperature levels. Therefore, this study evaluated the effect of PAA on a fermentative/methanogenic culture at room temperature (22±2ºC).

The objective of this phase of the study was to investigate the effect of PAA on the anaerobic treatment of poultry processing wastewater and compare the effect of pre-

decomposed and direct PAA addition to semi-continuously-fed, laboratory-scale anaerobic reactors, conditions reflecting normal operation and accidental spills, respectively, in poultry processing plants.

5.2 Ultimate Biodegradability of Poultry Processing Wastewater

5.2.1 Materials and Methods

The objective of this assay was to determine the ultimate anaerobic biodegradability of the dissolved air floatation (DAF) effluent obtained from the poultry processing plant, dextrin/peptone (D/P) mixture, and combination of DAF effluent and D/P mixture with a ratio of 44:3 (v:v; COD ratio was 1:9). Given the relatively low COD content of the DAF effluent (740 to 785 mg COD/L), D/P was chosen to increase the anaerobic reactors feed COD concentration, leading to biogas production higher than the reactor liquid waste needed to be removed and replaced by the feed to maintain a retention time of 21 days (see section 5.3.1). The ratio of DAF effluent and D/P mixture was chosen to arrive at a mean organic loading rate of 480 mg COD/L-day of the anaerobic reactors described in section 5.3.1.

The biodegradability batch assay included four culture series: seed blank, DAF effluent, D/P mixture, and DAF effluent + D/P mixture. Each series was prepared and monitored in duplicate 160 mL serum bottles. The composition of the four series, which contained DAF effluent, D/P stock solution (24 g/L dextrin, 12 g/L peptone), concentrated medium with 2-fold dilution (Table 5.1), and deionized water is summarized in Table 5.2. The substrate COD concentration is shown in Table 5.3. After the addition of all components, the serum bottles were then sealed with rubber stoppers and aluminum caps,

and flushed with helium gas for 15 minutes at 10 psig. Last, needles (Precision Glide™ Needle; 1.6mm × 40mm, Becton, Dickinson and Company, Franklin Lakes, NJ) were inserted to the rubber stoppers of all series to vent out excess gas, followed by the addition of 70 mL of seed obtained from the anaerobic reactor R1 (see Section 5.3.1). During the incubation, total gas production and gas composition were measured at appropriate time intervals. At the end of the incubation, the bottles were opened and the pH, tCOD, sCOD, TS, VS and VFAs were measured.

Table 5.1. Composition of the concentrated medium.

Component	**Concentration**
$CaCl_2 \cdot 2H_2O$	1.0 g/L
$MgCl_2 \cdot 6H_2O$	2.0 g/L
$FeCl_2 \cdot 4H_2O$	1.0 g/L
$NaHCO_3$	35 g/L
Vitamin Stock[a]	10 mL/L
Trace Metal Stock[b]	10 mL/L

[a] Vitamin stock solution composition described in Table 4.2
[b] Trace metal stock solution composition described in Table 4.3

Table 5.2. Component volume (mL) in the batch assay set up to evaluate the ultimate biodegradability of poultry processing DAF effluent and dextrin/peptone (D/P).

Component	Seed blank	DAF Effluent	D/P	DAF+ D/P
Anaerobic culture seed	70	70	70	70
DAF effluent	0	22	0	22
24 g dextrin/L, 12 g peptone/L	0	0	6	6
Concentrated medium (2-fold dilution)	1	1	1	1
DI water	29	7	23	1
Total volume	100	100	100	100

Table 5.3. Component of substrate COD concentration (mg COD/L) in the batch assay set up to evaluate the ultimate biodegradability of poultry processing DAF effluent and dextrin/peptone (D/P).

Component	Seed blank	DAF Effluent	D/P	DAF+ D/P
DAF effluent	0	163	0	163
24 g dextrin/L, 12 g peptone/L	0	0	2347	2347
Total COD	100	163	2347	2510

5.2.2 Results and Discussion

Incubation for the batch ultimate biodegradability assay lasted for 30 days. The initial and final characteristics of the four series are summarized in Table 5.4. Figure 5.1 shows the initial substrate (DAF effluent and D/P mixture) COD-normalized methane production. The CH_4 production rate decreased significantly after about 10 days of incubation. Based on the result of the DAF effluent and D/P mixture series, the calculated methane production of the DAF effluent and D/P combined series is shown in Figure 5.1 (dash line), which highly agrees with the measured methane production in the same series. All series had an initial biomass of 1.15 g VS/L. The total degradable COD was calculated as the sum of COD converted to CH_4 and the increase in biomass COD, which was regarded as the net VS increase in each series. The net VS increase was converted to COD equivalents by using a COD:VS ratio value of 1.42 g COD/g VS (Rittmann and McCarty, 2001) to represent the COD used for biomass growth. Taking into account the substrate COD converted to biomass, the biodegradability of the DAF effluent, D/P mixture and the combined DAF effluent and D/P mixture was 90.5, 93.2, and 86.9 %, respectively. Figure 5.1 shows the initial substrate (DAF effluent and D/P mixture) COD-normalized methane

production. The CH_4 production rate decreased significantly after about 10 days of incubation.

Table 5.4. Results of the batch ultimate biodegradability assay.

Parameter	Seed blank	DAF Effluent	D/P	DAF Effluent + D/P
Final pH	7.36	7.34	6.87	6.90
Initial tCOD (mg/L)	1866	2021	4259	4444
Final tCOD (mg/L)	1483	1580	2215	2337
Initial sCOD (mg/L)	214	318	1932	2163
Final sCOD (mg/L)	574	657	795	867
Final VFAs (mg/L)	ND	ND	ND	ND
Final TS (g/L)	3.63±0.07[a]	3.73±0.04	4.15±0.09	4.21±0.05
Final VS (g/L)	1.18±0.04[a]	1.21±0.05	1.56±0.04	1.47±0.05
CH_4 production (mL)[b]	14.8	18.6	77.9	82.3
COD balance (%)		-7.07	-1.13	-1.90
CH_4_COD/Initial COD (%)[c]		63.6	70.3	68.9
Total COD destruction (%)[c]		56.5	69.2	67.0
D/P biodegradability [c]		90.5	93.2	86.9

[a] Mean ± standard deviation (n = 3); [b] Gas data at 22°C and 1 atm
[c] After seed blank correction; ND, not detected

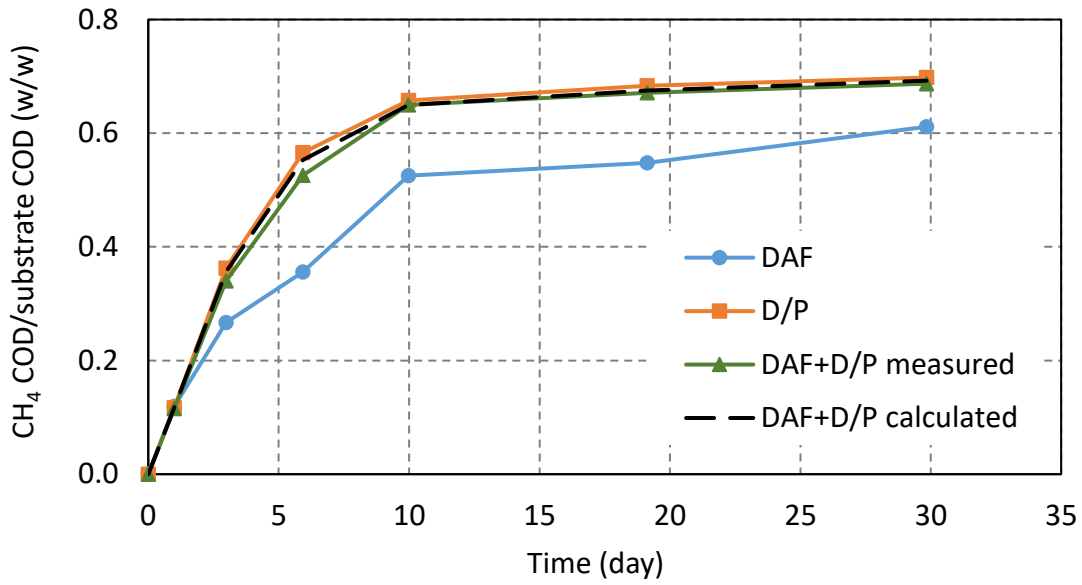


Figure 5.1. Cumulative CH_4 production in the biodegradability test normalized to initial substrate COD.

5.3 Effect of Peracetic Acid on Anaerobic Treatment of Poultry Processing Wastewater

5.3.1 Materials and Methods

The seed used in this assay was derived from the stock anaerobic digester described in section 4.2. To exclude the effect of the anaerobic culture medium and to reflect real poultry processing wastewater conditions, 4 L of the anaerobic digester mixed liquor were centrifuged at 10,000 rpm for 20 minutes, the supernatant was discarded and the pellet was re-suspended in 4 L of dissolved air floatation (DAF) effluent obtained from the poultry processing plant. The mixed liquor was transferred to four 2.5 L glass reactors, each with a total liquid volume of 1 L. The reactors' headspaces were connected to graduated cylinders containing an acid brine solution (10% NaCl w/v and 2% H_2SO_4 v/v) for gas

collection and measurement by liquid displacement. The reactors' contents were continuously mixed using a magnetic bar and stir plate. The reactors were maintained at room temperature (22±2^{o}C) with a hydraulic/solids retention time (HRT/SRT) of 21 days. The reactors were batch-fed every 3 and 4 days with 3 mL of dextrin/peptone (D/P) mixture (stock of 96 g dextrin/L, 48 g peptone/L), 44 mL of DAF effluent, and 1 mL of concentrated medium (Table 5.1) per day. The mean organic loading rate was 480 mg COD/L-day.

To investigate the effect of PAA on the anaerobic treatment of poultry processing wastewater, four reactors were set up, as follows: R1, PAA-free control; R2, fed with pre-decomposed PAA (40, 80, and 100 mg/L PAA); R3, fed with 5 mg/L PAA directly added to the reactor; and R4, fed with 40 mg/L PAA directly added to the reactor. For the pre-decomposed PAA addition, PAA working solution was added to the DAF effluent, holding it for 15 minutes and then fed to the reactor. On the other hand, for direct PAA addition, the DAF effluent was first added to the reactor and then the PAA working solution was added directly to the reactor. The PAA concentration mentioned in this section is the PAA concentration in the feed DAF effluent (pre-decomposed PAA) or the PAA concentration corresponding to the DAF effluent feed (direct PAA addition). Throughout the operation, gas production, pH, and VFAs were measured daily. Gas composition and sCOD were measured every three and four days.

Table 5.5 summarizes the feeding conditions and duration of each reactor. This portion of the study lasted for 63 days. R2 was fed in three different phases with pre-decomposed 40 mg/L, 80 mg/L, and 100 mg/L PAA, each phase lasting for 14, 28, and 14 days, respectively. R3 was fed in three different phases with direct 5, 20, and 40 mg/L PAA, each phase lasting for 14 days. R4 was fed with 40 mg/L PAA directly for 14 days;

however, because of 60 % inhibition in terms of total gas production compared to R1 (see Section 5.3.2.2), R4 was not fed for 35 days until its sCOD concentration decreased to approximately the same level as that in R1. After R4 had recovered, it was then fed again with 40 mg/L PAA directly and incubated for another 14 days.

Table 5.5. Feeding conditions and duration of four reactors used to assess the effect of PAA on the anaerobic treatment of poultry processing wastewater.

Parameter		R1	R2	R3	R4
Feed (mL/d)					
DAF effluent		44	42.9	42.9	42.9
PAA working solution		0	1.1	1.1	1.1
D/P mixture		3	3	3	3
Concentrated medium		1	1	1	1
Phase 1	Condition	PAA-free	Pre-decomposed 40 mg/L PAA	Direct 5 mg/L PAA	Direct 40 mg/L PAA
	Duration (d)	63	14	14	14
Phase 2	Condition		Pre-decomposed 80 mg/L PAA	Direct 20 mg/L PAA	Recovery
	Duration (d)		28	14	35
Phase 3	Condition		Pre-decomposed 100 mg/L PAA	Direct 40 mg/L PAA	Direct 40 mg/L PAA
	Duration (d)		14	14	14

5.3.2 Results and Discussion

Table 5.6 summarizes the performance of the four anaerobic reactors before PAA addition. All four reactors showed stable and similar performance in terms of gas production and gas composition.

Table 5.6. Performance of the four anaerobic reactors before PAA addition.

	R1	R2	R3	R4
pH	6.86	7.01	6.89	6.89
sCOD (mg COD/L)	544	354	534	388
Normalized gas production (%)[a]	100	101	103	103
CO_2 (%)	33.9	35.0	33.1	36.3
CH_4 (%)	66.1	65.0	66.9	63.7

[a] Normalized to R1 gas production

5.3.2.1 R1 Control

The mean R1 total gas production rate was 170 ± 19 mL/d (n = 19) (Figure 5.2 a), the pH was between 6.72 to 7.05, and the normalized CH_4 and CO_2 content of the biogas was 64.1 ± 1.4 % and 35.9 ± 1.4 % (n = 19), respectively (Table 5.6). The soluble COD before each feeding cycle was initially ca. 800 mg COD/L and gradually decreased to 400 mg COD/L (Figure 5.2 b), indicating the DAF effluent-re-suspended anaerobic culture gradually adjusted to the new feeding conditions. Figure 5.3 shows the VFAs evolution over 7 days; all VFAs decreased over time, indicating the acidogens and methanogens in R1 were functioning effectively.

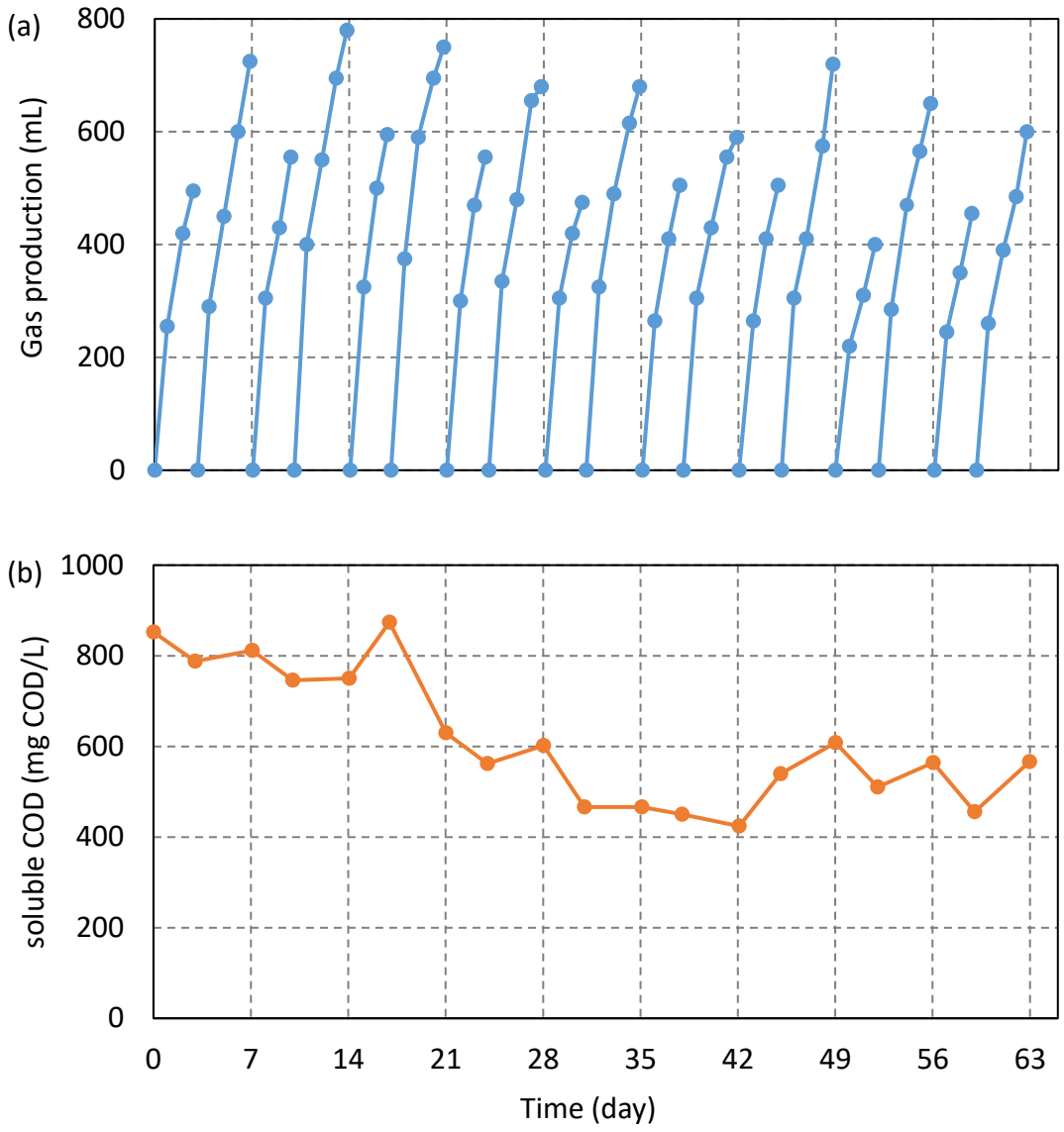


Figure 5.2. (a) Gas production (22°C, 1 atm) and (b) soluble COD concentration of the control reactor (R1) over 63 days of operation.

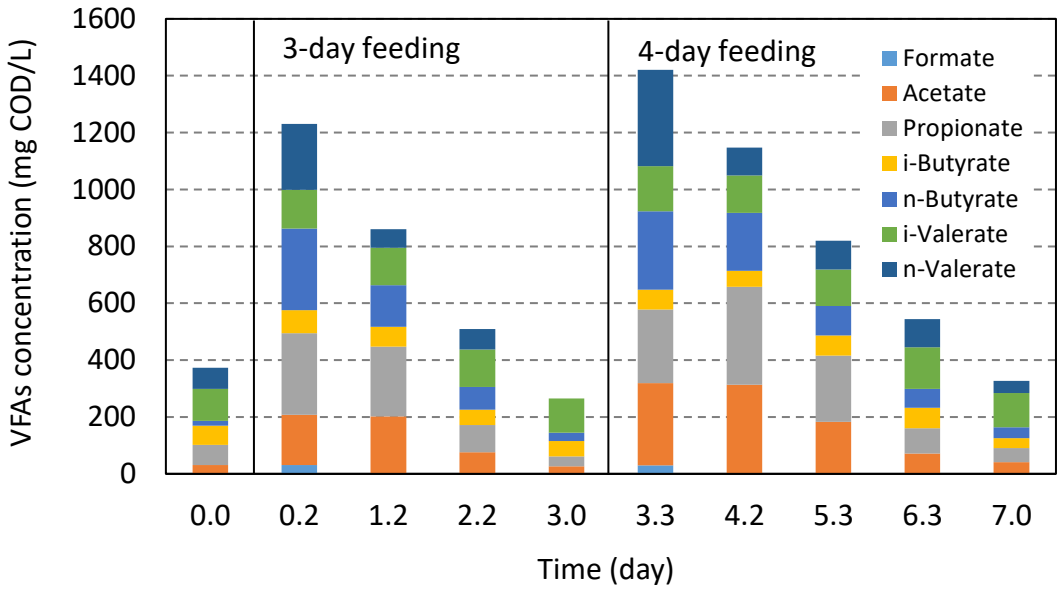


Figure 5.3. VFAs evolution over 3-day and 4-day feeding of the control reactor (R1) for the first 7 days of operation.

5.3.2.2 Pre-decomposed PAA addition

Table 5.7 summarizes the performance of R2 fed with DAF effluent, D/P and pre-decomposed PAA. During the first feeding phase (pre-decomposed 40 mg/L PAA), the R2 gas production was similar to that of R1 (Figure 5.4 b) and had no accumulation of sCOD and VFAs (Figure 5.4 a). From day 14 to 42, R2 was fed with pre-decomposed 80 mg/L PAA; during this period, R2 continued to have gas production similar to that of R1 (Figure 5.5 b). However, from day 39 to 42, accumulation of sCOD and VFAs, especially propionate, was noticed in R2 (Figure 5.5 a). Throughout the last feeding phase (Phase 3, pre-decomposed 100 mg/L PAA, from day 42 to 56), the biogas production in R2 was 78 % to 89 % of the R1's gas production (Figure 5.6 b). The biogas CH_4 content decreased as CO_2 increased, indicating methanogenesis was impacted. The sCOD before each feeding and propionate increased (Figure 5.6 a).

Table 5.7. Performance of anaerobic reactor R1 (PAA-free control) and R2 (pre-decomposed PAA addition) over time.

	R1 (control)	R2 (pre-decomposed PAA addition)		
PAA concentration (mg/L)	0	40	80	100
Phase duration (d)	63	14	28	14
Cumulative operational time (d)		1-14	14-42	42-56
pH	6.72-7.05	6.74-7.01	6.76-6.98	6.70-6.89
sCOD (mg COD/L)	424-874	354-812	770-870	1154-1748
Gas production rate (22^oC, 1 atm; mL/d)	170 ± 19 [a]	194 ± 10 [b]	162 ± 9 [c]	126 ± 11 [b]
Normalized gas production rate (%)[d]	100	96-108	91-100	78-89
CO_2 (%)	35.94 ± 1.43 [a]	36.35 ± 1.42 [b]	36.73 ± 1.02 [c]	40.66 ± 1.43 [b]
CH_4 (%)	64.06 ± 1.43 [a]	63.65 ± 1.42 [b]	63.27 ± 1.02 [c]	59.34 ± 0.74 [b]

[a] Mean ± standard deviation (n = 19)
[b] Mean ± standard deviation (n = 4)
[c] Mean ± standard deviation (n = 8)
[d] Normalized to R1 gas production

In conclusion, the biogas production in the DAF effluent-fed anaerobic digester R2 was not affected up to 80 mg/L pre-decomposed PAA addition. However, addition of pre-decomposed 100 mg/L PAA impacted the propionate-utilizers, leading to lower biogas production by 78% to 89%, compared to R1 performance.

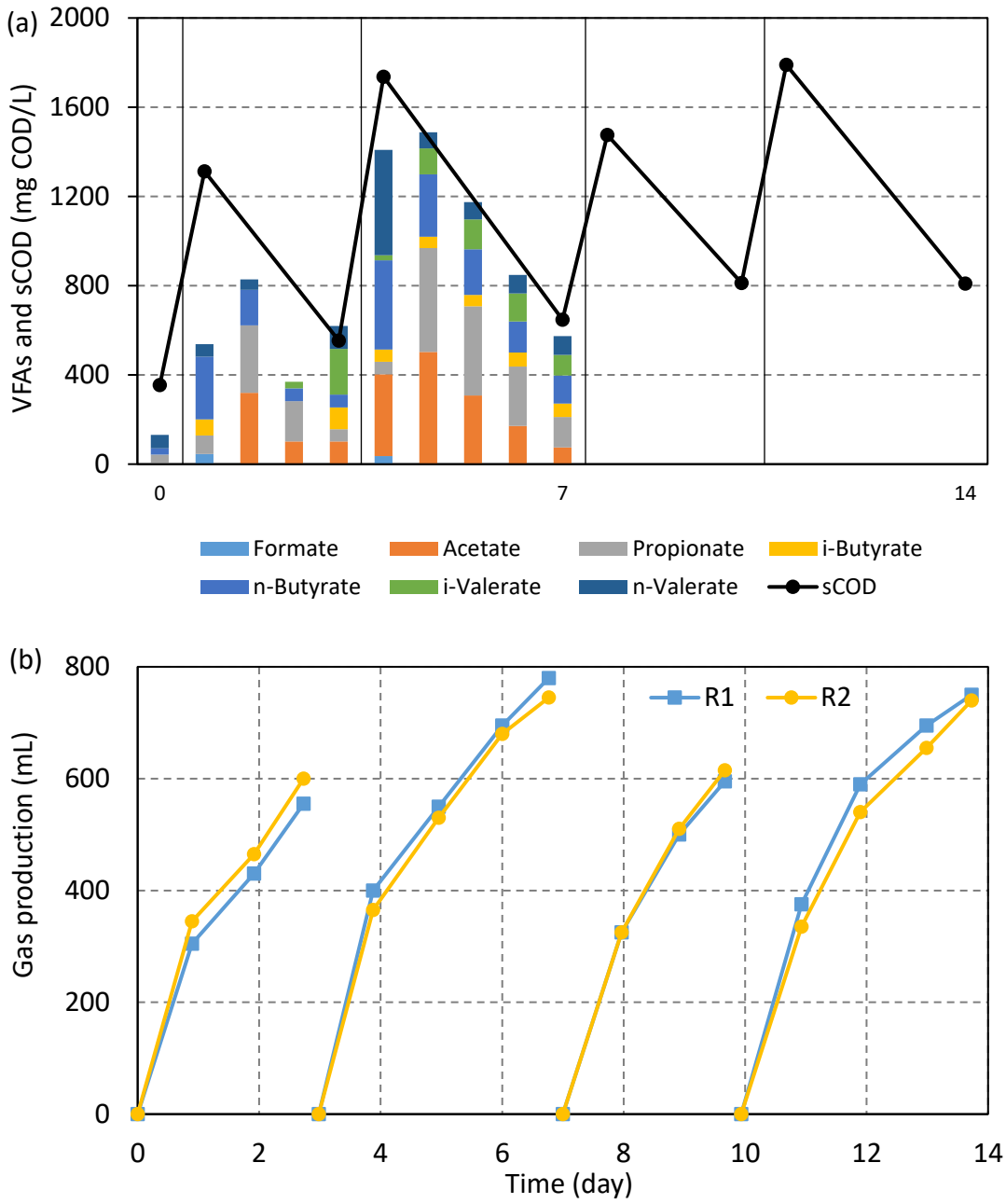


Figure 5.4. (a) VFAs and soluble COD concentration (vertical black lines represent feeding times) and (b) gas production (22°C, 1 atm) of R2 during Phase 1 (day 1 to day 14; feeding every 3 and 4 days; 40 mg/L PAA for R2).

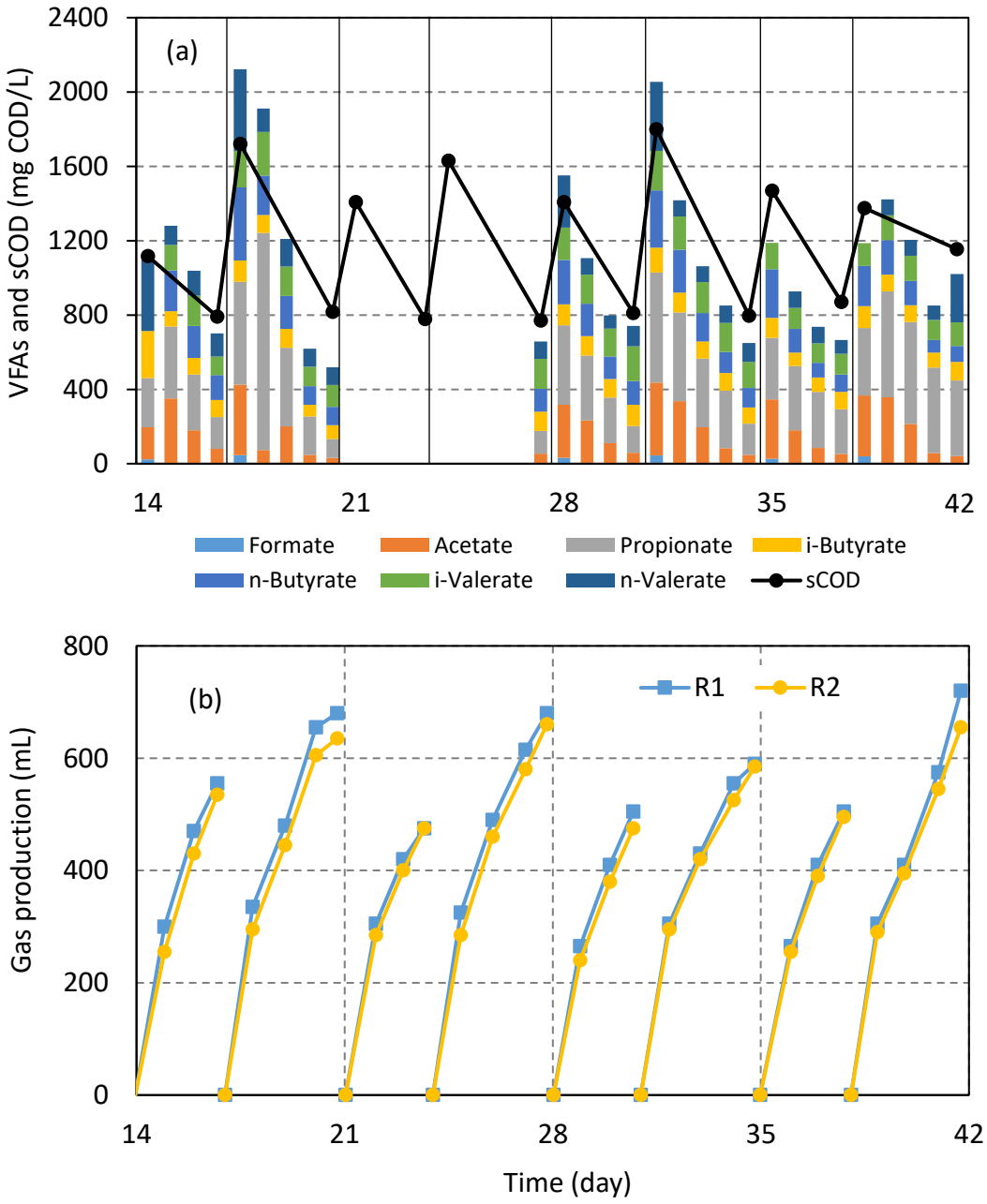


Figure 5.5. (a) VFAs and soluble COD concentration (vertical black lines represent feeding times) and (b) gas production (22°C, 1 atm) of R2 during Phase 2 (day 14 to day 42; feeding every 3 and 4 days; 80 mg/L PAA for R2).

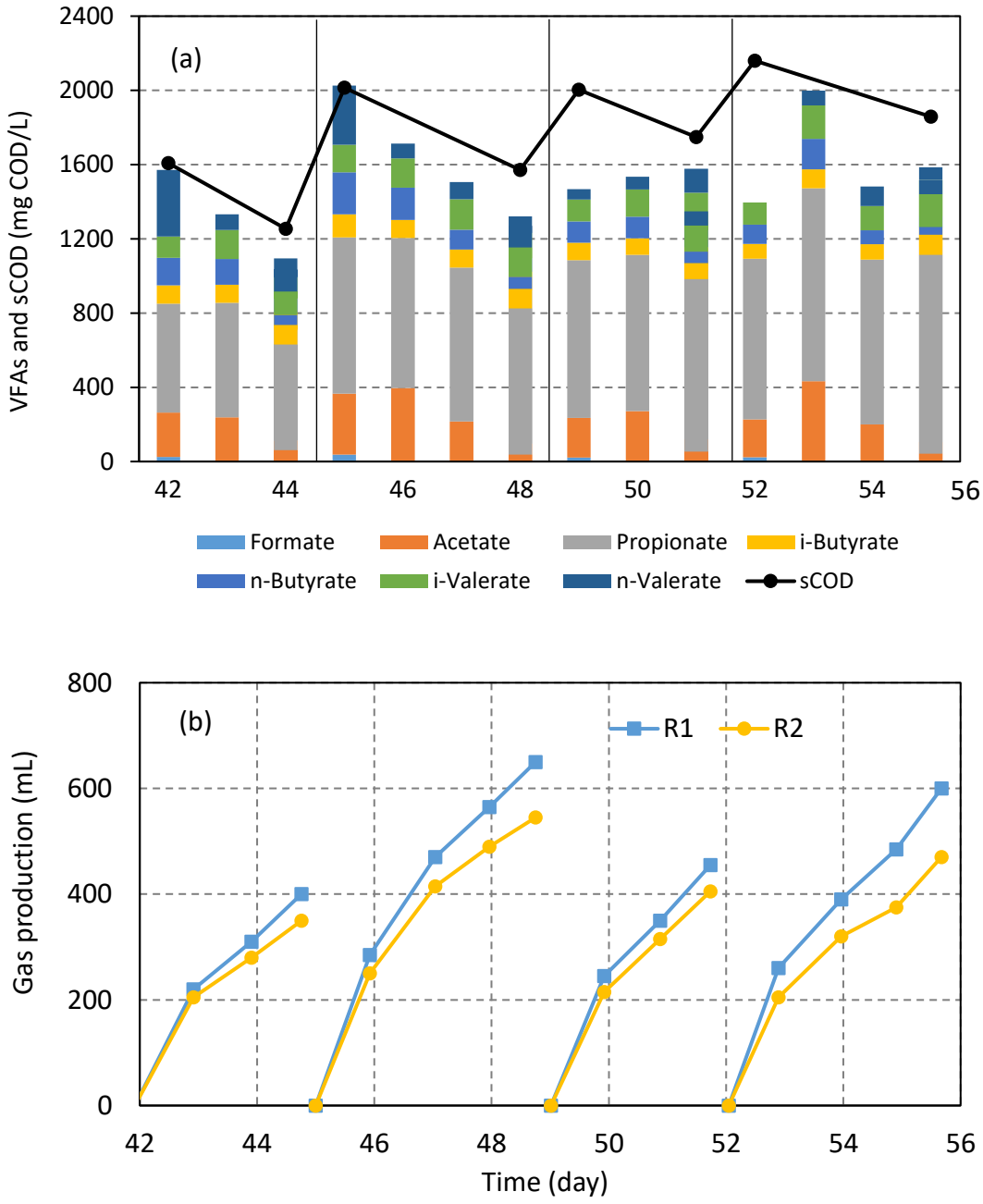


Figure 5.6. (a) VFAs and soluble COD concentration (vertical black lines represent feeding times) and (b) gas production (22°C, 1 atm) of R2 during Phase 3 (day 42 to day 56; feeding every 3 and 4 days; 100 mg/L PAA for R2).

5.3.2.3 Direct PAA addition

Table 5.8 summarizes the performance of R3 and R4 anaerobic reactors, fed directly with PAA as follows: R3 started with 5 mg/L PAA and then increased to 20 and 40 mg/L PAA. R4 started with 40 mg/L PAA.

Table 5.8. Performance of R3 and R4 anaerobic reactors fed directly with PAA.

Parameter	R3 (incremental direct PAA addition)			R4 (direct PAA addition)		
PAA concentration (mg/L)	5	20	40	40	0 (Recovery)	40
Duration (d)	14	14	14	14	35	14
Cumulative operational time (d)	1-14	14-28	28-42	1-14	14-49	49-63
pH	6.70-6.92	6.73-6.98	6.69-6.87	6.57-6.89	6.67-7.18	6.85-7.10
sCOD (mg COD/L)	388-528	442-482	628-892	896-2404	504-3100	554-1520
Gas production rate (22^oC, 1 atm; mL/d)	168 ± 9 [a]	157 ± 23 [a]	147 ± 9 [a]	107 ± 24 [a]	NA	96 ± 12 [a]
Normalized gas production (%) [c]	93-104	78-103	95-101	37-78	NA	59-67
CO_2 (%)	36.75 ± 0.75 [a]	37.75 ± 1.13 [a]	38.88 ± 3.64 [a]	39.84 ± 3.64 [a]	36.20 ± 7.99 [b]	32.71 ± 5.61 [a]
CH_4 (%)	63.25 ± 0.75 [a]	62.25 ± 1.13 [a]	61.12 ± 3.64 [a]	60.16 ± 3.64 [a]	63.80 ± 7.99 [b]	67.29 ± 5.61 [a]

[a] Mean ± standard deviation (n = 4)
[b] Mean ± standard deviation (n = 10)
[c] Normalized to R1 gas production
NA, not applicable

Figure 5.7 shows the gas production, sCOD, and VFAs in R3 over three different feeding phases. In Phase 1 (day 1 to day 14), during which 5 mg/L PAA was added directly, R3 showed little difference compared to R1. In Phase 2 (day 14 to day 28) the PAA concentration was increased to 20 mg/L PAA, directly added to the reactor. Except during day 14 to day17, the gas production in R3 was 78 % of that in R1; from day 18 to 28 R3 had similar gas production to that of R1. During Phase 3 (day 28 to day42; direct 40 mg/L PAA addition), R3 still had similar gas production to that of R1. Throughout the three phases, before each feeding, the VFAs concentration was below 500 mg COD/L, demonstrating that acidogens and methanogens in R3 were not impacted.

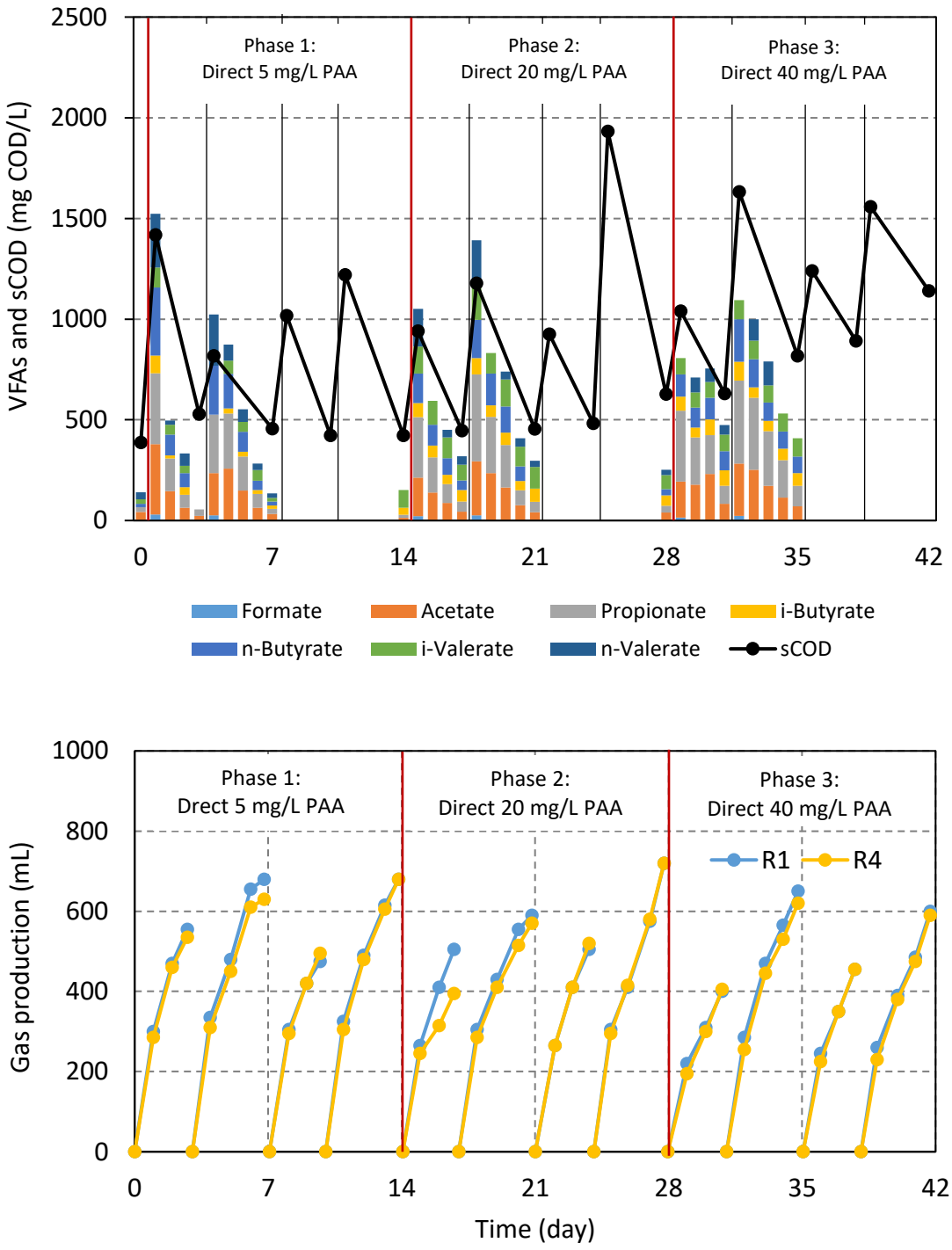


Figure 5.7. (a) VFAs and soluble COD concentration (vertical red lines represent switching to next phase; vertical black lines represent feeding) and (b) gas production (22^{o}C, 1 atm) of R3 (vertical red lines represent switching to next phase).

In Phase 1 (day 1 to day 14), compared to R1, R4 had a normalized gas production of 78%, which gradually decreased to 38% (Figure 5.8 a). The normalized CH_4 content decreased, as the CO_2 content increased (Figure 5.9), indicating that direct 40 mg/L PAA addition impacted methanogenesis, but not fermentation. Between day 14 and 49 (Phase 2), R4 underwent a recovery period for 35 days, during which the sCOD concentration gradually decreased to the R1 level.

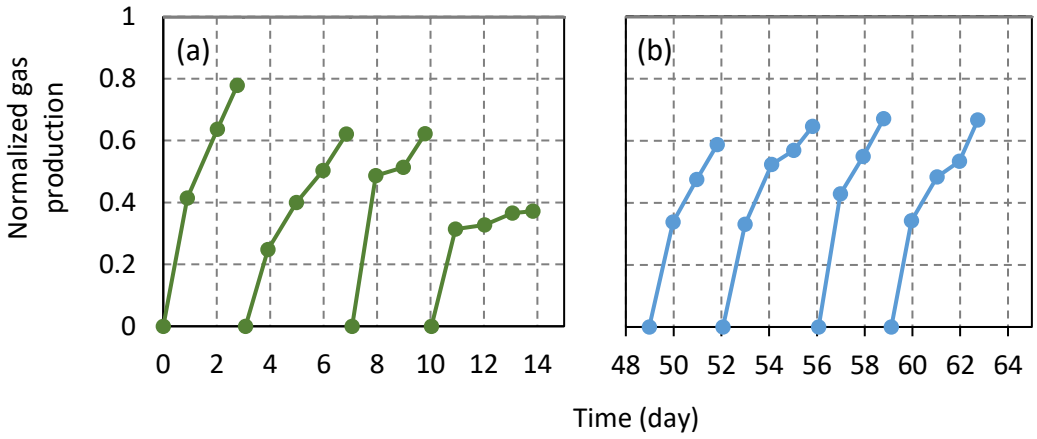


Figure 5.8. R4 gas production normalized to that of R1 for (a) first direct 40 mg/L PAA addition from day 1 to day14 and (b) second direct 40 mg/L PAA addition from day 49 to day 63 (Note: R4 was not fed between day 14 and 49; recovery period).

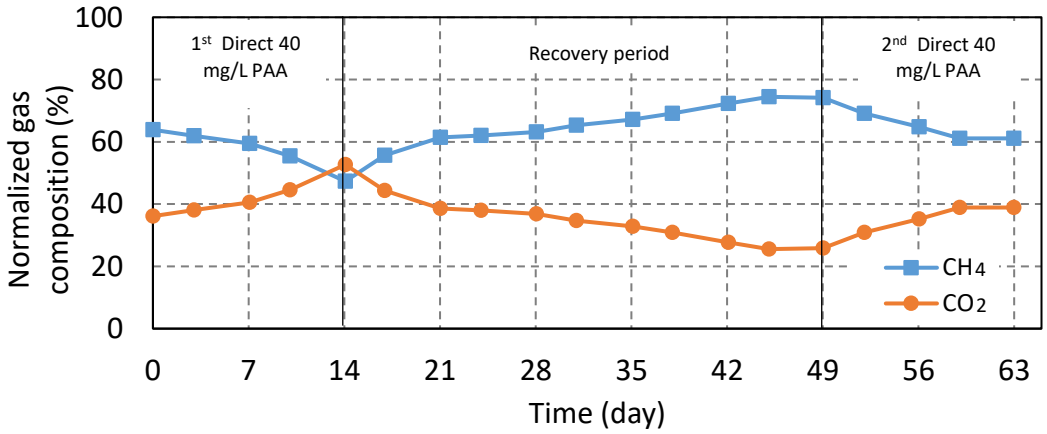


Figure 5.9. R4 gas composition over the three feeding phases (Phase 1, first direct 40 mg/L PAA addition; Phase 2, recovery period; and Phase 3, second direct 40 mg/L PAA addition).

Figure 5.10 shows the sCOD and VFAs evolution in R4 during Phase 1, in which both sCOD and VFAs gradually increased, showing that both acidogens and methanogens were impacted. During the first 3 days, n-valerate accumulated. However, n-valerate decreased from day 4 to day 14. On the contrary, acetate, propionate, n-butyrate, and i-valerate, accumulated from day 4 to day 14.

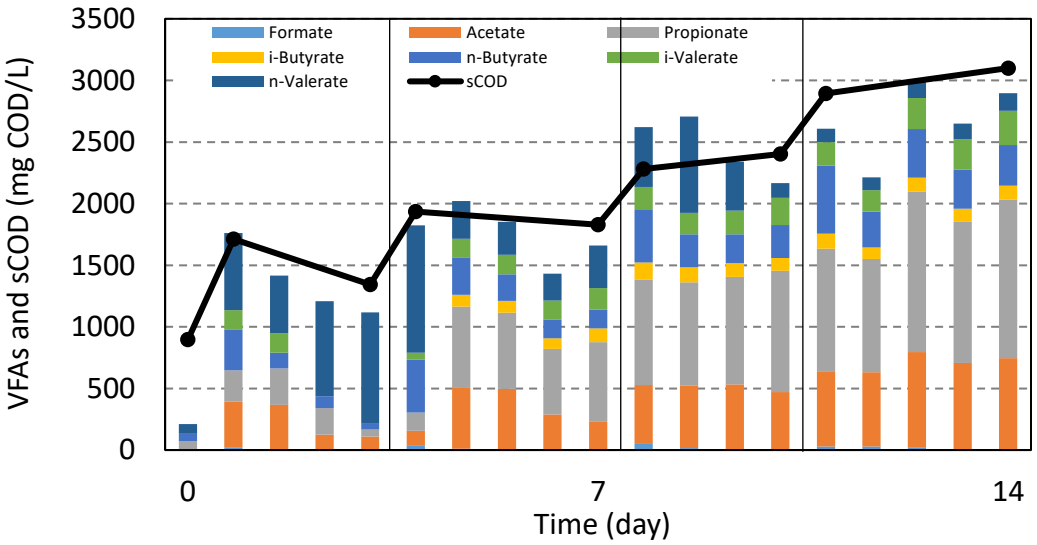


Figure 5.10. VFAs and soluble COD concentration in R4 during Phase 1 (day 1 to day 14; vertical black lines represent feeding times).

On day 14, feeding of R4 was stopped, while gas production, sCOD, and VFAs were monitored (Figure 5.11). Between day 14 and day 18, R4 produced 250 mL of biogas as the acetate concentration decreased. From day 19 to day 35, there was little gas production, as propionate, the predominant VFA, was kept between 1000 and 1200 mg COD/L, showing that propionate-utilizers were most impacted compared to all acidogens. From day 36 to day 49, R4 produced 500 mL of biogas as propionate and other VFAs decreased below the limit of quantification (ca. 30 mg COD/L).

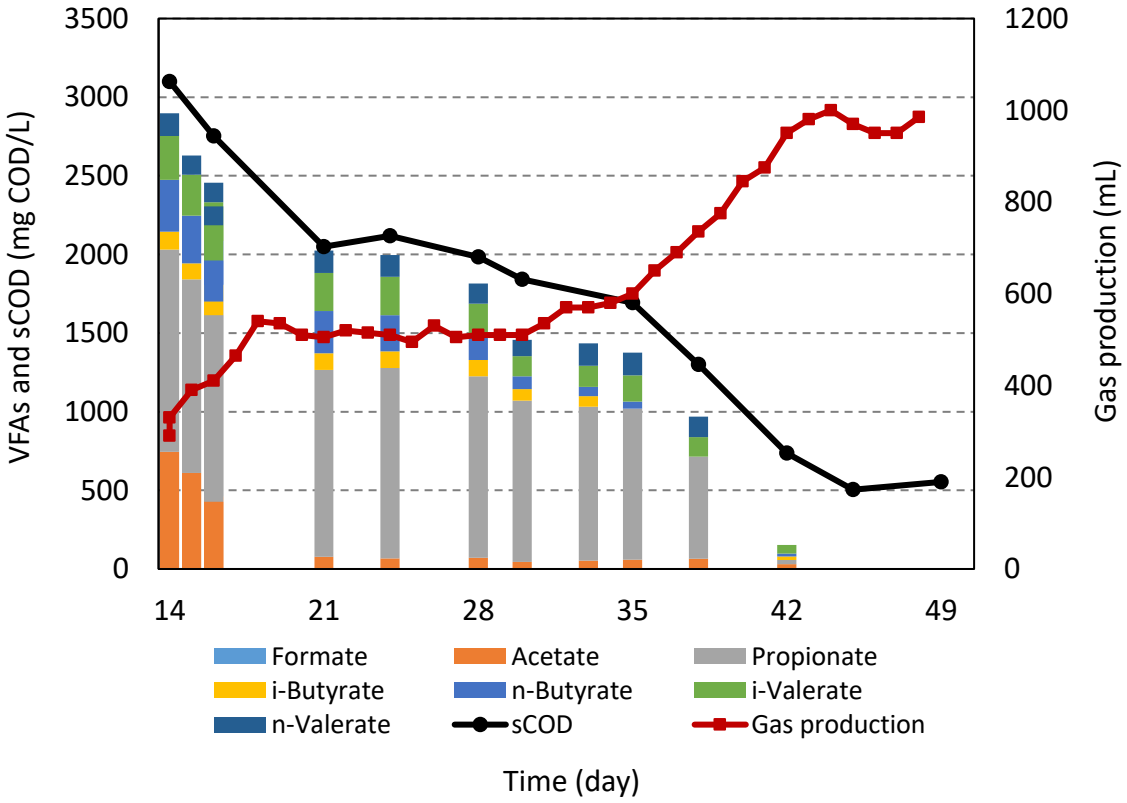


Figure 5.11. VFAs, soluble COD, and gas production (22°C, 1 atm) in R4 during the recovery period (day 14 to day 49).

After the recovery period, R4 was fed again with 40 mg/L PAA directly for 14 days (Phase 3, day 49 to day 63). R4 had a normalized gas production of 59 %, which gradually increased to 67 % compared to R1 (Figure 5.8 b). Compared to Phase 1, in which R4 had a normalized gas production of 37% compared to R1 at the end of Phase 1 (Figure 5.8 a), R4 was more resistant to PAA during Phase 3.

The normalized biogas CH_4 content decreased, as the CO_2 content increased (Figure 5.9), indicating that the direct 40 mg/L PAA second addition still impacted methanogenesis, but not fermentation. Figure 5.12 shows the sCOD and VFAs evolution in R4 during Phase 3, during which both sCOD and VFAs accumulated. The concentration of acetate and propionate, the predominant VFAs, was maintained between 400 to 600 mg COD/L during day 52 to day 63, demonstrating that both propionate-utilizers and acetoclastic methanogens were impacted.

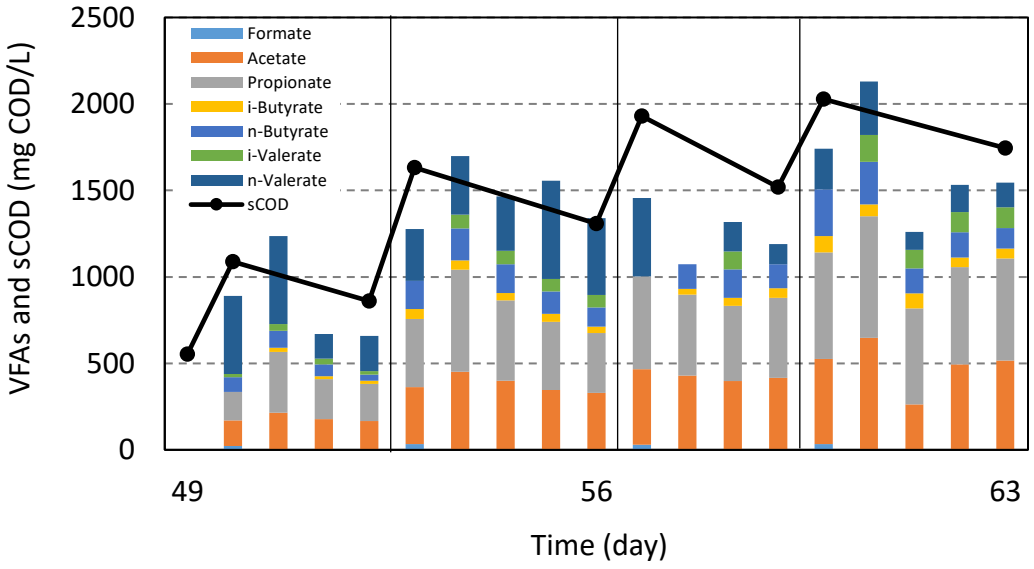


Figure 5.12. VFAs and soluble COD concentration in R4 during Phase 3 (day 49 to day 63; vertical black lines represent feeding times; 40 mg/L PAA direct addition).

Among fermentative/methanogenic species, propionate-utilizers have been shown to be the most vulnerable to several detrimental conditions. For example, under sudden change in salinity (40 g NaCl/L, 2-48 h), compared to acetate, propionate degradation was slower, demonstrating propionate-utilizers are more sensitive to sodium toxicity than acetoclastic methanogens (Vyrides et al., 2009). Acrylic acid, an intermediate product in the synthesis of resins, adhesives, and synthetic fiber, also completely inhibited propionate degradation while partially inhibited acetate degradation (Qu et al., 1996). High dosage of tetracycline (20 mg/L), a broad-spectrum antibiotic, added to an anaerobic reactor, reduced the population size of propionate-utilizers (e.g., *Syntrophobacter wolinii*), on the other hand, increased the population size of methanol-utilizing methanogens (Xiong et al., 2017).

Compared to R4, R3 showed an increasing resistance against PAA. The resistance could be explained by introducing low concentration (5 mg/L) of PAA during Phase 1 that induced the anaerobic culture to synthesize antioxidative enzymes without complete inhibition. The presence of the antioxidative enzymes helped R4 adapt to higher PAA concentrations (20 and 40 mg/L). PAA is decomposed to acetate and O_2 under neutral and acidic conditions. When strictly anaerobes are exposed to O_2, they generally produce hydrogen peroxide (H_2O_2), superoxide radical ($O_2^{\cdot -}$), and hydroxyl radicals ($OH\cdot$). These reactive oxygen species (ROS) are threat to the anaerobes if not immediately removed by the antioxidative enzymes catalase and superoxide dismutase (SOD). *Methanosarcina barkeri* showed increased catalase and SOD activity under H_2O_2 and an oxidative stress agent - paraquat (Brioukhanov et al., 2006). Other than catalase and SOD, the thioredoxin (Trx) system, which is a small redox protein, is also found in most methanogens. *Methanocaldococcus jannaschii*, a hydrogenotrophic methanogen, is found to possess Trx,

which is responsible for protecting a range of cellular processes against oxidative damage (Susanti et al., 2014).

In summary, direct 40 mg/L PAA addition impacted both acidogens and methanogens in R4. After feeding 40 mg/L PAA directly for 14 days, it took R4 35 days to recover and propionate-utilizers were the last to recover. After recovery, R4 was still significantly affected by direct 40 mg/L PAA addition. On the other hand, R3 showed greater resistance to PAA by incrementally increasing the dosage of PAA from 5 to 40 mg/L.

5.4 Summary

PAA pre-decomposed in dissolved air floatation (DAF) effluent (R2) did not affect the biogas production of the anaerobic reactor up to 80 mg/L. However, addition of pre-decomposed 100 mg/L PAA impacted the propionate-utilizers, leading to lower biogas production by 78% to 89%, compared to the PAA-free control reactor (R1).

Direct 40 mg/L PAA addition (R4) impacted both acidogenesis and methanogenesis, leading to lower biogas production. During Phase 1 (day 1 to 14), the total biogas decreased to 37% compared to the PAA-free control reactor (R1). Among all acidogens and methanogens, i-valerate-utilizers were the first to recover, while propionate-utilizers and acetoclastic methanogens were inhibited. After feeding 40 mg/L PAA directly for 14 days, it took 35 days for R4 to recover. Acetoclastic methanogens recovered over day 14 to 18, resulting in biogas production; propionate-utilizers recovered over day 36 to 49, resulting in increased biogas production. After 35 days of a recovery period, the anaerobic reactor was fed again with 40 mg/L PAA directly for 14 days. The biogas production in Phase 3 (day 49 to 63) declined to 59 % and gradually increased to 67 % compared to the PAA-free control reactor (R1). In contrast, direct PAA addition with incrementally increasing PAA concentration from 5 to 40 mg/L (R3) did not impact biogas production compared to the PAA-free control reactor (R1) and VFAs accumulation was not observed.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

Peracetic acid (PAA) is an effective disinfectant against a wide spectrum of microbes, used in healthcare, water treatment, and food industry. PAA gained its popularity because of its high redox potential and less possibility to form disinfection by-products (DBPs) compared to chlorine compounds (Luukkonen et al., 2016). In poultry processing, PAA is used in chicken chiller tanks to inhibit microbial growth as well as for the disinfection of processing equipment. However, residual PAA in poultry processing wastewater may be carried to biological wastewater treatment systems with potential adverse effects on the biological processes. The overall goal of this research was to evaluate the effect of PAA on anaerobic processes (i.e., fermentation/methanogenesis, acetoclastic methanogenesis, and hydrogenotrophic methanogenesis) typically used for the biological treatment of poultry processing wastewater. The following conclusions are reached based on the results of the present study:

1. The anaerobic culture medium and the dissolved air floatation (DAF) effluent have a great capacity to decompose PAA and showed an initial high PAA demand, followed by lower decomposition rates. As pH increased, the PAA decomposition rate increased.
2. Pre-decomposed 40 and 100 mg/L PAA addition had no effect on methane production, substrate COD conversion to CH_4, dextrin peptone mixture ultimate biodegradability, and specific methanogenic activity (SMA) compared to PAA-free,

control reactors under fermentative/methanogenic and acetoclastic methanogenic conditions. In the hydrogenotrophic methanogenic assay, the PAA-free control and both pre-decomposed 40 and 100 mg/L PAA addition had similar CH_4 produced/H_2 consumed ratios. PAA pre-decomposed in DAF effluent did not affect the biogas production of the anaerobic reactor (R2) up to 80 mg/L. However, addition of pre-decomposed 100 mg/L PAA impacted the propionate-utilizers, leading to lower biogas production compared to the PAA-free control reactor (R1).

3. Direct PAA addition did not affect the initial CO_2 production rate and extent in all batch assays, indicating fermentation was not impacted. Although the kinetics (i.e., SMA) of methanogenesis were impacted with direct 40 and 100 mg/L PAA addition in the assay which used D/P as the substrate and in the acetoclastic methanogenic assay, the extent of substrate COD conversion to CH_4 and the D/P biodegradability were similar to those achieved by the PAA-free reference series, demonstrating that methanogenesis recovered over time. In the hydrogenotrophic methanogenic assay, direct 40 mg/L PAA resulted in a CH_4/H_2 ratio similar to that of the PAA-free control. Direct 100 mg/L PAA addition impacted hydrogenotrophic methanogens, resulting in a lower CH_4/H_2 ratio compared to PAA-free control. Compared to the acetoclastic methanogens, hydrogenotrophic methanogens were not affected by direct 40 mg/L PAA addition, indicating they are more resistant to direct PAA addition than the acetoclastic methanogens.
4. Direct 40 mg/L PAA addition to a semi-continuously-fed anaerobic reactor (R4) impacted both acidogenesis and methanogenesis, leading to lower biogas production compared to the PAA-free, control reactor (R1). After feeding 40 mg/L

PAA directly for 14 days (four feedings), it took 35 days for R4 to recover without feeding. During the recovery period, acetoclastic methanogens recovered first followed by propionate-utilizers. After 35 days of recovery, the anaerobic reactor was fed again with 40 mg/L PAA directly for another 14 days (four feedings). Although the biogas production in Phase 3 (day 49 to 63) declined, as acetate and propionate accumulated, R4 produced more normalized biogas compared to R1 during Phase 3 than Phase 1.

5. Compared to direct addition of PAA at 40 mg/L (R4), direct PAA addition with incrementally increasing PAA concentration from 5 to 40 mg/L (R3) did not impact biogas production compared to the PAA-free control reactor (R1) and VFAs did not accumulate. Compared to R4, R3 showed an increasing resistance against PAA.

The findings of the present study suggest that anaerobic processes can be used to treat poultry processing wastewater carrying PAA, at least up to 80 mg/L of PAA. Even in the case of an accidental PAA spill, anaerobic treatment systems can recover over time providing that corrective measures are implemented (e.g., temporary wastewater diversion, pH adjustment).

To test the fermentative/methanogenic culture's mitigation capacity against PAA, future study can increase direct PAA addition with incrementally increasing PAA concentration from 40 to 100 mg/L or beyond. In addition to the mitigation capacity against PAA, the effect of hydrogen peroxide on a mixed fermentative/methanogenic culture is needed to better understand the effect of PAA solutions on anaerobic treatment, due to the fact that commercial PAA solutions are always mixtures of hydrogen peroxide and PAA. Additional research is also needed to understand the mitigation mechanisms of the

fermentative/methanogenic culture, e.g., microbial community change and synthesis of antioxidative enzymes under PAA stress conditions.

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