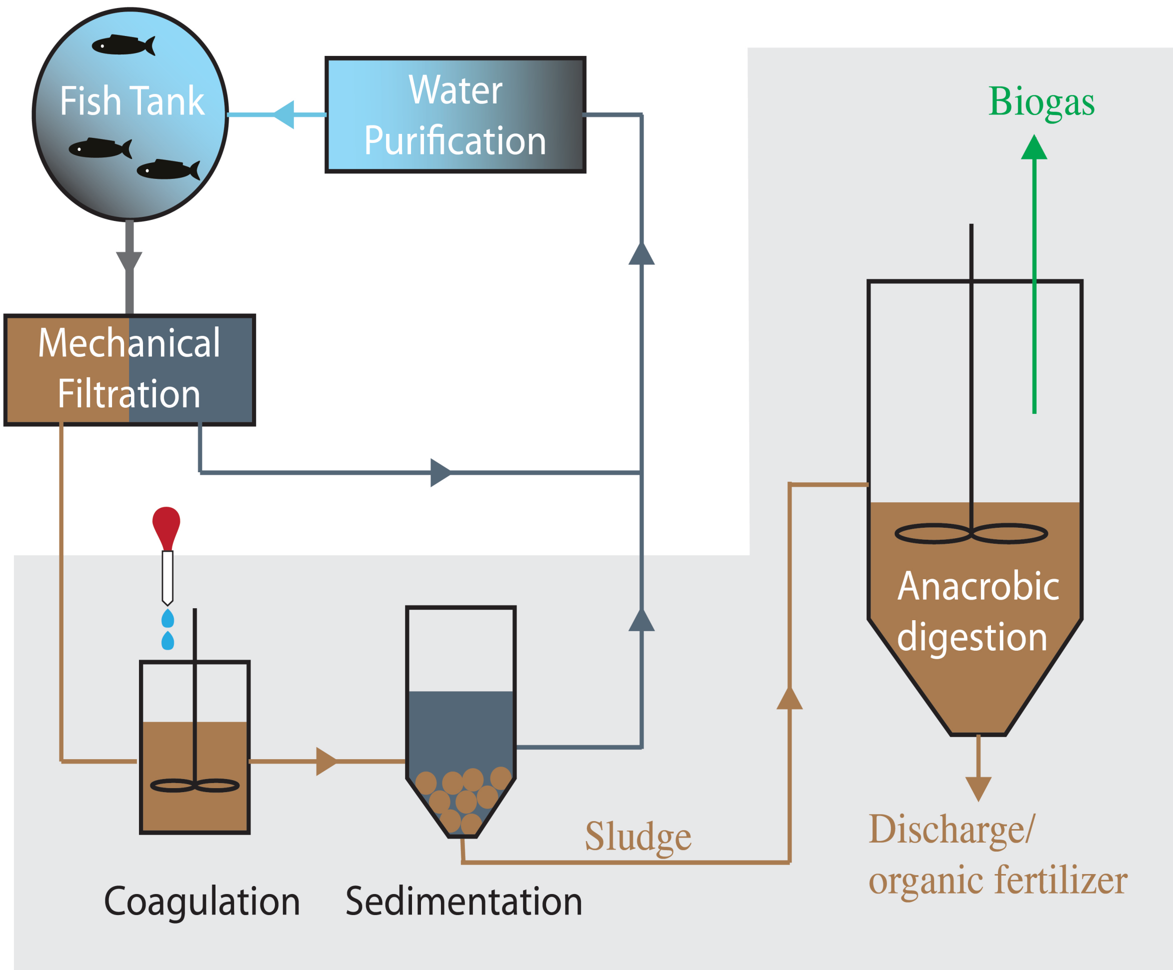
Anaerobic digestion of sludge from brackish RAS : CSTR performance, analysis of methane potential and phosphatse, struvite crystallization



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Challenge the future

Anaerobic digestion of sludge from brackish RAS: CSTR performance, analysis of methane potential and phosphatase, struvite crystallization

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Abstract

The marine/ brackish recirculating aquaculture system (RAS) is a sustainable and environment-friendly cultivating system to meet the increasing demand of aquatic animal protein for the human consumption. However, the increasing amount of aquaculture waste becomes problematic for further application and demands much research attention on it.

The emission minimization of marine/brackish aquaculture recirculation systems (EM-MARES) project aims to minimize the emission of brackish RAS and improve bio-energy yield as well as recovery phosphorus in the form of struvite. This project consists of three phases: coagulation of the sludge from brackish RAS; anaerobic digestion in the mesophilic continuously stirred tank reactor (CSTR); and struvite recovery from the digestate.

The present study included last two phases of the EM-MARES project. The involved objectives could be divided into four main parts : (1) to investigate the performances of CSTRs at different organic loading rates (OLR) and under different mixing regimes; (2) to investigate the effect of inorganic coagulants on methane potential of saline sludge, since inorganic coagulants were used in coagulation process prior to anaerobic digestion process, and to investigate the effect of compatible solutes on methane potential of saline sludge, since compatible solutes could be utilized in digesters to enable microorganism overcome sodium toxicity; (3) to investigate effects of compatible solutes and shear forces from biogas recirculation on phosphatase activity (PA), because PA is relevant to phosphate release from organic materials and subsequent struvite formation; (4) to investigate variation of particle size distribution of struvite at different Mg/P molar ratios, at different temperatures, or at different pH levels.

The steady state was obtained in all three CSTRs with inoculum adapted to high salinity already. The specific methane yields at steady state in three reactors were various within the range of 0.156-0.256L CH_4/g COD added. The COD and VS removal efficiency were respectively 40-83% and 45-85%. Compared to the previous study by Gebauer (2004) on simliar saline sludge, the performaces of CSTR were significantly improved. In addition, a higher OLR could cause an increase in specific methane yield. The impeller-stirring enhanced the specific methane yield compared with biogas recirculation.

Results from BMP tests showed that compared to the control group, methane potential of saline sludge was improved 5% at the presence of 0.5g/L trehalose, whereas it was inhibited 18% and 5% by addtion of PAS at 2.4gAl^{3+}/L and $FeCl_3$ at 6.0gFe^{3+}/L, respectively.

PA deactivation was a function of time. Addition of 1mM or 0.5mM trehalose enhanced acid PA in saline sludge during the digesting period of 0.25h-72h. A high level of shear stress from biogas recirculation could stimulate both acid and alkaline PA during the digesting period of 0.25h-12h.

The dominant diameters of struvite crystals were similar within the Mg/P molar ratio range of 0.12-2.19 excluding ratios of 0.91 and 1.09. Furthermore, it increased with increasing temperatures, while decreased with increasing pH levels. The optimum conditions in synthetic brackish solution for harvesting more struvite with large sizes and obtaining a higher P-removal efficiency were that Mg/P molar ratio approached to 1, and pH was maintained at 8, and temperature was kept at 25 or 35°C. However, the conclusion referring to Mg/P was not applicable to the filtrate from R2, and more research would be required to verify the application of other conclusions to the filtrate of digestate.

Contents

1 Introduction

1.1 Recirculating marine aquaculture system

The global appetite for fish is expanding as the growth of the urban population and income increment in developing countries (Delgado et al., 2003, Cahu et al., 2004). According to Food and agriculture organisation of the United Nations (FAO) capture fisheries cannot meet the increasing demand of aquatic animal protein for the human consumption anymore (FAO, 2009). Several conventional aquaculture methods are in use to response to this deficit, such as pond aquaculture, raceway aquaculture and net-pen aquaculture. However, currently they are heavily criticized due to the potential hazards they bring to the adjacent ecosystems, like eutrophication in nearby waters, transmission of parasites or diseases to native stocks, biodiversity loss in natural water-bodies since the escape of non-endemic species as well as chemical pollution from aquaculture facilities (Ackefors and Enell, 1994, Braaten, 1992, Erondu and Anyanwu, 2004). Then a sustainable and environment-friendly cultivating system-recirculating aquaculture system (RAS) was developed, which has striking advantages over other flow-through culture methods mentioned before.

The recirculating aquaculture system is relatively closed as it requires minimum water input and water discharge. Its water quality is always maintained in an adequate level by a series of reasonable treatment processes (Van Rijn, 1996). This system offers opportunities to reduce water usage(Verdegem et al., 2006), to improve waste management which contains dealing with aquaculture sludge and nutrients recovery (Piedrahita, 2003), and to better control biological pollution and diseases spread (Zohar et al., 2005), and also to reduce visual influence on fish farms (Martins et al., 2010). Despite of these advantages, its aquaculture production can hardly reach levels of other conventional methods. Moreover, the high initial investment makes this system adopted slowly in the moment (Schneider et al., 2006).

Due to the intensive culture in RAS, a substantial quantity of aquaculture waste is generated which should be treated prior to being disposal of (Sánchez et al., 2006). Normally it consists mainly of fish faeces and little uneaten food (Gebauer and Eikebrokk, 2006), and its characteristics may change widely in accordance with fish species (Timmons et al., 2010). The aquaculture waste cannot be simply discharged into municipal sewage systems, or directly into receiving waters (Mirzoyan et al., 2010). Besides, its bothersome odours and relevant hygienisation problems demand extra attention (Gebauer, 2004). So several options for treatment processes (Figure 1) are being applied in the current RAS. Choosing a rational treatment train should take many factors into accounts, for instance, waste mass, sludge concentration, and the degree of stabilization required (Chen et al., 1997). It has to be noted that the final effluent or sludge might cause soil and groundwater salinization once in use for irrigation or land spread, when originating from marine/brackish-water RAS (Boyd and Tucker, 1998, Primavera, 2006).

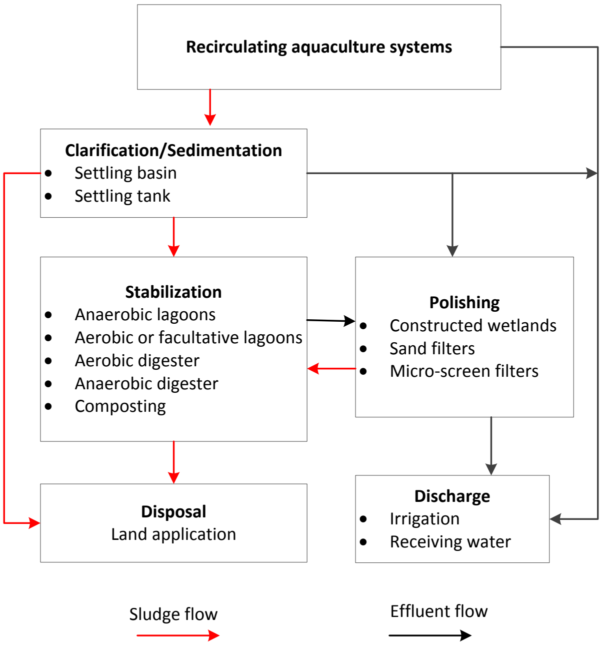

Figure 1 Options for aquaculture sludge treatment processes. Source:(Chen et al., 1997)

1.2 EM-MARES project

The project 'Emission Minimization of Marine/Brackish Aquaculture Recirculation Systems' (EM-MARES), which is the basis for this thesis, is depicted in Figure 2. EM-MARES aims at developing an approach to improve its waste management not only in minimizing the waste production but also in promoting bio-energy yield and recovering phosphorus in form of struvite. In this system, Water from fish facility goes through filtration and nitrification units and then finally back to the initial site. The backwash from filters is treated with separation units, and the separated liquid is handled by fish facility itself. The concentrated sludge that is high in chemical oxygen demand (COD) and salinity as well as total suspended solids (TSS), would be transported to digesters for further anaerobic degradation. At present the digestion part has been carried out on lab-scale, which is the focus of the thesis.

Sodium concentrations ranging from 0.1-0.2g/L were found to be favourable for growth of mesophilic anaerobes, while beyond this range sodium would inhibit the anaerobic digestion process. In terms of the studied sludge harvested from brackish RAS and with a sodium level of around 4.7g/L (Table 7, section 4.1.3), a reasonable method should be proposed to overcome this inhibition so as to enhance the methane yield of digesters.

A series of struvite recovery approaches have been established successfully for municipal wastewater, but methods for application to anaerobically digested fish waste remain economically impractical or technologically inefficient. The continuous production of enormous volumes of aquaculture waste in marine RAS requires further research to develop struvite recovery methods. High concentration of struvite component ions found in aquaculture waste, if recovered, could supply more struvite per unit of wastewater volume than when recovered from municipal wastewater. On the top of that, the high Mg^{2+} content found in aquaculture waste makes supplementation for struvite recovery unnecessary, thereby reducing process costs.

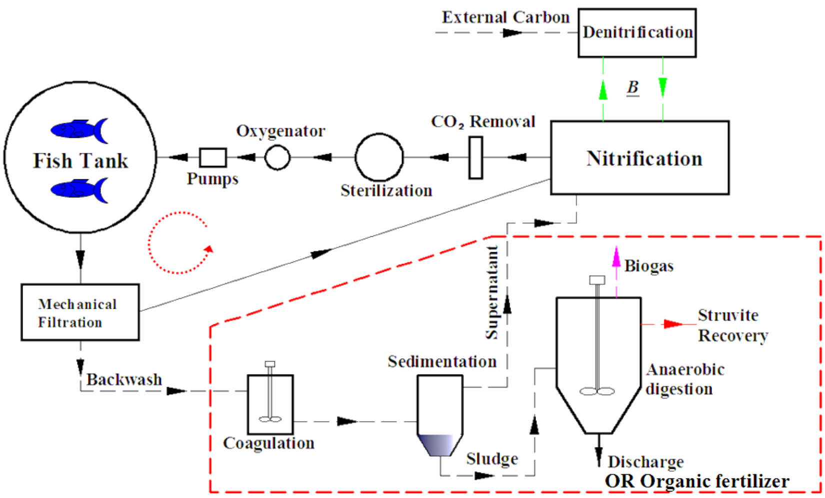

Figure 2 Scheme of recirculating marine/brackish aquaculture system in EM-MARES project. Source:(Ferreira, 2012)

1.3 Anaerobic digestion

The anaerobic digestion refers to degradation and stabilization of organic materials under anaerobic conditions by microbes, compatible with biogas production (composed of mainly methane and carbon dioxide) and microbial biomass formation (Kelleher et al., 2002).

1.3.1 Pros and cons of anaerobic digestion

As described earlier, stabilization and sanitation of aquaculture sludge may be achieved in several ways. While, among them anaerobic digestion may be preferable to others because of its numerous significant advantages. Table 1 simply illustrates features of different sludge stabilization options, and benefits anaerobic digestion can offer more than both points given there, for instance, low sludge production, low energy and chemical requirement (the produced biogas may be utilized as energy source in the plant or elsewhere), rapid start up within one week using granular anaerobic sludge as seed material, and positive market value of excess sludge as well as storage in a long period without feeding (Van Lier et al., 2008). Nevertheless, the major problems for anaerobic digestion is its sensitivity to toxic substances, potential production of odour and corrosive gases (Metcalf & Eddy. et al., 2003). In addition, the poor operational stability is also a limit for its wide application in commercial areas (Dupla et al., 2004). Typically, anaerobic digestion is commonly found in the stabilization of municipal, industrial and agricultural wastes, but not a totally new application like that in the RAS for treating aquaculture waste (Mirzoyan et al., 2010).

Table 1 Features of different sludge stabilization options. Source:(Chen et al., 1997)

Options	Advantages	Disadvantages
1. Anaerobic lagoon	High organic loading Low maintenance	Odour
2. Aerated lagoon	Space efficient High organic loading	Energy consumption Moderate maintenance
3. Aerobic digester	High loading	Energy consumption
4. Anaerobic digester	High loading Methane generation	Complex High maintenance
5. Composting	Useful end product	Dewatering required

1.3.2 Types of anaerobic digester

Different types of anaerobic digesters are employed in RAS for treatment of aquaculture waste, and three common ones are discussed briefly below (Figure 3).

- **Continuously stirred tank reactor** (CSTR)(Figure 3A): It is a suspended growth system, where the bacteria are suspended in the digester through intermittent or continuous mixing action. The complete mixing offers a good substrate-sludge contact, but consumes much energy and intensive-labour as well (Marchaim, 1992). Due to the recirculation of concentrated sludge from settler, the SRT does not equal to the HRT. Large digester volume is required to meet the relatively long SRT. This kind of reactor is suitable for treating the high-strength wastes (Gerardi, 2003).

- **Anaerobic membrane bioreactor** (AnMBR) (Figure 3B): AnMBR is usually divided into side-stream system and submerged system according to the position of membranes. In AnMBR, the biomass and particulate organic matter are physically retained inside the reactor by membrane filtration which provides superior effluent quality over conventional high rate anaerobic reactors. Compared to the submerged system, the side-stream could offer higher fluxes and more convenience of membrane-replacement, but demands more frequent cleaning and high-energy consumption (Lin et al., 2013). Normally the permeate through a membrane is driven by a pump or gravity.

- **Upflow anaerobic sludge blanket** (UASB) (Figure 3C): It is a high-rate anaerobic digester that is efficient in removing solids with low TS (3-4%) contents (Marchaim, 1992). Wastewater passes upwards through the anaerobic sludge bed where organic compounds are degraded by highly settable granules that mainly consist of microorganisms. The resulting biogas and upward inflow are responsible for the reactor mixing. Its most attractive characteristic is the distinctive gas-liquid-solid separation. Moreover, the simple operation, small footprint and low energy consumption make it an outstanding and widely-used technology in anaerobic digestion (Seghezzo et al., 1998).

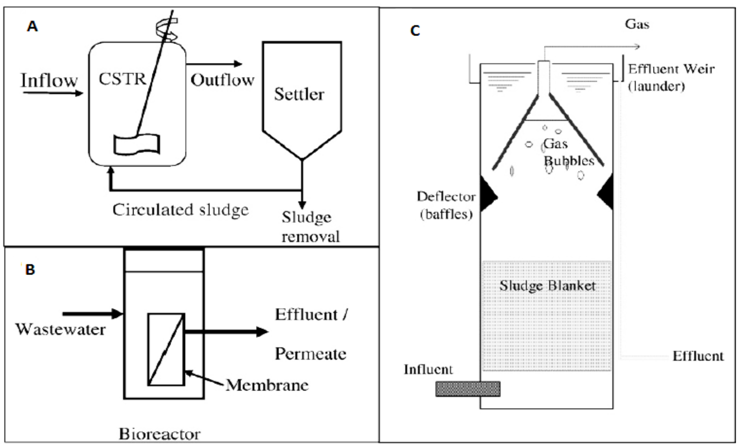

Figure 3 Commonly used anaerobic digesters for aquaculture sludge in RAS: (A)-CSTR; (B)-submerged MBR; (C)-UASB. Source:(Mirzoyan et al., 2010, Wang, 1994)

In the EM-MARES project CSTR is preferable to other three types for dealing with fish waste. Because around 60% of total solids in fish waste is suspended, and only by using CSTR can ensure high COD to bacteria contact. Moreover, the concern of inhomogeneous distribution of high salinity in reactors leads to the selection of CSTR over any other type of bed reactor.

1.4 Phosphate depletion and phosphorus recovery

Phosphorus is a basic chemical element on earth. It never appears in a free form since it is highly reactive and quickly binds to other elements. As part of the fundamental building block of all living organisms, phosphorus was found in cell membranes. It plays a decisive role in DNA and RNA, the storage and retrieval system for genetic information, and also in ATP the energy system. Furthermore, in vertebrate animals it is an important component of tissues such as bone (Butusov and Jernelöv, 2013). Phosphorus is a major nutrient required by crop growth as well. It is therefore obvious that phosphorus is essential for all life. According to an investigation (FAO, 2005) the recent consumption of phosphate fertilizers accounts for approximately 78% of the global phosphorus production from phosphate rocks. However, the continued growth in world population and the need for securing food supply enhance the importance of the phosphate fertilizers. Many studies gave a similar prediction that the annual phosphate consumption in 2050 will be 70 million tons, and at the same consumption rate, half of the phosphate reserves available now could be used out within around six or seven decades (EcoSanRes, 2005, Steen, 1998, Jasinki et al., 1999).

Besides the phosphate depletion, there are also some other factors attributing to the need of P recovery for sustainable development, for example, the P limitation in effluent to surface water that is sensitive to eutrophication becomes progressively stricter (Molinos-Senante et al., 2011) and also, the implementation of phosphorus recovery will assist in reducing about 8 to 31 percent of sludge mass under specific operational conditions compared to chemical phosphorus removal (Woods et al., 1999).

Phosphorus can be recovered from wastewater, sewage sludge and the ash of incinerated sewage sludge, as well as from animal manure, because they are the predominant carriers of phosphate (Driver et al., 1999). Certainly, P recovery from wastewater is comparatively simple due to lower TSS concentration in the liquid phase; whereas the higher P concentration in the solid phase, especially that comes from anaerobic digestion which gives rise to P release, may render its recovery economically feasible. Provided that the project involved here is intended to recover P from anaerobically digested fish faeces, emphasis of this section is placed on P recovery in anaerobically digested sludge or other relevant reject water.

Usually there are two kinds of chemical precipitation\crystallization available for P recovery from anaerobically digested sludge, namely calcium phosphate (CP) precipitation and magnesium ammonium phosphate (MAP, also called struvite) precipitation. In most cases CP precipitation is an alternative for removing phosphorus instead of recovering, and CP production in form of Slime is by no means as profitable as MAP to the industry (Driver et al., 1999). However, only CP extracted through DHV Crystalactor process can be regarded as a marketable end-product (Morse et al., 1998). It was proposed that struvite recovery may be superior to CP recovery in practice due to the great potential as an agricultural fertilizer at high application rates without any damage (Gaterell et al., 2000). The disadvantage of struvite precipitation is that its accumulation can result in the blockage of pipes in wastewater treatment plants, increasing pumping costs and reducing plant capacity (Doyle and Parsons, 2002).

At present, many approaches have been proposed already to improve P availability of anaerobically digested sludge through precipitating MAP, either in the developing stage, or in the pilot-scale and full scale implementation. The corresponding exemplary applications include Crystalator, SEABORNE and AirPrex. All of them are presented in Table 2.

Table 2 Exemplary P recovery techniques. Source:(Cornel and Schaum, 2009)

Technique	Applied on	Developing phase	Product	Main treatment process
Crystalactor PHOSNIX OSTARA PRISA	Reject water	Industry-scale Industry-scale Industry-scale Lab-scale	MAP	Fluidized-bed reactor
SEABORNE	Reject water	Full-scale	MAP	CSTR followed by centrifuge
AirPrex	Digested sludge	Industry-scale	MAP	Airlift reactor followed by sedimentation

2 Theoretical background

2.1 Anaerobic digestion

2.1.1 Conversion processes in anaerobic digestion

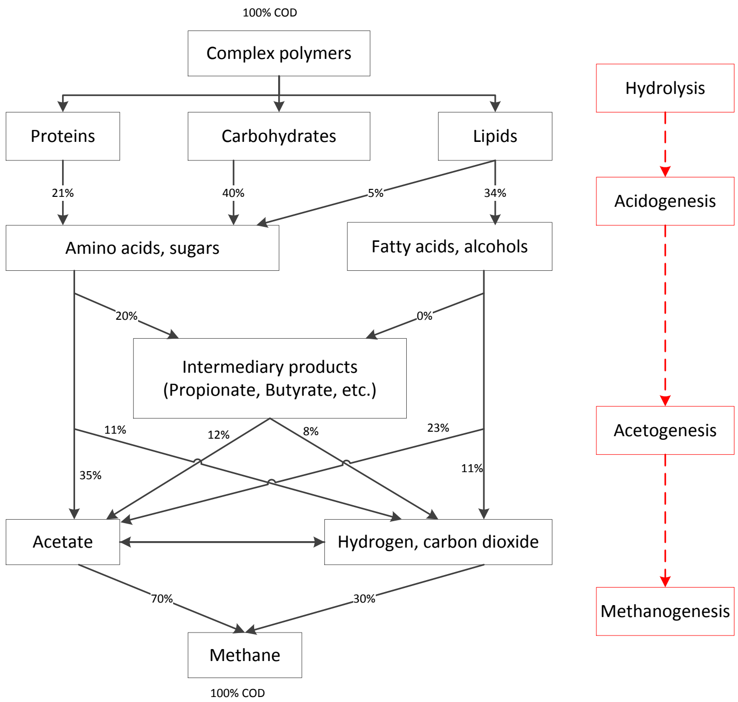

Figure 4 Reaction scheme for the anaerobic digestion of complex polymers and relevant carbon flow. Source:(Gujer and Zehnder, 1983, Appels et al., 2008)

The anaerobic digestion is a multi-steps biological process where complex polymers are progressively transformed into simpler and smaller sized materials by diverse bacteria, along with valuable biogas production. Four phases (Figure 4) involved have been identified and well accepted (Appels et al., 2008).

- **Hydrolysis**
  The first step in anaerobic digestion processes is to break down complex polymeric substrates into soluble compounds, like proteins, carbohydrates and lipids that are accessible for acidogenic bacteria in the next step through a number of exoenzymes produced by hydrolytic bacteria. Hydrolysis is very sensitive to temperature and usually considered as a rate-limiting step for the overall digestion process (Anderson et al., 2003).

- **Acidogenesis/Fermentation**
  The relatively small soluble compounds obtained from hydrolysis cross the cell wall and are continuously fermented or anaerobically oxidized in the cells of acidogenic bacteria. The acidic end-products include mainly volatile fatty acids, i.e. acetate and higher organic acids, hydrogen and carbon dioxide as well as other by-products such as ammonia. Acidogenesis is the fastest conversion step among all phases (Van Lier et al., 2008). Moreover, the facultative members of acidogens are capable of consuming traces of oxygen in feed so as to protect the oxygen-sensitive methanogens (Anderson et al., 2003).

- **Acetogenesis**
  Since only acetate, H_2 and CO_2 can server as direct substrates for methane-forming bacteria, other intermediate organic products from acidogenesis have to be further degraded by acetogens. When obligate hydrogen-producing acetogens (OHPA) produce acetate, hydrogen is also produced. Once hydrogen is accumulated and significant hydrogen partial pressure has built, it would terminate the activity of OHPA and affect acetate production. However, in practice the hydrogen partial pressure of a steady-state anaerobic digester is maintained at an extremely low level to stimulate OHPA due to hydrogen uptake by methanogens (Gerardi, 2003).

- **Methanogenesis**
  In the final phase of the anaerobic digestion processes, methanogens are responsible for forming methane by reducing CO_2 using H_2 as an electron donor or decarboxylating acetic acid. The latter path is predominant in producing methane up to 70% (Figure 4). Commonly the growth rate of aceticlastic methanogens (utilize acetate) is significantly low, thus results in a very long-time adaption for seed materials in anaerobic digesters. In addition, there is a competition between sulphate-reducing bacteria and methanogens for acetate and H_2, the result of which is decided by the substrate-to-sulphate ratio in digested medium (Gerardi, 2003).

2.2.2 Factors affecting anaerobic digestion

The effective anaerobic degradation of organic matters depends not only on the healthy population of relevant bacteria but also on the suitable environmental situations surrounding them. Hereby a summary is made to show the most important environmental factors impacting anaerobic digestion.

Temperature

It has been reported that anaerobic digestion can proceed under any temperature regime shown below (Marchaim, 1992), and characterized by its own advantages and disadvantages.

- **Thermophilic** (45-65°C): Higher methane production rate, higher organic loading rate and lower quantities of sludge as well as higher death rate of pathogens; whereas more heating energy requirement, higher instability and higher ammonia inhibition to cease methanogens' growth (Appels et al., 2008, Anderson et al., 2003)

- **Mesophilic** (25-45°C): The most widely used among the three regimes (Anderson et al., 2003). It is capable of supporting much more optimal biological activities and keeping more stable and adjustable to fluctuation in feedstock (Metcalf & Eddy. et al., 2003, EarthTech, 2003)

- **Psychrophilic** (10-25°C): lower energy input, having desirable economic trade-offs for anaerobic treatment of wastewaters in temperate acclimates (Anderson et al., 2003); lower methane production rate, longer retention time (Price and Cheremisinoff, 1981).

pH and alkalinity

The anaerobic bacteria normally work within the pH range between 6.6 to7.6, while the optimal appears to be around 7.0 to 7.2 (McCarty, 1964a). Except for the normal conditions, sometimes anaerobic digestion may even take place at extreme pH like 3 or 9.7 (Anderson et al., 2003). Some research indicates that the growth rate of methanogens declines dramatically below pH 6.6 (Mosey and Fernandes, 1989), and microbial granulation will be seriously destroyed at pH of 8.3 (Sandberg and Ahring, 1992).

To maintain digesters' pH within a stable range sufficient alkalinity is quite of importance. This buffer capacity is a combination of bicarbonate alkalinity, carbonate alkalinity as well as alkalinity provided by ammonia and amino groups (-NH_2). Generally, carbon dioxide released in the organic degradation process can be converted partially to carbonic acid, bicarbonate alkalinity and carbonate alkalinity. While other alkalinity with regard to ammonia and amino is a consequence of degradation of amino acids and proteins. Relevant equilibriums are shown below ([1.1] & [1.2]). The alkalinity is determined by the composition and concentration of substrates directly, hence, extra strong bases or carbonate\bicarbonate salts have to be dosed into the digester if the feed sludge cannot supply sufficient alkalinity (Gerardi, 2003).

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^{2-} \quad [1.1]$$

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^- \quad [1.2]$$

Retention time

Hydraulic retention time (HRT) and solid retention time (SRT) stand for the average time that liquid and sludge remain in the reactor respectively. HRT is equal to SRT when there is no sludge recycle or supernatant withdrawal in the digestion system. In general, the longer SRT exists in the digester, the better performance for organic removal is expected yet the larger reactor volume is requested. Appels et al. (2008) reported that anaerobic digestion on lab-scale became stable until SRT was extended to 10 days or more, which is in agreement with the general observation, that mesophilic anaerobic digestion needs a mean retention period of 15days and 20days for thermophilic digestion, eg. (EU, 2000).

Organic loading rate

Since the principal function of anaerobic digestion is to remove organic materials, the organic loading rate of digesters typically are expressed as the weight of volatile solids (VS) or chemical oxygen demand (COD) per unit of volume of digester capacity per unit of time. Designed and recommended loading rate is varying from 3.2 to 7.2kg VS/(m^3·d) for different types of digesters and feedstock (Gerardi, 2003). Metcalf & Eddy. et al. (2003) also suggested the OLR of 3.2-32kg COD/(m^3·d) for the anaerobic process. OLR is a particularly important control-parameter in the digestion systems, because the OLR above loading capacity would result in a low biogas yield due to accumulation of inhibiting fatty acids in the reactor.

Nutrients

Nutrients, like nitrogen (N), phosphorus (P) and sulphur (S), are essential to the function of anaerobic bacteria since they are the basic components of the cellular building blocks and assist in the synthesis of enzymes and relevant cofactors. Based on different amounts required by the cell, nutrients are classified into macronutrients and micronutrients. Both groups should not be present in excessive concentrations, which might lead to toxicity affecting bacterial metabolism (Table 3). The COD:N ratio most commonly recommended and adopted in anaerobic digestion of non-acidified organic matter is 100:2.5; the COD:P ratio proposed by most workers is somewhere between 80:1 to 200:1. (Anderson et al., 2003).

Mixing

The continuous rise of methane bubbles inside the working volume of the digester will supply an inherent mixing of substrate with microbes, which, however, is insufficient for efficient mass transfer. As a result, in order to enhance the uniform distribution of mixture and obtain an optimal reactor performance, several auxiliary mixing methods are being utilized in the digestion system, such as mechanical devices like propellers, gas recirculation and feed recycle. It has been pointed out that the differences in level and type of mixing have an influence on the size of sludge flocs, gas production, distribution of microorganisms within the sludge etc. (Anderson et al., 2003).

Toxicity and inhibition

There are many substances that can cause toxicity to the anaerobic digestion or inhibit the degradation process. They are either from outside input introduced with the feedstock or from by-products generated during the digestion process like ammonia and sulphide. Various essential nutrients are beneficial to microorganisms at low concentrations but detrimental at high concentrations (Figure 5). Common examples are included in Table 3 and 4.

Table 3 Toxic inorganic compounds for anaerobic digestion. Source:(Appels et al., 2008, Metcalf & Eddy. et al., 2003)

Compound	**Stimulating concentration (mg/L)**	**Moderately toxic concentration (mg/L)**	**Strongly toxic concentration (mg/L)**
Sodium (Na^+)	100-200	3500-5500	8000
Potassium (K^+)	200-400	2500-4500	12000
Calcium (Ca^{2+})	100-200	2500-4000	8000
Magnesium (Mg^{2+})	75-150	1000-1500	3000
Ammonia (NH_4^+)		1500-3000	3000
Sulfide (S^{2-})		200	200
Copper (Cu^{2+})			0.5 (soluble) 50-70 (total)
Chromium (Cr^{6+})		10	3.0 (soluble) 200-250 (total)
Chromium (Cr^{3+})		10	2.0 (soluble) 180-420 (total)
Nickel (Ni^{2+})			30 (total)
Zinc (Zn^{2+})			1.0 (soluble)
Arseniate and arsenite		>0.7	
Cyanide		1-2	
Lead- containing compounds		5	
Iron-containing compounds		>35	
Cupper-containing compounds		1	
Potassium chloride (KCl)		>10000	
Chloride (Cl^-)		6000	

Table 4 Toxic organic compounds for anaerobic digestion. Source:(Appels et al., 2008)

Compound	Concentration resulting in 50 % reduction in activity (mM)
1-Chloropropene	0.1
Nitrobenzene	0.1
Acrolein	0.2
1-Chloropropane	1.9
Formaldehyde	2.4
Lauric acid	2.6
Ethylbenzene	3.2
Acrylonitrile	4
3-Chloro-1,2-propanediol	6
Crotonaldehyde	6.5
2-Chloropropionic acid	8
Vinyl acetate	8
Acetaldehyde	10
Ethyl acetate	11
Acrylic acid	12
Catechol	24
Phenol	26
Aniline	26
Resorcinol	29
Propanol	90

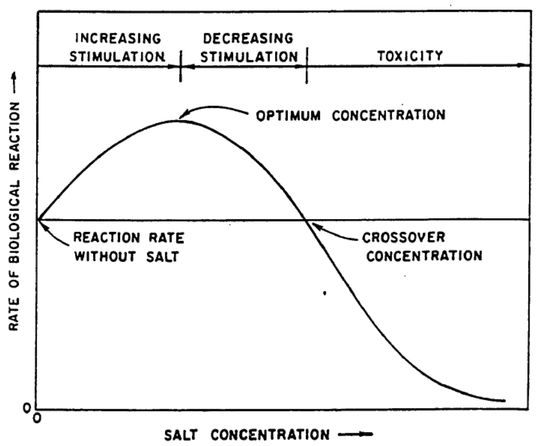

Figure 5 General effects of salts on biological reactions. Source:(McCarty, 1964b)

2.2 Biochemical methane potential

Optimal design and operation of anaerobic digesters are based on the knowledge of degradation kinetics and methane potential of mono or multiple substrates used. Therefore biological or biochemical methane potential (BMP) test as a relatively inexpensive and repeatable method is widely used in determining the anaerobic digestibility and methane potential of specific substrates, as well as biodegradation kinetics in lab-scale. Moreover it offers measurements for residual organic material amenable to further anaerobic treatment (Moody et al., 2009). In the case of co-digestion, BMP is effective as well for investigating effects of combined substrates on the performances of digestion processes (Esposito et al., 2012).

The principle of the BMP assay is to incubate a mixture of active anaerobic inoculum and substrate in sealed vessels at a specific temperature, and measure the biogas production and composition. The amount of inoculum must be enough to cope with unexpected accumulation of volatile fatty acids (Angelidaki et al., 2009). The duration of each substrate varies according to different anaerobic degradability. Usually after 30 days the accumulative biogas curve reaches a plateau phase (Labatut et al., 2011).

The conventional BMP assays following the above principle requires frequent manual sampling and determination of biogas volume released, and analysis of biogas composition using gas chromatography (GC). Compared to these aspects, AMPTS II is easy to operate and less labour-intensive, and is capable of supplying high quality data automatically. So this equipment was used in the experiments reported in this thesis, and will be descried in details in section 4.2.

The methane potential of a substrate is evaluated as the total volume of methane produced during the digestion processes over the amount of feeding substrate (i.e. mL CH_4/g VS added) (Labatut et al., 2011). Once the methane accumulation curve is obtained from experiments, the bio-methane potential (BMP) can be calculated as follow:

$$BMP = \frac{V_{substrate\&inoculum} - V_{inoculum}}{VS_{substrate\_added}} \quad [1.3]$$

2.3 Relevant compatible solutes

High salinity triggers both hypertonic and hyperosmotic stress and can lead to dehydration of the cytoplasm (Kempf and Bremer, 1998, Mahajan and Tuteja, 2005). Therefore, high salt concentrations in anaerobic digestion systems are strongly inhibitory to methanogens at mesophilic temperatures (Feijoo et al., 1995, Chen et al., 2008).

Usually two principal strategies are available for cells to survive the osmotic stress inherent in occurrence of high salinity: (a) the "salt-in" strategy. Cells maintain high ion concentrations to balance external osmotic pressure mainly through uptake of potassium, and all endo-enzymes have to adapt to the new conditions (Müller et al., 2005). (b) the "compatible-solutes (or osmoprotectants)" strategy. Cells maintain low ion concentrations, but with the help of accumulation of compatible solutes intracellularly to overcome osmotic stress, and the adaption of endo-enzymes are not necessary any more (Müller et al., 2005). These compatible solutes are either synthesized within cells or provided by the medium (Oren, 1999). Mostly uptake of compatible solutes from exogenous sources are energetically more favourable than synthesis (Martin et al., 1999).

Compatible solutes are defined as an group of chemically heterogeneous compounds that are highly soluble and carry no net charge at physiological pH (Kempf and Bremer, 1998). However the spectrum used by microorganisms is limited, including amino acids like proline, quaternary ammonium compounds such as glycine betaine, diverse sugar alcohols, trehalose, etc (Hincha, 2006). Aside from the premier role in withstanding high-osmolarity environments, osmoprotectants also can serve as effective stabilizers of enzyme function, protecting cells against high temperature, freeze-thaw treatment and even drying (Welsh, 2000). Figure 6 presents chemical structures of two types of osmoprotectants studied in this project.

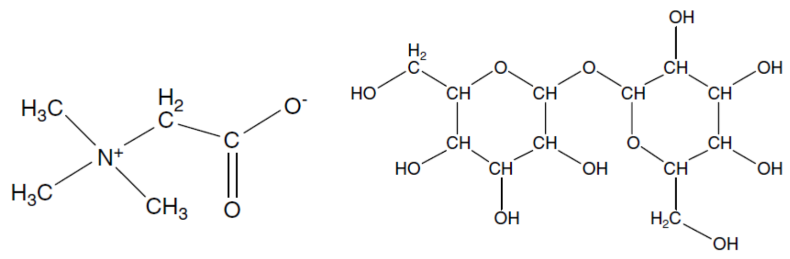

Figure 6 Chemical structures of two types of osmoprotectants

2.4 Phosphorus distribution

Phosphorus exists in wastewater in a number of different chemical forms, and has been described in general as dissolved and particulate phosphorus. Each fraction is made up of three components, reactive and acid-hydrolysable as well as organic parts (Spivakov et al., 1999).

Dissolved reactive phosphorus often refers to orthophosphate, occurs chiefly in four distinct species according to pH: H_3PO_4, $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-} (Warwick et al., 2012). It is the end product of bacterial mineralization of particulate and dissolved organic phosphorus forms, and can be directly assimilated by organisms (Ahlgren, 2006). When measuring total phosphorus organic part has to be converted into orthophosphates by acid digestion (Warwick et al., 2012).

To further investigate the phosphorus distribution, a new method was developed to determine orthophosphate, dissolved " organic" phosphorus, particulate "organic" phosphorus and metal bound phosphorus. Organic concept here, written between quotation marks, integrates acid-hydrolysable and organic phosphorus for both dissolved and particulate fractions. To reveal how these phosphorus fractions distribute, four measurements need to be conducted, namely total (P-total) and orthophosphate (P-ortho) in a sample without filtration, and P-total and P-ortho in the filtrate which passed through 0.45μm filter (Scherrenberg et al., 2008).

With results of four measurements, the amounts of four phosphorus fractions are determined and presented in Figure 7. In most cases only orthophosphate and total phosphorus are necessary for monitoring performances of bioreactors.

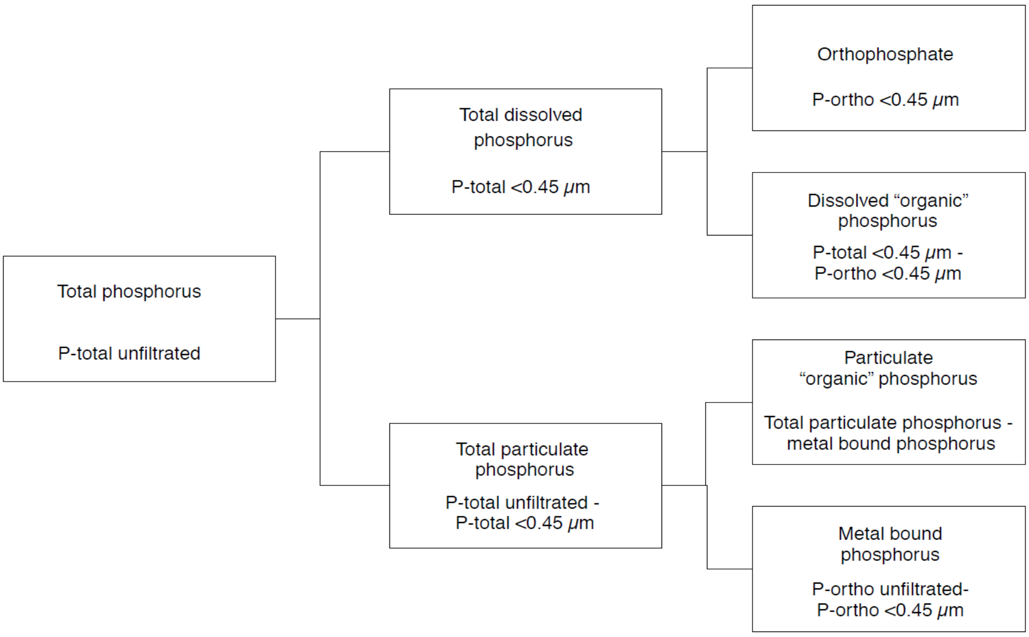

Figure 7 Phosphorus distribution. Source:(Scherrenberg et al., 2008)

2.5 Phosphatase enzymes

Phosphatases are a class of hydrolytic enzyme that catalyse the liberation of orthophosphate from organic phosphorus compounds. They are basically categorized into five groups: (1) phosphomonoesterases (E.C. 3.1.3.) (E.C. stands for enzyme commission number, which is a numerical classification scheme for enzymes); (2) phosphodiesterases (E.C. 3.1.4.); (3) triphosphoric monoesterases (E.C. 3.1.5.); (4) phosphoryl-containing anhydrides hydrolases (E.C. 3.6.1.) and (5) P-N bonds hydrolysing phosphatases (E.C. 3.9.) (Criquet et al., 2004). Among these types phosphomonoesterases are often identified as the primary phosphatases in soil or litter due to their low substrate specificity (Turner et al., 2002). In accordance with pH-optima of enzyme activity, phosphomonesterases is subdivided into acid and alkaline phosphatases (E.C. 3.1.3.2. and E.C. 3.1.3.1. respectively). Both types of phosphatase can be released by bacteria, fungi and yeast (Criquet et al., 2004).

Acid phosphatases function well in the pH range of 4 to 6. As intracellular enzymes they free bound phosphate groups from internal P-containing compounds to meet relevant requirements of energy yielding phosphorylation relations in cells. Alkaline phosphatases, very effective in neutral and basic pH conditions, typically appear in the cell envelope as extracellular hydrolytic enzymes and account for providing cells with an alternative source of orthophosphate, and will be induced by the presence of inorganic phosphorus at growth limiting concentrations (Fintan Van Ommen and Gill, 2004). It has been observed that phosphatase activity highly correlate with population of acid producing and phosphatase producing bacteria in the anaerobic digesting sludge (Ashley and Hurst, 1981).

Some studies imply that phosphatase is an indicator of imminent failure or instability of the anaerobic digestion process because of its easier detection than other enzymes and quicker feedback over pH, VFA and gas analysis under an unstable state (Ashley and Hurst, 1981, Zhenglan et al., 1990).

2.6 Struvite

2.6.1 Chemistry of struvite crystallization

Struvite is a white crystalline substance with orthorhombic structure, composed of magnesium, ammonium and phosphorus in stoichiometric concentrations ($MgNH_4PO_4 \cdot 6H_2O$) (Doyle and Parsons, 2002). Its precipitation reaction is expressed as follows:

$$Mg^{2+} + NH_4^+ + PO_4^{3-} + 6H_2O \leftrightarrow MgNH_4PO_4 \cdot 6H_2O \, [1.4]$$

The spontaneous formation of struvite crystal takes place in supersaturated solution where the combined concentrations of three components exceed the struvite solubility product. The solubility product is temperature and pH dependant, and for solubility of struvite pH is of great concern. As pH increases, struvite solubility declines (Marti et al., 2008). It may encounter an apparent decrease as well due to foreign ions in solution (Le Corre et al., 2005).

There are mainly three other possible phosphate magnesium salts co-existing with struvite in a solution containing Mg^{2+}, $NH_4^+$and PO_4^{3+}, namely newberyite ($MgHPO_4 \cdot 3H_2O$) and trimagnesium phosphates ($Mg_3(PO_4)_2 \cdot 22H_2O$ and $Mg_3(PO_4)_2 \cdot 3H_2O$). The precipitation of which salt depends on pH level of solution. Struvite is favoured at neutral and higher pH and at Mg/Ca molar ratios more than 6; newberyite is always found at pH lower than 6; two states of trimagnesium phosphates precipitate outside the range of 6 to 9 (Marti et al., 2008). Therefore struvite is considered as the main precipitate formed in anaerobic digestion processes.

2.6.2 Factors influencing struvite crystallization

The struvite crystallization is separated into two stages: crystal nucleation (primary and secondary) and crystal growth. Primary nucleation occurs when constituent ions combine to form the first crystal nuclei in solution; and further formation of nuclei in presence of other struvite crystals (secondary nucleation); and then enlargement of struvite crystals until equilibrium (Mehta and Batstone, 2013).

Several parameters attribute to struvite crystallization, in terms of its growth rate and crystal size distribution, which are discussed here.

pH

pH has a direct influence on solubility of struvite and supersaturation during the precipitation process. The solubility of struvite decrease as pH level increase in solution. Many papers confirmed that the optimum pH for struvite precipitation ranges from 8.5 to 11 (Nelson et al., 2003, Bouropoulos and Koutsoukos, 2000, Doyle and Parsons, 2002, Kim et al., 2009). Supersaturation, the thermodynamic driving force for crystallization, is found to reach maximum value itself between pH 9 and pH 10 (Ronteltap et al., 2010).

Moreover, the speciation of struvite constituents is pH dependent. The change in this speciation from a slight variation of pH will result in more or less favourable conditions for struvite precipitation. For example, more ammonium will be transformed to ammonia due to the increasing pH, which seriously affects the production of struvite. It goes in the reaction [1.2] (section 2.2.2).

Temperature

The effect of temperature on struvite crystallization is associated with the solubility of struvite. It is generally accepted that as temperature increases struvite becomes more soluble. Nevertheless, the maximum solubility of struvite at a certain temperature varies greatly between different studies. (Borgerding, 1972) working on anaerobic digestion system of a wastewater treatment plant concluded that 20°C is the temperature of maximum struvite solubility. However, Burns and Finlayson (1982) obtained a maximum struvite solubility at 38°C instead of 20°C during their experiments.

Mg:P ratio

Struvite forms in the theoretical Mg:N:P molar ratio of unity, but the solution should be in supersaturation initially in order to ensure crystallization proceeding, as supersaturation has a tremendous impact on mechanisms appearing in the crystallization process.

To initiate struvite precipitation, supplementation of magnesium from an external source is always necessary because Mg^{2+} concentration is relatively low compared to the others in wastewater or sludge. Various researches have verified that at a given pH and N:P molar ratio, an increase of Mg:P ratio induced a rise in removal efficiency of phosphorus (Stratful et al., 2001, Adnan et al., 2003b, Rahaman et al., 2008). The suitable ratio of 1.3:1 (Mg:P) is suggested at full scale to guarantee maximum phosphorus removal as struvite given possible competing reactions (Jaffer et al., 2002).

Seeding

Addition of seeding materials in solution, like pre-formed struvite, is an incentive to the struvite crystallization by providing sufficient contact surface. According to Kim et al. (2007) both crystal nucleation and growth are crystallization-control at low level of seeding material, while at high level of seeding material the mechanism of crystal growth is predominant for struvite precipitation. However, some observations indicate that seeding has little impact on struvite crystallization because fresh nucleus were produced with larger surfaces compared to seeding crystals and equilibrium reached quickly before the growth could happen on the surface of seeding crystals (Rahaman et al., 2008).

Foreign ions

Anaerobic digested sludge contains far more than three fundamental constituents of struvite. And their presence in solution impacts struvite crystallization from several aspects, including variation in solubility due to ionic strength, inhibition of crystal growth from competing ions, as well as the replacement of struvite constituents in the crystal structure.

Generally speaking, struvite solubility is apparently enhanced in saline solution of both inorganic and organic salts, with the increasing normality of solution (the gram equivalent weight of a solute per liter of solution) (Andrade and Schuiling, 2001). A further relevant investigation has proved that the presence of salts of strong acids and weak bases promotes struvite solubility, leading to a decrease in struvite precipitation (Andrade and Schuiling, 2001).

Ca^{2+} usually exists in anaerobic digestion system, so its competition for orthophosphate with Mg^{2+} is the main concern in struvite crystallization. Because calcium ions are more likely to interact with orthophosphate to form the amorphous hydroxyapatite ($Ca_5(PO_4)_3OH$) at expense of struvite, with molar ratios of Ca : Mg of 1:1 and above (Le Corre et al., 2005).

In addition, it has been found already on lab-scale that divalent ions for instance Mn^{2+}, Fe^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+} and Cd^{2+} can take the position of Mg^{2+} in the struvite structure at ambient temperature

(Bridger et al., 1962). Also the replacement of NH_4^+ by K^+ in crystals became possible in laboratory experiments (Banks et al., 1975).

Mixing

In the precipitation process, both struvite formation and removal efficiencies of nitrogen and phosphorus are directly influenced by mixing types and intensity(G) as well as duration(t_d). The degree to which impact can reach is closely dependent on individual situation.

It is believed that low turbulence reduces dissipation of precipitants and brings a local higher supersaturation favourable for crystal nucleation (Mersmann, 1995). So that is why smaller crystal sizes of struvite were distributed with the weak magnetic stirrer compared with propeller in urine (Ronteltap et al., 2010). On the other hand, high turbulence is helpful in stripping CO_2, which leads to an increase in pH and therefore an increase in struvite precipitation (Doyle and Parsons, 2002).

Moreover, the product of G and t_d Gt_d was in use to describe the mixing energy on the struvite precipitation process by Kim et al. (2007). He found in his study that sufficient mixing energy (Gt_d) was instrumental in inducing the potential of struvite crystallization and growth, and the removal of nitrogen and phosphorus. More specifically, struvite was the only end precipitate once the dimensionless Gt_d was over 10^5.

3 Research problem

- Few research was carried out regarding anaerobic digestion of concentrated sludge with high salinity and COD from fish farms. In an recent study on digesting undiluted sludge from saline fish farm effluents the mesophilic CSTR was never stabilized throughout the whole operational period (Gebauer, 2004). This failure came from the usage of an impropriated inoculum in their digesters, which was developed from a mixture of digested municipal sewage sludge and cow manure and only adapted to low-salinity surroundings. Therefore, the **first** research question was formulated : With the inoculum already adapted to high salinity can the CSTR performance be improved during anaerobic digestion of saline sludge?

  To answer this question, the sludge from a full-scale anaerobic digester was collected as the inoculum in the studied reactors. That digester has been operated for more than 4 years at a salinity of 17g/L. The CSTR performance in the current investigation would be evaluated by comparing with relevant results published by Gebauer (2004).

- Current anaerobic digesters are often very simple and running at a low OLR, far from the maximum capacity of the digestion process, to prevent overload. So it would be quite beneficial if this extra capacity could be utilized in practice. According to Gómez et al. (2011) increasing OLR of digesters improved the efficiency of organic conversion to methane and led to higher organic matter removal and volumetric methane production rate when dealing with municipal sewage sludge. Therefore, the **second** research question was proposed : When CSTRs are fed with highly saline sludge, can the increasing OLR still enhance the methane yield and to what extent?

  To response this question, two CSTRs were run at same conditions except OLR. By means of doubling OLR in one of reactors, the potential difference between both CSTRs on biogas and methane production would be expected and monitored.

- Mixing is very helpful in distributing organisms uniformly over the reactor, transferring heat and reducing particle sizes as well as removing biogas from the mixture. Many regimes exist, such as mechanical mixers, recirculation of digester contents and biogas recirculation. The mixing regime is capable of affecting the anaerobic digestion process by supplying various degrees of contact between substrate and a viable microbial population. A research confirmed that the influence of mixing regimes became substantial when digesting thicker manure slurry with TS of 100 and 150g/L (Karim et al., 2005). Thus in terms of the studied sludge with similar TS (Avg.103.9 ± 19.5g/L), the **third** question was clear : Whether CSTR behaves differently under different mixing regimes and which mixing regime can supply a higher specific methane yield?

  To solve this question, two CSTRs were operated under same conditions excluding the mixing regime. One used impellor-stirring, while the other used biogas recirculation. The resulting specific methane yield of both reactors would be compared in the following discussion part.

- Highly saline organic matter is often poorly biodegradable in conventional anaerobic digesters with non-adapted biomass because of sodium toxicity. However, Vyrides et al. (2010) discovered in his research that the usage of compatible solutes can improve the anaerobic digestion process when sewage sludge was directly exposed to high salinity conditions. This report drives the **fourth** research question : Whether these compounds can enhance methane potential of saline substrate together with inoculum already adapted to high salinity? The compatible solutes in the current study refer to betaine and trehalose.

- The inorganic coagulant, ferric chloride ($FeCl_3$) is being utilized to coagulating sludge prior to sedimentation process in the EM-MARES project. However, iron would be considered as one of toxic heavy metals because it is non-biodegradable and can accumulate to a potentially toxic concentration. Consequently, the **fifth** question should be investigated : Whether addition of $FeCl_3$ influences the methane potential of saline sludge from brackish RAS, and is it a better inorganic coagulant for the EM-MARES project than the other common coagulant poly aluminium sulphate (PAS)?

  To reply the fourth and fifth question, several groups of BMP assays were designed and conducted in accordance with the principals described in section 4.2.

- Besides improving biogas yield of the digester, the EM-MARES project also aims at recovering phosphorus in the form of struvite. During the anaerobic digestion process phosphatase is responsible for PO_4^{3+} release. As a consequence, it is of great importance to understand the potential factors influencing phosphatase activity (PA) in the digester, such as compatible solutes and shear stress from biogas circulation. The **sixth** research question involving PA consists of two sub-questions : 1) If and how compatible solutes affect PA? and 2) If and how shear stress from biogas recirculation affect PA?

  To answer these sub-questions, a series of PA batch tests were performed. More details about test design are given in section 4.3.3.

- The size of struvite crystal produced was various depending on operational conditions of anaerobic digesters (Wang et al., 2006, Münch and Barr, 2001). Until now information is lacking regarding particle size distribution of struvite crystal in anaerobic digestion with saline sludge from brackish RAS. As a result, the **last** research question was of interest : How struvite size distribute in the saline sludge? Depending on different operational parameters the question can be studied in three aspects : 1) How will struvite size distribution vary with Mg/P molar ratio? 2) How will struvite size distribution vary with temperature? and 3) How will struvite size distribution vary with pH?

  To solve these sub-questions, the filtrate of CSTR digestate should be prepared during experiments. However, due to the filtrate limitation, the synthetic brackish solution were mainly utilized instead of filtrate to simulate the possible particle size distribution in the following jar tests.

4 Materials and methods

4.1 Routine analysis of reactors

4.1.1 Experimental setup

Three lab-scale continuously stirred tank reactors (CSTR) were employed in this study, all of which were round-bottom cylindrical, jacketed glass chambers. Temperature of each digester was maintained within the mesophilic range (35±1°C) by circulating temperature-controlled water through a water bath (Tamson TC16). The operational volumes were 7L for reactor 1 and 2 and 5L for reactor 3. First two reactors were continuously mixed by recirculation part of the biogas, which was collected at the top of reactor and pumped through a variable speed centrifugal pump (KNF Neuberger PM25370-86) into the bottom. Reactor 3 was stirred continuously using an impeller instead of recycling biogas. The produced biogas firstly entered a demi-water bottle for immediate cooling to the ambient temperature so as to ensure accurate counting in the following biogas meter (Ritter MiliGascounter MGC-1 PMMA). Then the gas was passed through alkaline conditions to capture CO_2 and H_2S, where 200mL of 3M NaOH solution and 0.5mL of 0.4% Thymolphthlein pH indicator were added. So the remaining biogas was nearly pure methane, and its production was measured by another identical gas meter. The biogas-recycling pump or impeller motor (Micromotors series HL149), biogas meter and methane meter in each reactor were connected to the individual custom central controlling system (R1and R2 share LabVIEW 2010-Version 10.0.1; DASYLab 11 for R3), with which all reactors could be controlled independently and data could be continuously recorded. The feeding and sampling of all reactors were done manually, though a syringe of 100mL. Figure 8 and 9 present the system schemes of three reactors.

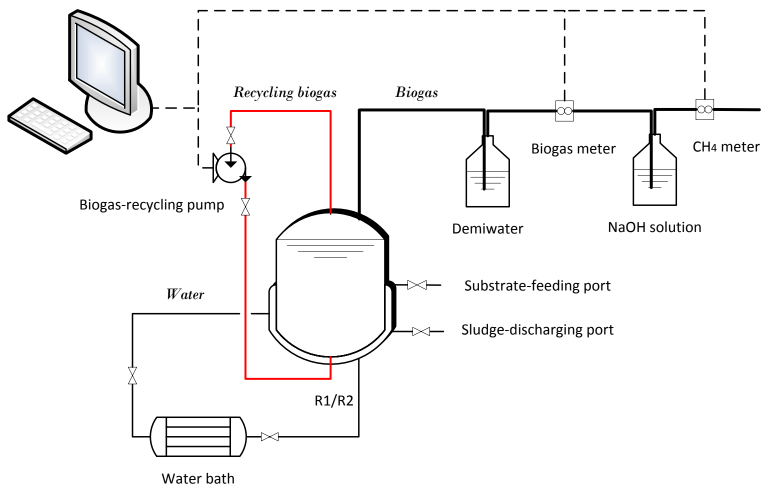

Figure 8 System scheme of Reactor 1 or 2

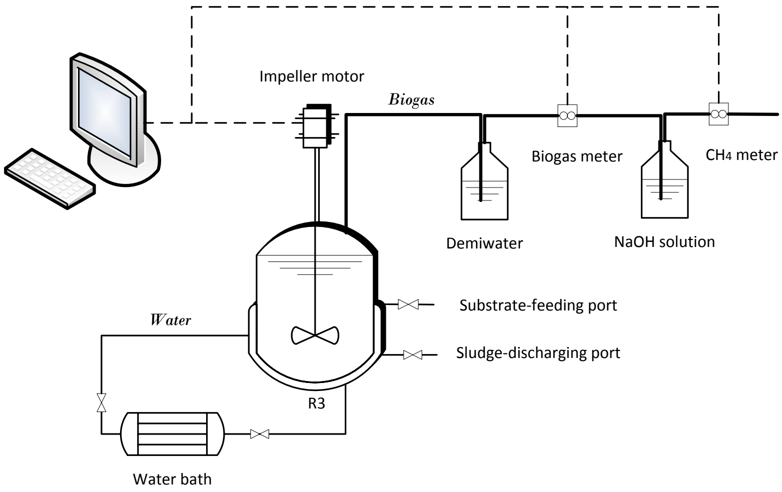

Figure 9 System scheme of Reactor 3

4.1.2 Operational conditions

All reactors were operated in the semi-continuous mode where feeding and effluent withdrawal were carried out every two days. Both volumes of feeding and discharging were 250mL each time. Sludge was discharged at the base of digesters, prior to substrate addition at the middle port. The pH levels of all reactors were maintained within the range of 7.51 to 8.15 as a result of internal alkalinity. Table 5 is a summary to display different operational conditions of all reactors, containing hydraulic retention time (HRT), OLR, and total operational period, etc. Here, only ranges of OLR for each reactor are given, and more details about its variation are reported in section 5.1.

Table 5 Operational conditions for three digesters

No.	Sludge volume (L)	Mixing intensity	HRT (d)	OLR (kg COD/(m^3·d))	Operational period (d)
R1	5.2	80L biogas/h	42	0.93 – 2.97	360
R2	5.2	80L biogas/h	42	1.87 – 3.44	358
R3	4.0	40rpm	32/42	1.86 – 4.42	400

4.1.3 Substrate for reactors

The substrate (sludge) was collected every three months from the brackish fish farm GrovisCo, located in Stavenisse, Netherlands, where a recirculating brackish aquaculture system is applied. It was stored frozen at -20°C till being used in lab. Basic analyses were taken for this substrate and the brackish water that was collected from the same site and used to dilute substrate for R1. The salinity of brackish water was assumed to be as same as substrate. Results are presented in Table 6 and 7.

Table 6 Substrate composition from direct measurement of substrate

Parameter	Range	Mean value
pH	5.75-6.31	6.14 ± 0.18
Salinity (g/L)	13.5-14.7	14.0 ± 0.36
EC (mS/cm)	20.4-24.7	22.4 ± 1.46
ORP(mV)	-196 – -217	-206 ± 6.11
TS (g/L)	82.0 ± 1.0 – 136.3 ± 0.6	103.9 ± 19.5
VS (g/L)	57.7 ± 3.1 – 110.4 ± 4.4	75.9 ± 18.5
TSS (g/L)	50.1 ± 2.2 – 110.7 ± 5.2	77.0 ± 19.6
VSS (g/L)	45.5 ± 1.8 – 94.3 ± 4.4	66.6 ± 16.5
tCOD (gO_2/L)	77.2 ± 5.4 – 143.1 ± 9.8	104.0 ± 23.4
sCOD (gO_2/L)	7.2 ± 0.6 – 24.3 ± 0.1	13.9 ± 5.73
TN (gN/L)	2.05 ± 0.21 – 6.65 ± 0.49	4.00 ± 1.31
TP (gP/L)	0.81 ± 0.08 – 1.79 ± 0.35	1.25 ± 0.32
NH_4^+ (mgN/L)	172.0 ± 4.0 – 406.7 ± 15.1	327.3 ± 86.5
PO_4^{3-} (mgP/L)	95.0 ± 1.1 – 242.0 ± 0.0	173.7 ± 56.2

Table 7 Ion composition from direct measurement of brackish water

Ion composition	Concentration (ppm)
B	6.1
Br	29.0
PO_4	0.2
C	81.4
Ca	270.1
Cl	9080.0
Fe	1.3
K	133.7
Mg	266.0
Na	4674.0
SO_4	962
Sr	9.2
Si	6.5

4.1.4 Methods for routine analysis

The performances of the reactors were monitored by analysing sludge samples weekly. The relating measurement items include total and soluble chemical oxygen demand (tCOD and sCOD, respectively), total phosphorus (TP) and reactive phosphorus (RP), total nitrogen (TN), ammonium (NH_4^+), total and volatile suspended solids (TSS and VSS respectively), as well as total solids and volatile solids (TS and VS, respectively). TSS, VSS, TS and VS were determined according to standard methods provided by APHA (2005), and the rest were analysed through direct usage of Merck Spectroquant® cell test kits. All of them were done in triplicate for statistical significance. Some weekly off-line measurements were also necessary for the studied sludge in digesters, for instance the pH, electrical conductivity, salinity and temperature.

4.2 Biomethane potential (BMP) assay

4.2.1 Instrument setup

BMP assay was performed by using Automatic Methane Potential Test System II (AMPTS II), which is developed by BPC to measure ultra-low biogas and Biomethane flows generated from anaerobic digestion of biological degradable substrates and display real-time data.

The AMPTS II is made of three parts, sample incubation unit, CO_2-fixing unit and gas volume measuring device (Figure 10). In sample incubation unit, maximum 15 vials of 500mL can be run simultaneously. Each vial is filled with certain quantities of substrate and anaerobic inoculum, and incubated at desired temperatures. Periodical mixing in each vial is produced by a slow rotating agitator. In order to measure methane production, the generated biogas passes through a NaOH solution in a CO_2-fixing unit, removing several acid gas fractions such CO_2 and H_2S by chemical reactions. This highly alkaline solution in each vial is monitored by a pH indicator Thymolphthalein. The third unit is to analyse the exact methane production through a wet gas flow measuring device, and record and display the dynamic degradation profile of any substrate by means of a connected computer or laptop.

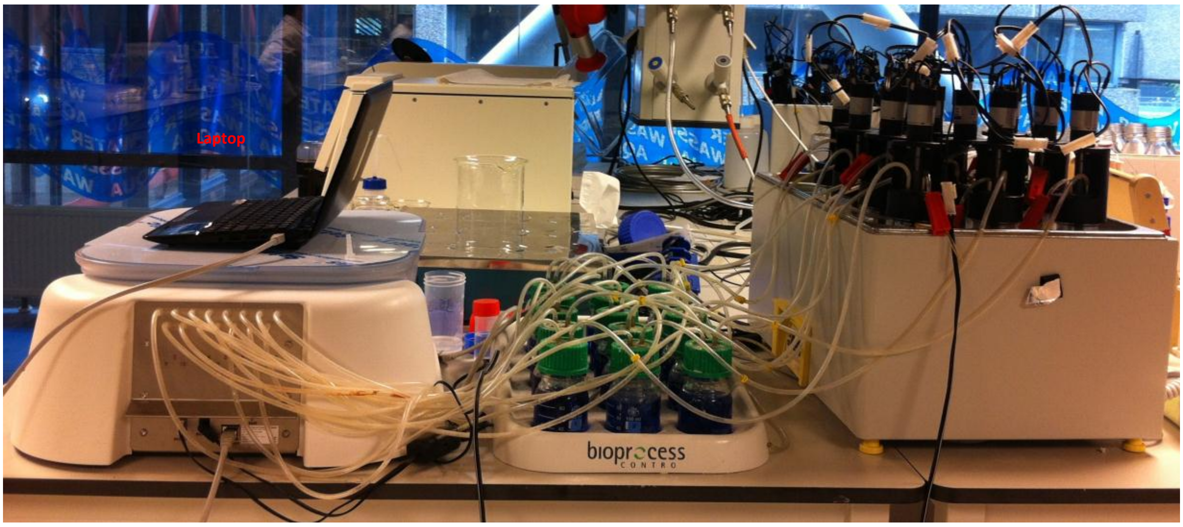

Figure 10 The instrument setup of AMPTS II

4.2.2 Procedure and materials

The inoculum used in BMP assays was collected from R2 under steady-state condition. Depending on the ratio of inoculum to substrate and total volume of both materials in a vial, the amount of substrate and inoculum in a sample were calculated:

$$\frac{V_{Inoculum} \times VS_{Inoculum}}{V_{Substrate} \times VS_{Substrate}} = 2 \ [1.5]$$

$$V_{Inoculum} + V_{Substrate} = 200mL \ [1.6]$$

Also phosphate buffer, macronutrients and trace elements shown in Table 8 were added to each vial together with inoculum and substrate. The headspace of each vial was flushed with nitrogen gas for 2min. Then vials were sealed with installed agitators and put in the sample incubation unit which had been prepared at 35°C. The NaOH bottles in CO_2-fixing unit contained 100mL of 3M NaOH solution and 0.5mL of Thymolphthlein (0.4%). Once all tubes and motor connections were completed the AMPTS II was started where all motors were switched on and methane production was recorded and dynamically displayed. Normally the experiments were run for a period of about 20 days until accumulated methane production remained stable. All samples were done in triplicate.

For all samples of batch tests, sources of inoculum and substrate were the same. Table 9 demonstrates VS values of substances used in batch tests.

Table 8 Dosages and constituents of phosphate buffer, macronutrients and trace elements used in BMP tests

Solution	Dosage (per vial)	Constituents	
Phosphate buffer	10mL	$K_2HPO_4·3H_2O$	0.2 M
		$NaH_2PO_4·2H_2O$	0.2 M
Macronutrients	1.2mL	NH_4Cl	170 g/L
		$CaCl_2·2H_2O$	8 g/L
		$MgSO_4·7H_2O$	9 g/L
Trace elements	0.12mL	$FeCl_2·4H_2O$	2 g/L
		$CoCl_2·6H_2O$	2 g/L
		$MnCl_2·4H_2O$	0.5 g/L
		$CuCl_2·2H_2O$	30 g/L
		$ZnCl_2$	50 g/L
		HBO_3	50 g/L
		$(NH_4)6Mo_7O_2·4H_2O$	90 mg/L
		$Na_2SeO_3·5H_2O$	100 mg/L
		$NiCl_2·6H_2O$	50 mg/L
		EDTA	1 g/L
		HCl 36%	1 mL/L
		Resazurine	0.5 g/L

Table 9 Material VS and volume requirements per vial in batch tests

Batch test		VS (g/L)	Volume(mL/vial)
Compatible solutes dosage	Inoculum	24.9	168.2
	substrate	66.0	31.8
Inorganic coagulants dosage	Inoculum	24.9	162.8
	substrate	66.0	31.8

4.2.3 Batch tests

- Compatible solutes dosage
  To investigate the effect of compatible solutes on BMP, BMP test was conducted as previously described, in which four samples were conducted: sludge with 0.5g/L betaine, sludge with 0.5g/L trehalose, sludge with 0.25g/L betaine and 0.25g/L trehalose, and sludge without any compatible solute. Each sample was performed in triplicate.

- Inorganic coagulants addition
  The second run of BMP assay was carried out as above but with the objective of studying the effect of inorganic coagulants on BMP. Again four different samples were conducted: sludge with 6.0gFe/L (dose $FeCl_3$), sludge with 2.4gAl/L (dose PAS), sludge without any inorganic

coagulant, and sludge where inoculum remained same but substrate was replaced by the equal volume of cellulose. Each sample was done in triplicate.

4.2.4 Hydrolysis constant estimation

The first-order kinetic model is a simple and useful model that frequently applied to describe the hydrolysis progress during anaerobic digestion. The model defines substrate utilization rate as function of substrate concentration only. Many aspects such as microbial growth and decay or concentration are not considered. The basic equation is :

$$dS / dt = -k_h \bullet S \, [1.7]$$

Where k_h is the first-order kinetic constant ($time^{-1}$), t is the digestion time and S represents the biodegradable substrate concentration. AS S is a difficult parameter to measure, it is preferable to derive the model by using the measurement of methane, which is much easier to determine:

$$B = B_{max} \bullet \left(1 - e^{-k_h t}\right) [1.8]$$

Where B (mL CH_4/g VS) represents the cumulative methane yield, B_{max} (mL CH_4/g VS) is the maximum methane yield of substrate, k_h (d^{-1}) is the first-order hydrolysis rate constant and t (d) is the time.

The first-order hydrolysis rate constant was determined by minimizing the difference in methane yield between the measured and modeled values through using the Solver Tool in EXCEL worksheet.

4.2.5 Biodegradability based on methane yield

Biodegradability of substrate is defined as percentage of ultimate methane yield measured by BMP test to theoretical methane production (Raposo et al., 2011), as follows :

$$Bio\,deg\,radability\,(\%) = \frac{V_{max}}{V_{Th}} * 100 \, [1.9]$$

Where V_{max} (mL) is the ultimate methane yield, V_{Th} (mL) is theoretical methane yield, which can be calculated in the following equation :

$$V_{Th} = VS_{added} \bullet (gCOD / gVS) \bullet 350 \bullet 10^3 \, [2.0]$$

Theoretically, 1kg COD can be converted into $0.35m^3$ CH_4. The value of gCOD/gVS was determined in experiments.

4.3 Phosphatase activity assay

4.3.1 Mechanism

Phosphatase hydrolyzes the organic monophosphate ester p-nitrophenyl phosphate (p-NPP) with the formation of a p-nitrophenol (p-NP) and a phosphate group. The phosphate group is adopted by phosphate acceptors present in the reaction medium. Under the alkaline condition the released p-nitrophenol is stained as p-nitrophenolate, and measured spectrophotometrically at 405nm (Figure 11).

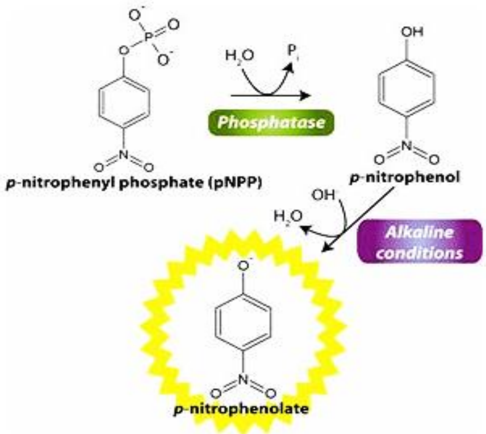

Figure 11 Mechanism for PA assay. Source: (G-Biosciences, 2013)

4.3.2 Procedure of PA assay

The procedure for PA assay followed the description from Anupama et al. (2008). Firstly, 2mL of sludge sample is added to 8mL acetate-acid buffer of 0.2M (pH=4.8) for acid PA analysis, or 4mL carbonate-bicarbonate buffer of 0.1M (pH=9.6) for alkaline PA analysis. Then the mixture is sonicated with a Ultrasonic Cleaner (Cole Parmer EW-08895-01) for 1min at an ambient temperature. To initiate the reaction, 2mL p-NPP was added as substrate to the solution and mixed thoroughly. Before incubation in Innova 44 Incubator Shaker at 130 rpm and 35.5° C, the mixture was deoxygenated with nitrogen gas for 1min. After one-hour incubation, 2mL of 1M NaOH was used to end the reaction and stain p-NP. The resulting sample was then centrifuged (Sorvall ST 16R) at 9000rpm for 20 min and the absorbance of supernatant was read at 405nm from a spectrophotometer (GENESYS 6).

Assuming that blank absorbance of all chemical reagents are insignificant, only the blank absorbance of sludge must be determined for each sample to account for the absorbance of sludge. The previous procedure was repeated and each chemical reagent was substituted with an equal volume of demi-water. This blank absorbance of sludge should be subtracted from results of acid PA or alkaline PA measurements.

The final absorbance associated with acid or alkaline PA was interpreted by p-NP calibration curve (Appendix 1) to the corresponding concentration of p-NP in solution. Then dividing the resulting concentration by the incubating period of 1h resulted in acid or alkaline PA in $\mu mol/(L \cdot h)$. The total PA is the sum of acid and alkaline PA. All the samples were analysed in triplicate for statistical significance. The scheme of PA assay is illustrated in Figure 12.

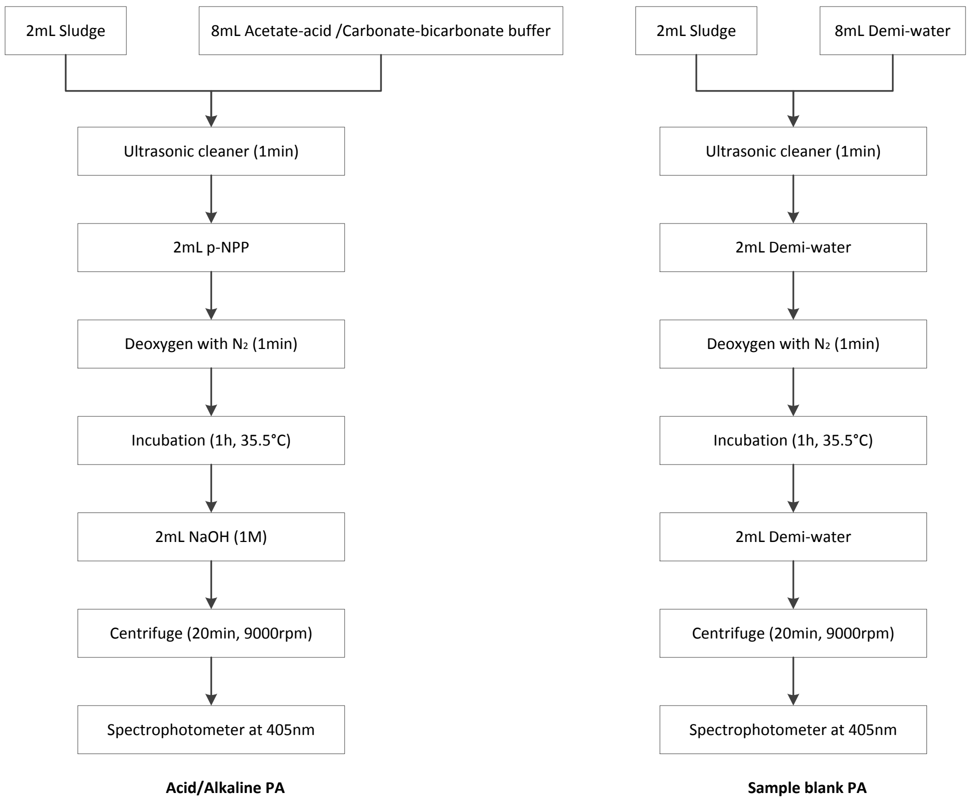

Figure 12 Scheme of PA assay

4.3.3 Batch tests

To study the effect of compatible solutes, $MgCl_2$, and shear forces from biogas recirculation on PA, three groups of batch test were performed in accordance with PA assay mentioned previously. The sludge in R3 was employed as inoculum and substrate from brackish RAS was added in case of substrate limitation during testing. All tests were carried out in triplicate.

- Effect of compatible solutes on PA

  Four samples were studied here : sludge with 1mM trehalose, sludge with 1mM betaine, sludge with 0.5mM trehalose and 0.5mM betaine, and sludge without any compatible solute. All the samples were anaerobically incubated at 130rpm and 35.5°C for 72h. The PA was monitored during PA course.

- Effect of Shear forces on PA

  Shear force from biogas recirculation was divided into three classes on the basis of gas velocity in the research : 0.018m/s, 0.035m/s and 0.057m/s biogas. At the beginning of the experiments, 150mL of sludge and substrate were fed into a glass tube that was 1m in length and 20mm in diameter and temperature-controlled at 35.5°C, and purged with N_2 for 2min to create an anaerobic condition. Part of the biogas collected in the gas container was transferred to the bottom of the tube by a gas-recycling pump. The pump was controlled through voltage, on the basis of biogas flow-voltage characteristic of the pump. PA changes were tracked in 12h. Table 10 presents the voltages required and shear rates generated at three gas velocities.

Table 10 Voltage and shear rate at the corresponding gas velocity

v_{gas} (m/s)	D (mm)	Q_{gas} (L/h)	Voltage (V)	γ(1/s)[a]
0.018	20	20.5	1.12	1.76
0.035	20	40	1.42	9.36
0.057	20	65	1.81	31.98

[a] Shear rate (γ) was calculated following the equation :

$\gamma = 3.26 - 3.51 * 100 * v_{gas} + 1.48 * 10^4 * v_{gas}^2$ while v_{gas} is in range of 0.004m/s to 0.06m/s (Cerri et al., 2008).

4.4 Particle size distribution

4.4.1 Procedure

The particle size distribution of struvite was measured using HIAC Particle Counter-Model 3000, which covers a range of 2 to 400μm. The container with a sample of 50mL and a stirring bar was put on the stirring plate, and by setting speed knob at level 5 the sample was well mixed. Dead volume that passed the sensor as a retention volume and sample volume were checked at 2mL and 5mL, respectively. First flushing with sample to rinse the sampler system was necessary in order to avoid undesired influence from the previous measurement. The analyses started with pressing [F9] and ended with [F10] on keyboard. In terms of resulting data, it was saved as an ASCII file in the floppy disk and could be processed using EXCEL. Each sample was measured in triplicate. Some reasonable dilution for samples might be required since the maximum detection concentration is 18.000 particles per mL for the sensor.

Original results were displayed on a number basis, describing particle quantity in each particle size class. By assuming that every particle is a sphere, the number distribution of each sample was converted to the volume distribution of particles, as a percentage of total volume of solids. This data interpretation made it straightforward to tell which particle size class the majority of total particle volume came from.

In addition to the volume distribution of particles, the average particle diameter and struvite production should be determined as well. The struvite production is expressed as the mass of struvite produced per unit of liquid volume, and can be measured in the same way as TSS. To obtain the average particle diameter the average particle volume was initially calculated:

Average particle volume= total volume of a sample/total number of particles [2.1]

4.4.2 Jar tests

Following experiments were carried out with a jar test apparatus (VELP-JLT6). 500mL of the synthetic brackish solution or filtrate of R2 supplemented with varying amount of magnesium source ($MgCl_2$) was mixed into the beaker which was covered with a piece of parafilm to reduce NH_3 volatilization. Solution were stirred with paddles at 300rpm over 75min. At the end of the experiments, samples in the steady state were taken to analyse the particle size distribution.

- ***Particle size distribution at different Mg/P molar ratios in synthetic brackish liquid***
  A set of jar tests were undertaken using the synthetic brackish liquid, where Mg/P molar ratio varied from 0.12 to 2.19. All synthetic brackish solution was prepared by using the following

stock solution: 5mol/L NaCl, 3mol/L KCl, 3mol/L NH_4Cl, and 0.1mol/L $Na_3PO_4 \cdot 12H_2O$. The amount of $MgCl_2$ added was determined by the Mg/P molar ratio designed. The pH was maintained at 9.0 with 3M NaOH solution. Solution was kept at the ambient laboratory temperature during experiments. More details about the composition of synthetic brackish solution are provided in Table11. Each time before adding $MgCl_2$ to the synthetic brackish liquid, its salinity was measured and varied from 20.9 to 36.6 g/L.

Table 11 Initial values of batch experiments performed to determine particle size distribution at various Mg/P molar ratio

Sample No.	Ionic strength (M)	$[NH_4^+]$ (mM)	$[P_{ortho}]$ (mM)	Mg/P initial	K (mM)	Na (mM)
1	0.50	243	16	0.12	5.12	150
2	0.59	286	20	0.41	5.12	150
3	0.67	357	19	0.58	5.12	157
4	0.44	179	12	0.91	5.12	150
5	0.43	179	10	1.09	5.12	150
6	0.41	179	8	1.37	5.12	150
7	0.37	143	5	2.19	5.12	157

- ***Particle size distribution at different Mg/P molar ratios in the filtrate of R2***
  First, a 2L filtrate of digestate from R2 was prepared through centrifuging digestate at 12000rpm for 15min, followed by the filtration with 0.45μm filters. The ion composition of filtrate from R2 was analysed, and relevant results are displayed in Table 12.
  Four groups of jar tests were performed on the basis of filtrate. The Mg/P molar ratio was adjusted to 0.5, 1.00, 1.44 and 2.19 by dosing $MgCl_2$ and $Na_3PO_4 \cdot 7H_20$. The pH was maintained at 9.0 with 3M NaOH solution. Tests were performed at a room temperature. The initial salinity of the filtrate of R2 varied in the range of 27.1-30.7g/L before dosing $MgCl_2$.

Table 12 Initial values related to filtrate from R2

Sample No.	Ionic strength (M)	$[NH_4^+]$ (mM)	$[P_{ortho}]$ (mM)	Mg/P initial
1	0.51	257	11	0.50
2	0.61	286	22	1.00
3	0.44	243	4	1.44
4	0.46	243	5	2.19

- ***Particle size distribution at different temperatures in the synthetic brackish liquid***
  Experiments in this part made use of the synthetic brackish solution with Mg/P molar ratio of 1.37 and kept it running at 10°C, 25°C, 35°C, 45°C and 55°C for 75min. pH level was controlled at 9.0 with 3M NaOH solution.

- ***Particle size distribution at different pH levels in the synthetic brackish liquid***
  Again, the synthetic brackish solution with Mg/P molar ratio of 1.37 was used as a source in four jar tests, but at room temperature, and pH was adjusted to 7, 8, 9 and 10, respectively. The analysis were conducted 75min later.

4.4.3 PHREEQC modelling on saturation index for struvite in synthetic brackish liquid

The saturation index (SI) is a measure of the saturation condition, whether a solution is undersaturated or supersaturated, and the magnitude of that saturation condition. The SI is the logarithm of the ratio of ion activity product (IAP) to the minimal solubility product (K_{sp}). Calculating struvite solubility is a complex process because it is affected by multiple parameters. The computer model PHREEQC 2.18.00 was used in this research to perform the equilibrium calculations based on sample data and to determine the molarities and activities of struvite constituents as well as struvite SI for the sample, based on equations [2.2] and [2.3].

$$IAP = \{Mg^{2+}\}\{NH_4^+\}\{PO_4^{3-}\} = \gamma_{Mg^{2+}} C_{Mg^{2+}} \bullet \gamma_{NH_4^+} C_{NH_4^+} \bullet \gamma_{po_4^{3-}} C_{PO_4^{3-}} \text{ [2.2]}$$

$$SI = \log(\frac{IAP}{K_{SP}}) \text{ [2.3]}$$

Where, γ_i is activity coefficient of free ion i; C_i is concentration of free ion i. $\gamma_i C_i$ is specie activity.

PHREEQC 2.18.00 was developed to simulate geochemical reactions in the liquid, gas and solid phases. It utilizes the initial conditions of the system and a database of equilibrium information to calculate the concentration of all species in solution. In its database all the thermodynamic data used to make saturation calculations and parameters for estimating the activity coefficients are incorporated and editable. The standard database employed in the current study was phreeqc.dat, where the minimal solubility product of struvite and the enthalpy of struvite formation DeltaH was set at $5.49*10^{-14}$ (pK_{sp}=13.26) and 5.401Kcal/mol respectively, according to Ronteltap et al. (2007) study on struvite in urine. The outputs of PHREEQC on struvite SI was used as theoretical backgrounds to analysis the different particle size distribution in various synthetic brackish liquids.

5 Results and discussion

5.1 Performances of three anaerobic digesters (CSTR)

To give a complete evaluation of the performances of three CSTRs on lab-scale, the results shown below contain partial data obtained earlier from the same experimental setup (Ferreira, 2012): day 0-90. During the entire operational period, some commonly used indicators were weekly measured for monitoring the anaerobic process, such as total COD, soluble COD, NH_4-N, TN, TP,RP, TSS, VSS, TS, VS, pH and salinity. The flow rate of biogas or methane was expressed as liter per day, and averaged over each week of operation.

5.1.1 performances of Reactor 1

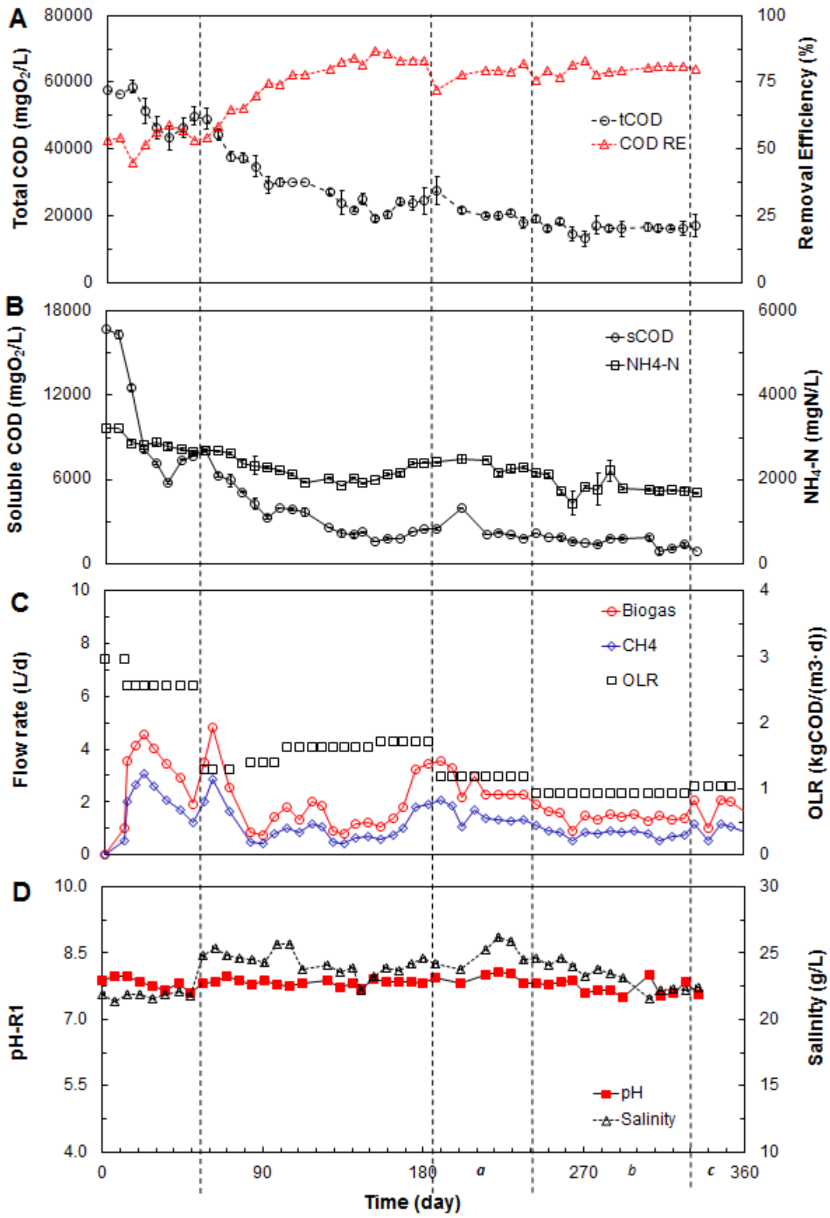

Figure 13 Performance of Reactor 1, A: total COD of sludge & COD removal efficiency, B: soluble COD & NH_4-N of sludge, C: CH_4 & biogas flow rates, D: pH & salinity of sludge

Reactor one (R1) was operated for a period of 360 days with a SRT of 42 days. The operating conditions and performance over the whole period are depicted in Figure 13. It is evident that the pH and salinity in R1 remained stable during 360 days compared to other analysed parameters, and varied within the range of 7.51-8.06 and 21.4-26.2g/L, respectively. Before day 53 undiluted substrate was used for feeding R1; from day 53 onward, substrate was diluted with an equal volume of brackish water in order to make possible a long-term comparison on performance between R1 and R2 at same operational conditions excluding different OLRs.

During the initial 52 days R1 was in start-up with unstable biogas production rates and COD removal efficiencies due to adjustment of microorganisms. Biogas and methane production started at the 10^{th} day of operation.

In the next phase (day 53-186), as OLR rose gradually from 1.29 to 1.72kg COD/ (m^3·d)), COD removal efficiency was improved from 59% to 83%, and total COD and soluble COD decreased by 51% and 16% accordingly. The flow rate of biogas and methane were variable all the time. Despite of a 50% reduction in OLR at the beginning, the methane production continued increasing in the first 8 days due to the remaining substrate in R1, and then sharply decreased from 2.9L/d to 0.5L/d in the following 21 days. During the period of day117-128 the flow rate of methane suffered a 58% of decline from 1.2L/d to 0.5L/d, which was probably caused by the decreasing ratio of COD/SO_4^{2-}. This was related to the competition between SRB and the other anaerobic bacteria involved in the anaerobic digestion process. Low ratio of COD/SO_4^{2-}could lead to the inhibition of methanogenesis and induction of substantial sulphide production in the digester. Subtil et al. (2012) reported that a successful anaerobic treatment was obtained with COD/SO_4^{2-}ratio > 10, however in the present investigation the methane production may already have been influenced at a COD/SO_4^{2-}ratio of 73. Because unlike other CSTRs treating municipal sewage sludge, R1 was fed with highly saline sludge, which could inhibit the activities of methanogens and require methanogens to synthesize or take up more compatible solutes at a substantial energetic cost. Furthermore, the sulphate reduction would be an prevailing terminal-electron-accepting process in the saline environment (McGenity, 2010). The methane-forming bacteria spent approximately 37 days (day 129-166) in adapting to this condition rich in sulphate forms. Later methane and biogas yield was steadily recovered.

During the last phase (day 187-360) the feeding load was maintained at around 1kgCOD/(m^3·d). The digester was in steady state, which was demonstrated by a constant COD removal efficiency of about 80%. The average flow rate of biogas and CH_4 was 1.9 and 1.0L/d, respectively, throughout overall phase. Also the total COD and soluble COD as well as NH_4-N concentration showed a slight variation during the last phase, with average values of which in R1 was 17977mg O_2/L, 1823mg O_2/L and 1982mg N/L, respectively. There was an obvious fluctuation in methane and biogas yield between day195 and day215. Due to the result of a sudden accumulation of VFA in the reactor during day 187-201,followed by its consumption during the next two weeks. The period corresponding to the VFA accumulation and its subsequent consumption was consistent with the initial drop followed by an increase in the methane flow rate. Also the pH decreased by 0.13 from day 187 to day 201.

5.1.2 Performances of Reactor 2

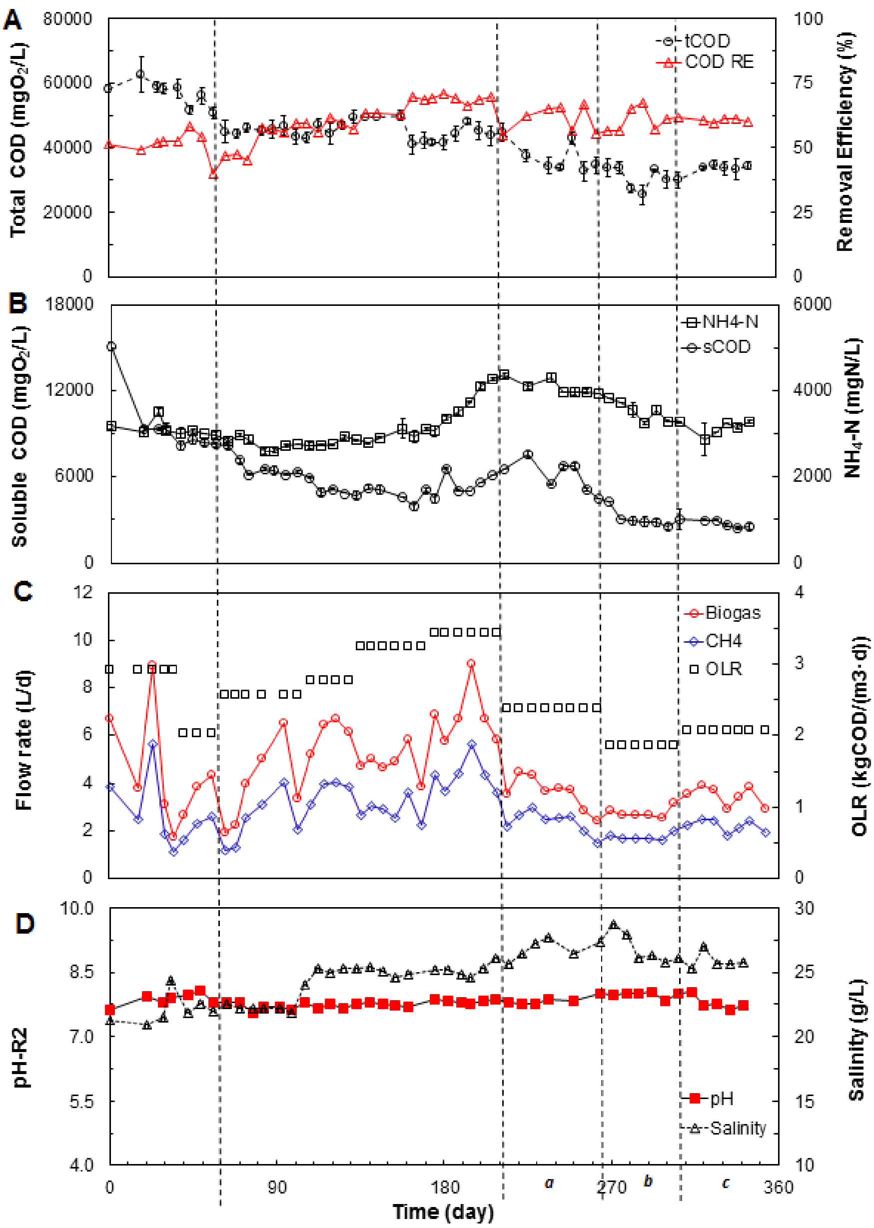

Figure 14 Performance of Reactor 2, A: total COD of sludge & COD removal efficiency, B: soluble COD & NH_4-N of sludge, C: CH_4 & biogas flow rates, D: pH & salinity of sludge

Figure 14 illustrates the operational conditions and performance of R2 over 358 days. The reactor was operated with a constant SRT of 42 days. The pH level was between 7.56 and 8.06. Before day 92 the salinity in reactor stabilized at 23g/L, whereafter it slightly increased to 25-28.7 till the end.

Within the total operation period the first 64 days served as a start-up period. The reactor was firstly fed with the normal range of OLRs applied in sewage digester (2.2-6.7kg COD/ (m^3·d)). Due to the short operational period the stability of R2 was not achieved within the initial phase.

During the second phase (day 70-210) a stepwise increase of OLR was applied from 2.57 to 3.44kg COD/ (m^3·d) so as to improve the methane capacity of R2. COD removal efficiency and ammonium concentration became higher with increasing OLRs, and were resp.70% and 4280mgN/L at the end. There were obvious fluctuations in flow rates of methane and biogas during OLR-transition periods, because substrates used at different OLRs were not completely the same and microorganisms needed time to get adapted. Besides the biogas and methane meters were not in a good condition all the time. For these reasons the results of biogas and methane production never presented an ideal increasing trend as OLRs increased. The salinity after day 92 was around 10% higher than during the previous period, which was most likely caused by the high concentration of ammonium in R2. Thus high levels of ammonium salts of VFA could result in an increase of salinity in R2.

The last phase ran from day 211 to 358, where the reactor was stable already with OLRs controlled between 1.86 and 2.38kg COD/ ($m^3 \cdot d$). The average value of COD removal efficiency and CH_4 production was approximately 60% and 2.1L/d, respectively, during whole period. In the middle of first sub-phase, the methane flow rate showed a 14% decrease relative to its production at day 227, probably due to VFA accumulation in digester. This temporal accumulation of VFA could be implied by the increase of sCOD concentration from 6560 to 7540mg O_2/L in day 236-264. Over the entire phase, the NH_4-N concentration in R2 varied within the range of 2852 to 4316mgN/L, corresponding to the total COD range of 25333 to 43667mgO_2/L.

5.1.3 Performances of Reactor 3

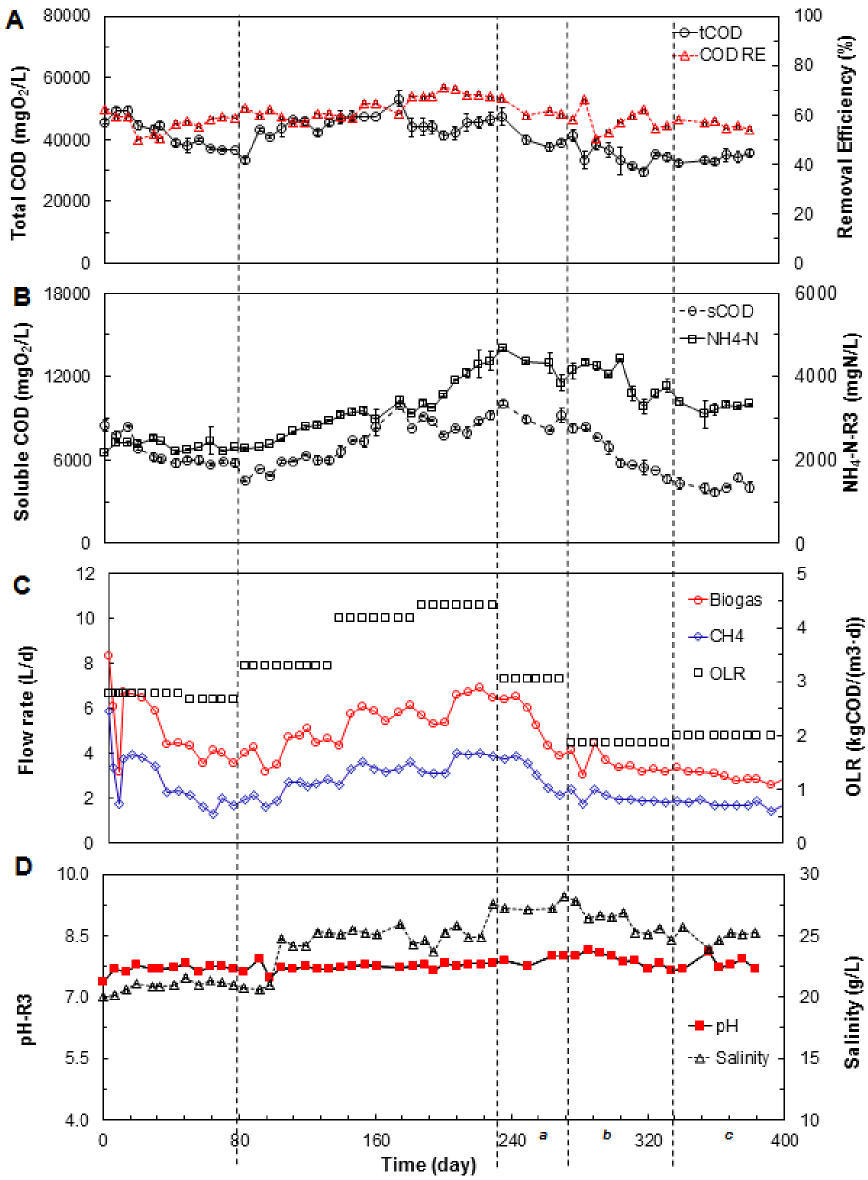

Figure 15 Performance of Reactor 3, A: total COD of sludge & COD removal efficiency, B: soluble COD & NH_4-N of sludge, C: CH_4 & biogas flow rates, D: pH & salinity of sludge

Reactor 3 was operated during a total period of 400 days. A SRT of 32 days was applied in the digester before day 228, and then a SRT of 42 days till the end of experiments. The performance and operating conditions with respect to R3 are shown in Figure 15. The pH was quite stable within the range of 7.64-8.15. Salinity in R3 stabilized at 21g/L before day98, and then increased to around 26 due to high concentration of NH_4-N in the reactor.

In the first 74 days the OLR was controlled at around 2.8kg COD/(m^3·d). The biogas and methane flow rate stabilized at 4.1 and 1.9L/d, respectively, after 35 days. The COD removal efficiency and the concentration of NH_4-N as well as soluble COD also remained constant at ca.57% and 2250mgN/L and 5662mgO_2/L, respectively, since then.

The second phase (day 75-228) was divided into three sub-phases with different OLRs. The maximum OLR of 4.42kg COD/(m^3·d) was applied in the last sub-phase. As seen in Figure 15 the reactor was always at steady state throughout the whole phase. Each sub-phase was featured by a quite constant CH_4 flow rate, which was 2.7, 3.4 and 4.0L /d in sequence. The COD removal efficiency was slowly improved from 57% to 68% with time. Concentration of NH_4-N and soluble COD increased as the increasing OLRs.

During the last phase (day 229-400) the biogas and methane flow rate became stable after the first sub-phase, because of microbial adjustment to the reduction in feeding load. In the sub-phase b, the average CH_4 flow rate was 1.8L/d at the OLR of 1.9kg COD/(m^3·d), which was 3% greater than that at OLR of 2.66kg COD/(m^3·d) (day 48-74), demonstrating that the capacity of R3 in methane production was improved when a longer SRT was adopted in the digester. The longer the SRT in the reactor, the larger proportion of organics could be converted into CH_4. Moreover, the average value of COD removal efficiency and NH_4-N concentration reached 57% and 3671mgN/L in the last two sub-phases, respectively.

5.1.4 Evaluation of Performances of three reactors

Following performance-evaluation of three CSTRs is based on the comparison between results of the current investigation and those of the previous study by Gebauer (2004). In his experiments sludge from saline fish farm effluents was anaerobically digested in mesophilic CSTRs, which is similar to the present study. Table 13 summarizes performances of three CSTRs at steady state in present investigation.

The OLR in R1 and R2 was within the operational range of 0.93-3.14kg COD/(m^3·d)), similar to those applied in the Gebauer's study (1.24-3.12kg COD/(m^3·d)). Meanwhile R3 experienced a slightly wider range of OLR during the entire operation period, which was 1.86-4.42kg COD/(m^3·d)). Compared to Gebauer's results, operation of three CSTRs were quite successful because steady state conditions were attained in all three CSTRs at the middle (R3) or last phase (R1 and R2) of the operational period, as indicated by constant pH, salinity, COD removal efficiency, methane content in biogas and production. The failure in reaching stable condition in his study was attributed to a higher salinity of 35g/L.

The three CSTRs in the present investigation were operated at STR of 32 or 42 days, whereas in Gebauer (2004) study STR ranged from 24 to 65 days for undiluted sludge. From Table 13 the COD and VS removal efficiency were improved in the current study, compared to results of Gebauer obtained in undiluted sludge that 36.7-55.2% for COD and 47.4-61.9% for VS.

Moreover, the methane percentage in biogas and production were considerably higher in the present study (Table 14 and 15), compared to the corresponding range of 49-54% and 0.114-0.184LCH_4/g COD added resulting from Gebauer (2004) study.

In line with Table 3 (section 2.2.2) anaerobic digestion process would be substantially inhibited at a concentration of NH_4-N beyond 3000mgN/L. However, when its concentration was around 4gN/L in the current investigation (Table 13), no significant inhibition appeared in the CSTRs, which indicated that anaerobic microorganisms could adapt to the medium with a toxic level of ammonium.

Table 13 Summary of performances of three reactors at steady state. RE is short for removal efficiency.

	Salinity (g/L)	TS (g/L)	VS (g/L)	TSS (g/L)	VSS (g/L)	TCOD (g O_2/L)	SCOD (g O_2/L)	TP (mgP /L)	TN (mgN/L)	NH_4-N (mgN /L)
R1	21.4-26.2	28.2-40.1	9.1-18.8	8.9-23.3	8.9-19.0	13.1-30.1	0.9-4.0	163-400	2100-3500	1440-2486
RE(%)	-	60-73	79-85	71-86	71-85	72-83	-	-	-	-
R2	20.9-28.7	37.9-66.3	17.5-35.7	18.9-50.3	16.9-33.3	25.3-62.7	2.4-15.1	353-1533	3467-6633	2579-4368
RE (%)	-	33-61	45-72	38-70	45-72	40-71	-	-	-	-
R3	23.7-28.2	44.3-60.3	18.1-32.9	25.0-52.7	17.5-31.2	29.5-53.3	3.6-10.1	1240-2153	4550-7133	2978-4664
RE (%)	-	41-60	60-72	37-69	45-73	50-71	-	-	-	-

5.1.5 Effect of OLR on reactor

The last phase of R1 and R2 were selected to evaluate the effect of OLR on CSTR performance. Both reactors were at steady state during that period, and with same operational conditions except OLRs and ratios of COD/SO_4^{2-} in the feeding substrate. The OLRs in R2 were always double as high as in R1, while the resulting specific CH_4 yield in R2 was only 10% and 14% higher than those in R1 during last two sub-phases (Table 14). In terms of the CH_4 content, it was above usual 60% in R2 whereas below 60% in R1, which indicated an inhibition of lipid degradation in R1 (Gebauer and Eikebrokk, 2006) or a high flow rate of undesired gas like H_2S in R1. Moreover, average COD removal efficiency in R2 was 60%, which was 20% less than in R1. The main cause for this is the great COD consumption by SBR in R1. R1 was fed with diluted substrate, where the COD/SO_4^{2-} was as half as that in R2. Much more proportion of organic compounds in R1 will be utilized by SBR to convert into H_2S, which resulted in a higher COD removal efficiency but a lower specific CH_4 yield in R1 at the same time. Throughout the whole phase, the average pH in both reactors was similar and between 7.51 and 8.06, indicating that operation of the high-loaded CSTR at 2.38kg COD/(m^3·d) did not cause any pH-related problem, which was a proof of good buffering in R2.

5.1.6 Effect of mixing regime on reactor

The unique difference between R2 and R3 in their last two sub-phases was the application of different mixing regimes. R2 used biogas-recirculation, whereas R3 used impeller-stirring. Comparing Table 14 and 15 specific CH_4 yield during phase b and c was significantly improved by an impeller, and respectively 43% and 7% compared to biogas recirculation. 43% increase was probably associated with continuous consumption of undigested substrate from previous sub-phase (R3-a) in R3. Under stirring condition much smaller particles were distributed in reactor, which could offer a larger contact surface between substrate and microbes and hence increase biogas production (Sreekrishnan et al., 2004). However, the result is contrary to what Karim et al. (2005) observed on the digestion of animal wastes. The different ways of biogas circulation might account for this disagreement. In addition, the average CH_4 content in biogas dropped from 63% to 58% when biogas recirculation was replaced by impeller-stirring. Biogas recirculation recycled biogas produced into the reactor, which increased the amount of CO_2 available for hydrogenotrophic methanogenesis, and thereby may lift the CH_4 percentage in biogas to some degree.

Table 14 Specific methane yield and average methane content of R1 and R2 at steady state with a SRT of 42d

Stage	OLR (kg COD/(m^3·d))	COD:SO_4	Specific CH_4 yield ($mLCH_4$ /g COD added)	Average CH_4 content (%)
R1-a	1.19	46	207	57
R1-b	0.93	33	163	56
R1-c	1.03	38	186	54
R2-a	2.38	85	201	65
R2-b	1.86	56	179	63
R2-c	2.00	67	212	63

Table 15 Specific methane yield and average methane content of R3 during the entire operational period

Stage	OLR (kg COD/(m^3·d)	SRT (day)	Specific CH_4 yield ($mLCH_4$ /g COD added)	Average CH_4 content (%)
	2.77	32	-	-
	2.66	32	156	51
	3.30	32	186	53
	4.18	32	194	58
	4.42	32	203	59
R3-a	3.05	42	255	58
R3-b	1.86	42	256	57
R3-c	2.00	42	226	59

5.2 Variable impacts on BMP

5.2.1 Addition of compatible solutes

Figure16 presents all methane accumulation curves from BMP tests about dosage of compatible solutes. Based on equation [1.3] (section 2.2), the accumulative methane production was corrected taking into account the methane production from the inoculum, and expressed as normal mL per gram of VS added at standard temperature and pressure conditions.

For all groups, after 13-day digestion the methane production was close to the maximum obtainable. As both compatible solutes are biodegradable, their CH_4 production should be withdrawn from results, which were totally 57.4mL from betaine, 39.3mL from trehalose, and 48.4mL from the combination. Therefore, the exact methane potential from the studied substrate was 323, 315, 307 and 306 mL/gVS$_{added}$ for groups with 0.5g/L trehalose, a combination of 0.25g/L betaine and 0.25g/L trehalose, 0.5g/L betaine as well as control group, respectively. These values are similar to results reported in literature on anaerobic digestion of marine biomass. Samson and Leduyt (1986) observed methane yield ranging from 250mL to 340mL/gVS$_{added}$ on the blue-green alga Spirulina maxima, whereas on co-digesting fish waste with sisal pulp the methane yield was 310/gVS$_{added}$ (Mshandete et al., 2004).

Compared to the control group, the methane potential increased 5% and 3% respectively in trehalose group and combination group, and the corresponding biodegradability increased 3% and 2% as well (Table 16). However, for betaine group nothing was improve. This result suggests that either 0.5g/L or 0.25g/L trehalose is capable of slightly promoting methane yield of the studied saline sludge. An early study by Vyrides et al. (2010) had revealed that the addition of 1mM betaine or 1mM trehalose improved the adaptation of anaerobic sewage sludge to 35g NaCl/L, and also methane yield but to different degrees. Similarly, for the acclimated biomass to high salinity, trehalose may still enable microorganisms to increase their intrinsic hydrolysing capacity, driving them to decompose more hardly degradable substrate.

Apart from more accessibility to hardly degradable substrate, the hydrolysis rates were also boosted but with the help of betaine rather than trehalose. Table 16 shows hydrolysis rates in four groups that were derived from the first-order kinetic model. Around 16% increase in hydrolysis constants happened in betaine group and combination group. These higher hydrolysis rates confirmed that during anaerobic digestion of saline sludge 0.5g/L or 0.25g/L betaine is effective in reducing sodium inhibition and stimulating activities of hydrolytic enzymes.

Table 16 Hydrolysis constants and biodegradability for four groups of tested sludge

Sludge sample	**First order kinetics**		**Biodegradability**
	K_h (d^{-1})	R^2	
Betaine 0.5g/L	0.205	0.979	61.43%
Trehalose 0.5g/L	0.170	0.972	64.76%
Betaine 0.25g/L+Trehalose 0. 25g/L	0.212	0.974	63.11%
Control group	0.173	0.965	61.40%

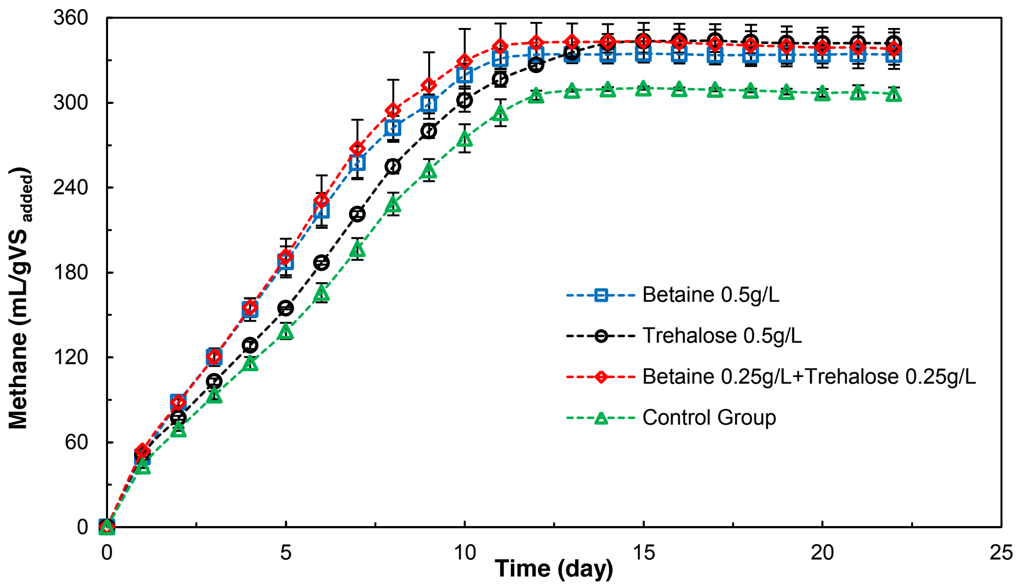

Figure 16 Cumulative methane production as a function of time in sludge with or without CS addition

5.2.2 Addition of inorganic coagulants

Figure 17 shows four methane accumulation curves from BMP tests on addition of inorganic coagulants. All curves are each means of three triplicates. The methane potential after 23-day incubation was 297, 283, 268 and 240mL/gVS$_{added}$ for cellulose group, control group, $FeCl_3$ group and PAS group, respectively. Compared to the control group, the methane potential was down by 5% and 18% respectively in $FeCl_3$ group and PAS group, so it is apparent that the addition of PAS at 2.4gAl^{3+}/L is more inhibitory to methane yield of the studied sludge than $FeCl_3$ at 6.0gFe^{3+}/L. Moreover, from Table 17 about 3% and 9% decline in substrate biodegradability took place in $FeCl_3$ group and PAS group, respectively.

One possible mechanism responsible for these reductions in anaerobic digestibility is that a portion of substrate became less accessible and /or less reactive to bacteria or relevant exoenzymes, due to interaction between inorganic coagulants and substrate and subsequent enmeshment of substrate within the floc structure (Dentel and Gossett, 1982). In addition, ferric ion is the electron acceptor during the reduction process. The iron reducing bacterial could outcompete with SRB and methanogens, using oxidized iron as an electron acceptor for carbon mineralization in anaerobic conditions. In the case of the defined sludge, the theoretical CH_4 reduction is 29mL/gVS$_{added}$ under the complete reduction of Fe^{3+}, however, which is larger than 15mL/gVS$_{added}$ obtained from experiments, meaning that a part of Fe^{3+} added was reduced to Fe^{2+} either chemically or biologically and the effect of chemical interaction and physical enmeshment did exist during anaerobic digestion.

Aluminium as a species of light metals is very toxic to anaerobic microorganisms in excessive amounts because of its association to the cell membrane or wall, which may impact microbial growth (Cabirol et al., 2003). The heavy-metal iron is also identified to be of particular concern in anaerobic digestion due to its disruption of enzyme function and structure through connecting Fe^{3+} with thiol and other groups of proteins or through replacing existing metals in enzyme prosthetic groups (Vallee and Ulmer, 1972). However, it did not seem that the presence of 2.4gAl^{3+}/L and 6.0gFe^{3+}/L showed notable toxicity to the anaerobes in the studied sludge, since both $FeCl_3$ group and PAS group were distinguished by the higher CH_4 production rates during the first three days (Figure 17) and also the reasonable hydrolysis constants simulated from the first-order kinetic model (Table 17). It was found that a UASB system was able to function normally in the presence of an Fe^{3+} concentration of up to 5.65g/L (Jackson-Moss and Duncan, 1990).

From Figure 17 all test groups began with a rapid methane production rate, with the exception of cellulose group which took three days to decompose insoluble cellulose into monomers and then turned to CH_4 generation. Table 17 gives the largest hydrolysis rate of 0.273d^{-1} in the coagulated sludge with 6.0gFe^{3+}/L, which was 43% higher than the control group and reached about 8 days earlier stable digestion than the control group. This faster hydrolysis process may be attributed to a decrease in H_2S gas. The activity of ion-reducing bacteria inhibited the production of sulphide by SRB and the growth of these bacteria in the anaerobic conditions, thus decreasing the inhibition of methanogens by H_2S.

Commonly, the cellulose group is prepared as a reference to evaluate the biodegradability of substrate during BMP analysis. Only a 3% biodegradability decrease in control group relative to the reference (Table 17) hinted that anaerobic digestion is viable for treating the studied sludge harvested from brackish RAS.

Table 17 Hydrolysis constants and biodegradability for four groups of tested sludge

Sludge sample	**First order kinetics**		**Biodegradability**
	K_h (d^{-1})	R^2	
6.0g Fe/L	0.273	0.984	53.68%
2.4g Al/L	0.193	0.991	48.15%
Cellulose group	0.103	0.981	59.46%
Control group	0.191	0.980	56.70%

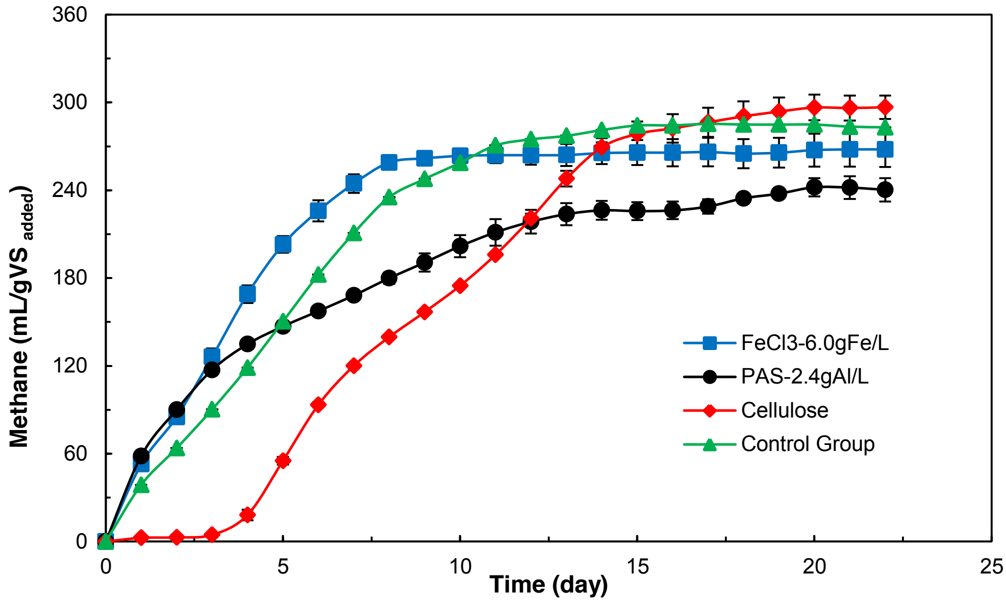

Figure 17 Accumulated methane production in coagulated or uncoagulated sludge as a function of time

5.3 Variable impacts on PA

5.3.1 Addition of Compatible solutes

To analyse effects of compatible solutes on PA in the studied sludge collected from R2, four groups of batch test were carried out, namely control group without addition of any compatible solute, group with 1mM betaine, group with 1mM trehalose, and group with a combination of 0.5mM betaine and 0.5mM trehalose. Because the limited supply of the studied sludge from R2 makes it impossible that four groups of tests are done in a run, they were conducted in two runs (betaine and control group; trehalose and betaine & trehalose), and the initial PA of two runs slightly differed from each other accordingly due to the dynamic operation for reactors. Therefore, it made sense to compare the deactivation of PA in different groups, where deactivation was defined as a decrease in PA from beginning (after 15mins) to the steady state on the basis of initial values.

Figure 18 depicts how PA changed during 72h of substrate-depletion with or without addition of compatible solutes. A similar variation of PA was only found in co-composting of green wastes and sewage sludge by Albrecht et al. (2010), but for 150d observation. This unstable process can be separated into five distinct phases, two for increase and three for decrease. The mechanism behind each phase is not clearly known, but might be explained as follows: In the first six hours, phosphatase activities were repressed by excess availability of inorganic P at the start of substrate-feeding. Within the following two hours, the successive hydrolysis of complex organic compounds and consumption of free inorganic P by organisms supplied a favourable condition for synthesis of phosphatases. The high PA stimulated the release of orthophosphate from organic materials, which in turn induced a PA decrease during the next phase. Several researches discovered negative correlations between phosphatase activities and the availability of inorganic P in soil and sludge (Criquet et al., 2007, Moscatelli et al., 2005). After 12h of anaerobic digestion, phosphorus became a limiting factor for growth and metabolism of bacteria, so PA had to be enhanced again to free phosphorus from organic materials to meet P requirement in cells. From 16h to the end of experiment PA remained stable after a short-time decline.

From Figure 19-a addition of 1mM betaine had no significant influence on PA during the period of 0.25h-72h. From Figure 19-b PA behaved similarly in trehalose group and the combined group, indicating addition of 1mM trehaloe or a combination of 0.5mM betaine and 0.5mM trehalose brought same effects on PA during the entire measuring period. Table 18 gives the accurate data for PA deactivation. Compared to the initials at 15mins, alkaline PA after 72h deactivated 76%-79% in four groups. For acid PA, this loss was less, i.e. 52% for the control, 54% for betaine alone, and 36% for trehalose alone and the combination.

In comparison with the control, 16% decrease in deactivation of acid PA in trehalose group and combination group suggests that 1mM trehalsoe alone or the combined did enhance acid PA in the studied saline sludge during the period of 0.25h-72h. Trehalose is capable of stabilizing cell structures and protecting cellular proteins under stress conditions (Ocón et al., 2007), so it is likely that activity of acid phosphatase (intracellular enzyme) was improved in the studied sludge where addition of trehalose help anaerobes better adapt to high salinity. Moreover, similar deactivation of acid PA in control group and betaine group indicates that addition of 1mM betaine was ineffective to acid PA in the studied sludge, which, however, is not in accordance with the report that betaine was an inducer of acid phosphatase activity by supplying carbon and nitrogen sources in high inorganic P medium (Lucchesi et al., 1989). This disagreement might be due to different amounts of betaine addition.

In Table 18 around 76% deactivation of alkaline PA in all groups suggests that alkaline phosphatase was hardly impacted by input compatible solutes. This is because cells depends on the accumulation of compatible solutes intracellularly to overcome osmotic stress, hence, the adaption of endo-enzymes (alkaline phosphatase) are not necessary any more (Müller et al., 2005).

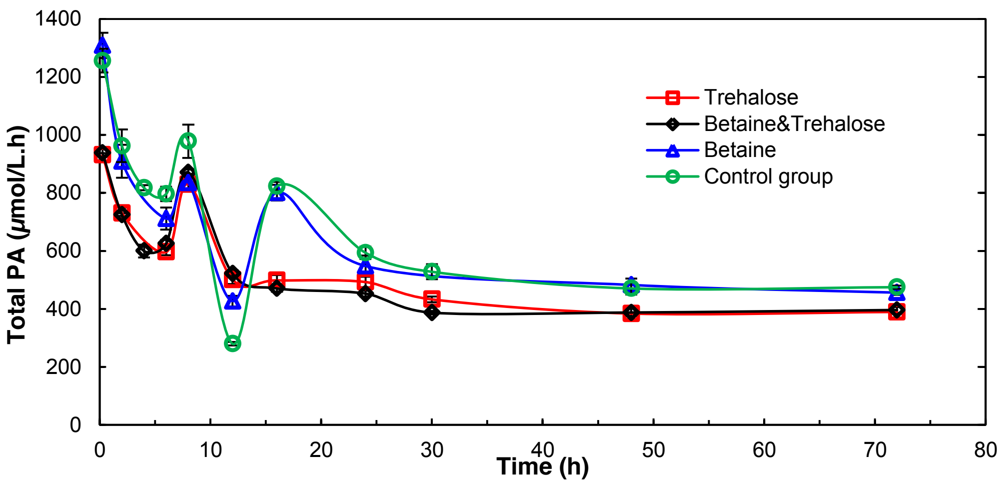

Figure 18 Total PA as a function of time for different doses of compatible solute

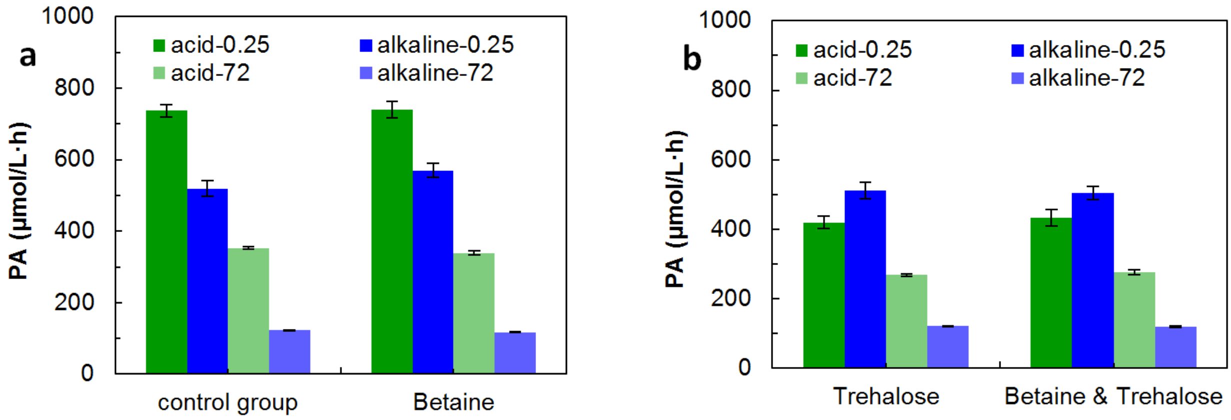

Figure 19 Comparison of PA at 0.25h and 72h with or without compatible solutes dosage

Table 18 PA deactivation between 0.25h and 72h in sludge with or without compatible solutes dosage

Sludge sample	Acid PA	Alkaline PA	Total PA
Control group	52%	76%	62%
1mM Betaine	54%	79%	65%
1mM Trehalose	36%	76%	58%
0.5mM Betaine & 0.5mM Trehalose	36%	76%	58%

5.3.2 Shear forces from biogas recirculation

Morphology, size, growth velocity and enzyme biosynthesis of microorganisms were affected when exposed to shear rates ranging from 0.028 to 1482 s^{-1} (Garcia-Soto Mariano, 2006). As a result, phosphatase must be impacted at testing shear rates of 1.76s^{-1}, 9.36s^{-1} and 31.98s^{-1} generated from biogas recirculation. According to Charm and Wong (1970) enzyme loses activity as a function of time and shear rate when subjected to shear forces. Therefore, PA at 0.25h and 12h were analysed in each case to figure out how phosphatase inactivates at different shear stresses.

From Figure 20 acid and alkaline PA at 12h were apparently lower than those at the start of experiments in all cases, and the extent of phosphatase deactivation decreased upon the increasing shear rates. Table 19 presents that after 12h the loss in total PA was 79%, 59% and 52% at 1.76s^{-1}, 9.36 s^{-1} and 31.98s^{-1}, respectively. A possible cause behind it is that high shear stresses broke up the original flocs into smaller particles which were easily attached to gas bubbles (Huang et al., 2011), leading to a reduction in contact area between substrate and microorganisms and subsequent a decrease in available P and substrates of phosphatase, thus stimulating both acid and alkaline PA. Also high shear forces offer more opportunities to disrupt the secondary or tertiary structure of enzyme exposed to shear forces directly (Thomas and Geer, 2011). In this way much extracellular alkaline phosphatase could be destroyed by higher shear forces, and the activity of undamaged alkaline phosphatase would be motivated. Additionally, the possibility of enhancing cell lysis under high shear stresses may promote acid PA in the living cells left.

The loss in alkaline PA after 12h was 88% at 1.76 s^{-1}, and 70% at 9.36 s^{-1} or 31.98s^{-1}, which were always higher than those in the corresponding acid PA. So the decrease in alkaline PA was proved to be a major contributor to phosphatase deactivation with shearing. Further, the less variation in deactivation of alkaline PA than acid PA among three cases indicated that alkaline PA was more tolerant with shear forces produced by biogas recirculation than acid PA.

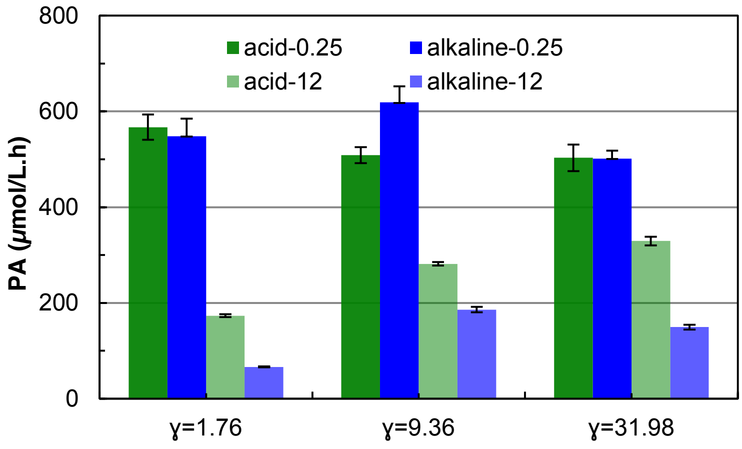

Figure 20 Comparison of PA at 0h and 12h under different stress forces

Table 19 PA deactivation between 0.25h and 12h in sludge at various shear stresses

Sludge sample	Acid PA	Alkaline PA	Total PA
ɣ=1.76 S^{-1}	69%	88%	79%
ɣ=9.36 S^{-1}	45%	70%	59%
ɣ=31.98 S^{-1}	35%	70%	52%

5.4 Variation of particle size distribution

5.4.1 At different Mg/P molar ratios in synthetic brackish liquid

To investigate how Mg/P molar ratio influence struvite size distribution in the synthetic brackish liquid, following seven jar tests were conducted in lab. The availability of struvite component and saturation condition were modelled by using PHREEQC to support this research.

During experiments the initial concentration of NH_4^+ varied in tests from 146 to 369mM, but was still one or two order larger in magnitude than Mg^{2+} and PO_4^{3-}. Therefore, the NH_4^+ concentration was always in significant excess compared with the other two components of struvite. From Table 21 values of struvite SI in all samples simulated by PHREEQC model are positive, meaning all systems were supersaturated and struvite could nuclear and grow, returning systems to equilibrium. This theoretical result was in line with the experimental observations that struvite crystals appeared in all tests.

The initial Mg^{2+} concentrations in first two jar tests were minimal compared to the other constituents of struvite, indicating a limiting element for struvite precipitation in each test (Table 20). The more Mg^{2+} were added into solution, the more phosphate were precipitated in form of struvite. This explains the occurrence of only 0.75g and 0.22g struvite production per gram of PO_4^{3-} added in first two tests (Table 21). From ratio of 0.58 to 2.19, the initial $NH_4^+$and Mg^{2+}concentrations were nearly kept constant, while PO_4^{3-} concentration at start was reduced with increasing ratio values, and the struvite supersaturation declined accordingly based on modelling results. In theory, high supersaturation prefers crystal nucleation over crystal growth (Hutnik et al., 2013, Ronteltap et al., 2010, Mersmann, 1995). As a consequence, the great amount of struvite may precipitate as undetectable nuclei, leading to a low specific struvite production in high supersaturation, such as 2.80g struvite /g PO_4^{3-} added at Mg/P of 0.58.

Figure 21 illustrates the particle size distribution of struvite crystals at different Mg/P molar ratios. The leading crystal sizes (corresponds to differential volumetric distribution maximum) were close to 30μm in most cases, except ratios of 0.91 and 1.09, where the presence of two major populations of particles was indicated, one in the diameter size range of 40 to 50μm and another located near D70μm. The secondary dominant crystals was roughly the double size of the most dominant crystals in each scenario. It seemed that some small crystals were bound together as a result of collisions among them while in suspension. Adnan et al. (2003a) discovered that struvite crystals in large sizes (over 2.5mm) consisted of a tightly packed inner core and an outer thick coating of fine aggregates. Therefore, regarding very fine crystals of less than 400μm, it is also possible that they were solidly packed together in diverse natural morphological forms including orthorhombic, wedge, or short tabular forms. However, these second peak fractions at D70μm should be further analysed by scanning electron microscopy (SEM) to confirm agglomeration of fine crystals. Moreover, the average size of struvite was around 20μm within the ratio range of 0.58-2.19, which was slightly affected by the molar ratio of Mg/P (Table 21).

At Mg/P molar ratio of 1.09 and above, high struvite production and high P-removal efficiency were achieved in synthetic brackish solution, i.e. larger than 4g/gPO_4^{3-} and 99%, respectively (Table 21). As a result, the ratio optima in the synthetic brackish solution was around one in order to harvest much struvite with large sizes and obtain a high P-removal efficiency at the same time.

Table 20 Component availability and struvite SI at tested Mg/P molar ratios

Mg/P	**Con. (M) before mixing**			**Con. (M) after mixing from PHREEQC**			**Struvite SI**
	NH_4^+	Mg^{2+}	PO_4^{3-}	NH_4^+	Mg^{2+}	PO_4^{3-}	
0.12	2.49E-01	2.05E-03	1.64E-02	1.15E-01	1.79E-04	1.19E-06	2.65
0.41	2.94E-01	8.43E-03	2.06E-02	1.34E-01	7.73E-04	1.10E-06	3.32
0.58	3.69E-01	1.13E-02	1.96E-02	1.66E-01	1.20E-03	8.83E-07	3.50
0.91	1.83E-01	1.11E-02	1.23E-02	8.57E-02	1.59E-03	5.41E-07	3.13
1.09	1.83E-01	1.11E-02	1.02E-03	8.58E-02	1.80E-03	4.26E-07	3.08
1.37	1.83E-01	1.11E-02	8.17E-03	8.58E-02	2.04E-03	3.20E-07	3.01
2.19	1.46E-01	1.11E-02	5.10E-03	6.91E-02	2.49E-03	1.81E-07	2.75

Table 21 Struvite production and P-removal at different Mg/P molar ratios (TSS: struvite production)

Mg/P	**TSS (g/L)**	**TSS/P (g/gPO_4 added)**	**P-removal**	**Avg. D (μm)**
0.12	0.37	0.75	13.77%	18.0
0.41	1.38	2.22	42.12%	18.9
0.58	1.65	2.80	57.97%	19.4
0.91	1.54	4.14	89.78%	20.0
1.09	1.45	4.65	99.07%	20.5
1.37	1.11	4.40	99.68%	20.5
2.19	0.74	4.84	99.67%	19.5

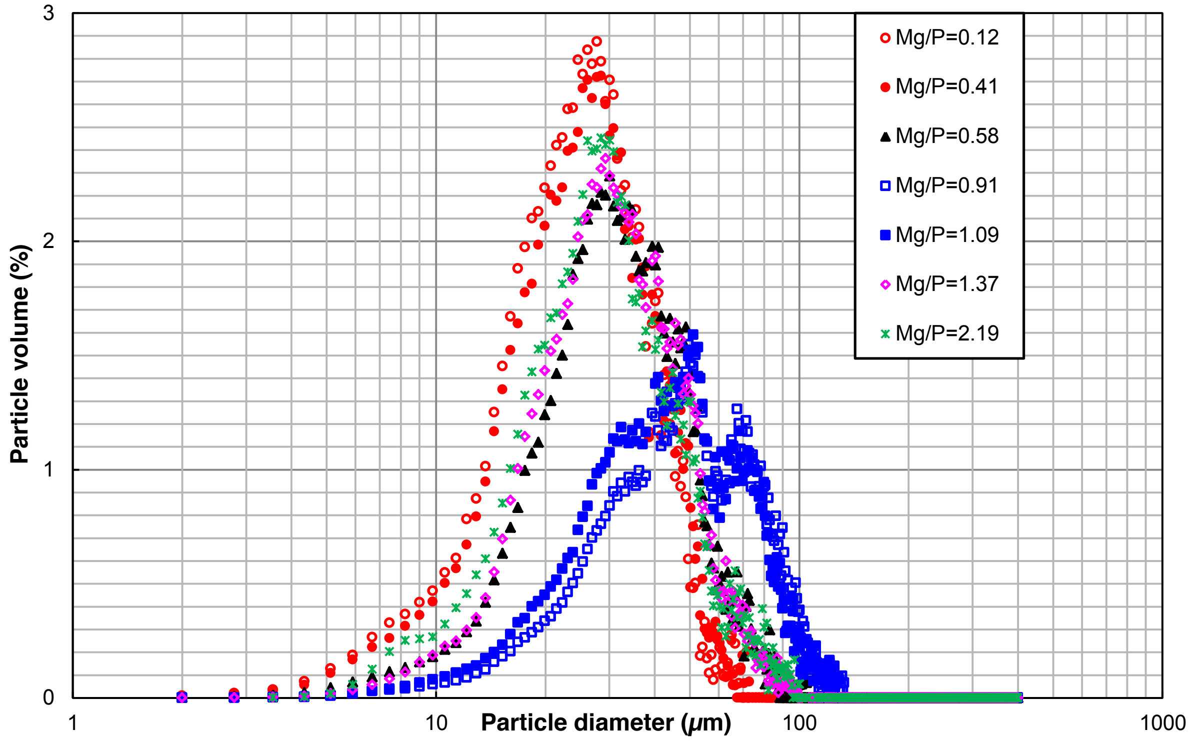

Figure 21 Particle size distribution of struvite at different Mg/P molar ratio in synthetic brackish solution

5.4.2 At different Mg/P molar ratios in the filtrate of R2

Four groups of particle size distribution were investigated at different Mg/P molar ratios by using filtrate from R2, in order to examine if the ratio optima of around one was valid for the studied sludge from mesophilic CSTR.

Results (Figure 22) indicate that the diameter of predominant particles became smaller as the Mg/P molar ratio increased, excluding the ratio of 1.44. The largest diameter of main particles was 16μm in the group of Mg/P=0.50. It reduced 44% compared to the similar ratio group of 0.58 in section 5.4.1, while the corresponding volume percentage increased 1.6%. The average diameter of struvite crystal in all groups was 15μm or below (Table 22). It is apparent that more particles with smaller sizes were produced in filtrate of R2 than the synthetic brackish solution in section 5.4.1. A possible explanation could be the competition between Ca^{2+} and Mg^{2+} to react with PO_4^{3-}, because Ca^{2+} cation existed in the filtrate with the initial concentration of around 40mg/L and was likely to generate $Ca_5(PO_4)_3OH$ in the filtrate of R2. Besides, humic acids in the filtrate could be another cause for the reduction in struvite diameter. According to Burns et al. (2001) struvite solubility is increased in the presence of organic acids. Therefore, high COD levels in R2 correlated to high concentration of humic acids, which may interfere with the process of struvite crystallization.

From Table 22 all P-removal efficiencies below 90%. Especially at Mg/P of 1 only 66% of the phosphorus was removed in the filtrate, which was 24% lower than that at Mg/P of 1.09 in the synthetic brackish solution. When Mg/P ratio was larger than one, above 80% of P-removal was achieved, meaning much more Mg^{2+} was required in the filtrate to precipitate PO_4^{3-} in the form of struvite crystal compared to the synthetic brackish solution.

Table 22 Struvite production and P-removal in filtrate of R2

Mg/P	initial P(mg/L)	final P (mg/L)	P-removal	Avg. D (µm)
0.50	35.5	-	-	15
1.00	34.5	11.65	66.23%	14
1.44	33.7	4.95	85.31%	10
2.10	33.2	3.57	89.23%	10

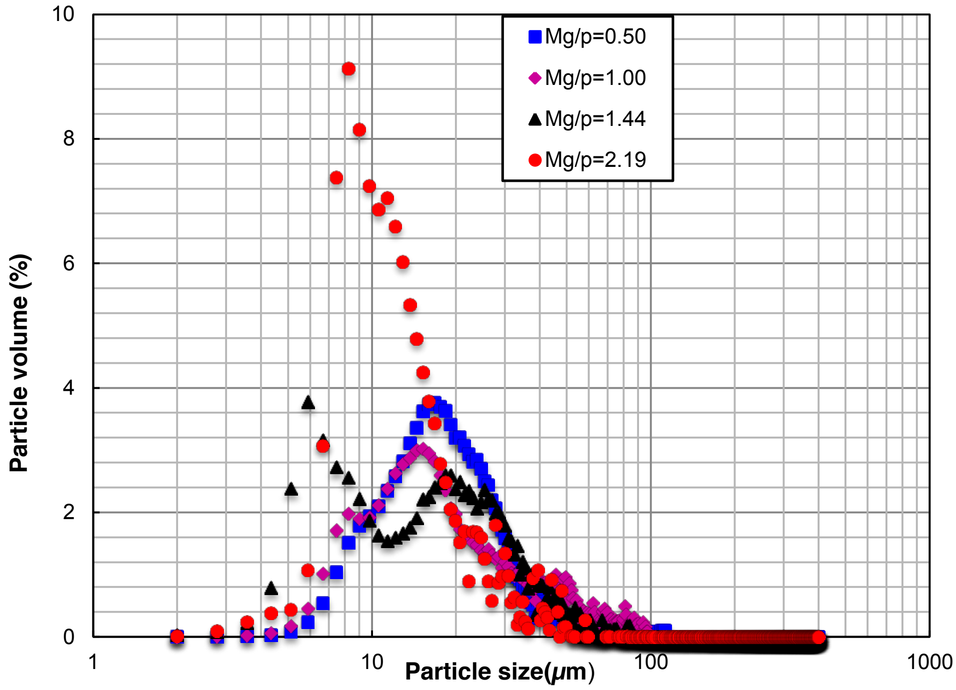

Figure 22 Particle size distribution at different Mg/P molar ratio in filtrate from R2

5.4.3 At different temperatures in synthetic brackish liquid

To analyse the effect of temperature on struvite particle size distribution in synthetic brackish solution, five jar tests were performed at respective 10°C, 25°C, 35°C, 45°C and 55°C. The initial saturation condition of struvite were calculated by PHREEQC model, which uses Van't Hoff equation to estimate struvite solubility products at different temperatures.

There is an indication that prevailing particles shifted towards larger sizes with increasing temperatures (Figure 23). Normally the increasing temperature could enhance the struvite-solubility and thereby decrease supersaturation, which was confirmed by values of struvite SI as derived from PHREEQC model (Table 23). The low supersaturation caused by high temperatures benefits crystal growth over nucleation (Ronteltap et al., 2010). So much more struvite were promoted to form on already present crystals rather than occurring as a nuclei. On the other hand, temperature has a direct impact on the stripping of ammonia which is associated with NH_4^+ availability. In the equation [1.2] (section 2.2.2), the high temperature help drive the reaction to the left, leading to the transformation of much ammonium into gaseous ammonia and a decrease in pH. The initial NH_4^+ concentration applied in experiments was 179mM, which was substantially higher than those of Mg^{2+}

and PO_4^{3-}; that is: its remaining concentration was always abundant for struvite precipitation during the entire course. Consequently, merely the reduction in pH resulted from the temperature-increase positively affected the formation of larger particles, which is in accordance with observation in the next section.

From Table 23 the average particle size increased with increasing temperature as well. Similar conclusion on synthetic wastewater was already reported by Ronteltap et al. (2010), although the temperature ranged from 5°C to 30°C in their research. The variation of average particle size within the range of tested temperature is less compared to that in the pH group (section 5.4.4), confirming that temperature has a lower impact on struvite precipitation than pH (Durrant et al., 1999).

Both struvite production and P-removal efficiency were maintained at a quite higher level at 35°C and below (Table 23). It is clear that there is a kind of trade-off between particle size and P-removal efficiency. 25°C or 35°C is recommendable for operation to precipitate more phosphorus and produce great quantities of struvite with larger sizes in the synthetic brackish solution.

Table 23 Struvite production, SI and P-removal at tested temperatures

Temp.	TSS (g/L)	TSS/P (g/gPO$_4$ added)	P-removal	Struvite SI	Avg. D (μm)
10	1.19	4.90	99.87%	3.31	16
25	1.11	4.44	99.68%	3.01	19
35	1.16	4.60	99.19%	3.03	20
45	1.09	4.31	97.73%	2.99	19
55	1.11	4.46	97.21%	2.91	22

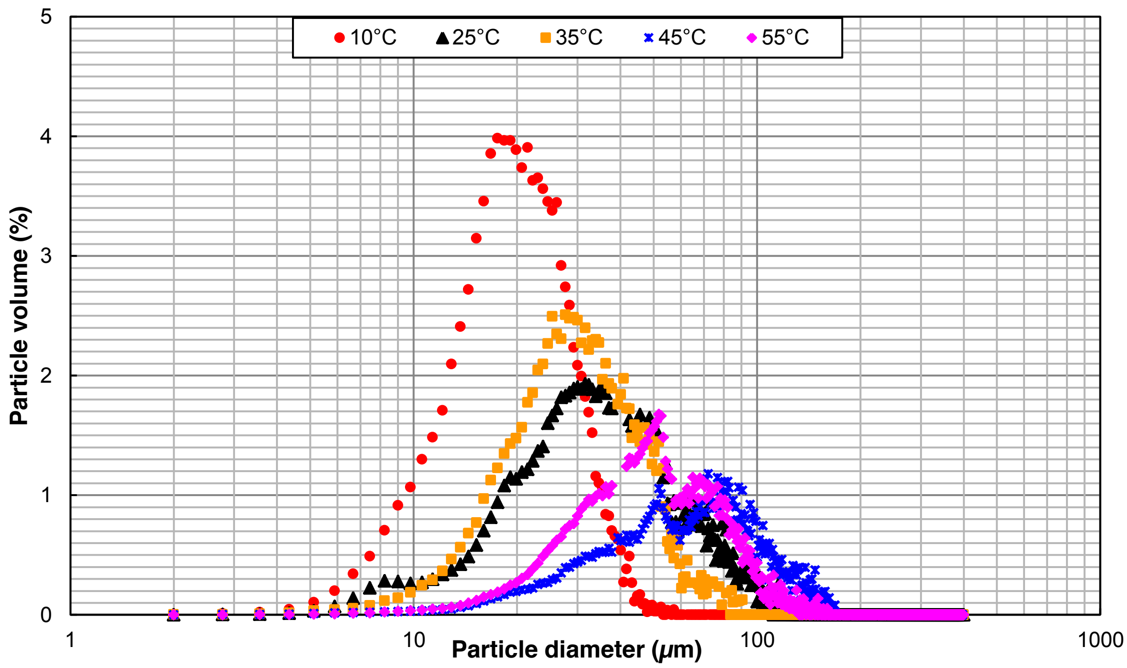

Figure 23 Particle size distribution of struvite at different temperatures

5.4.4 At different pH levels in synthetic brackish liquid

To investigate how pH affects particle size distribution of struvite crystals, the synthetic brackish solution with Mg/P molar ratio of 1.37 were used to examine struvite size distribution at different pH levels. The theoretical component availability and struvite SI was modelled by using PHREEQC.

Table 24 shows the very start saturation condition of struvite in the synthetic brackish liquid. As SI value higher than zero indicates a state of supersaturation, all tests were in supersaturation which was essential for struvite crystallization. Moreover, compared to the other free ions, the concentration of PO_4^{3-} is minimal, meaning PO_4^{3-} was a limiting ion for struvite precipitation. Normally, PO_4^{3-} mainly exists in form of $H_2PO_4^-$ and HPO_4^{2-} at pH below 10 due to thermodynamics (Ronteltap et al., 2007), which would release H^+ in order to constantly form struvite, thus lowing pH during the nucleation process.

From Figure 24 and Table 25 both dominant and average diameter of struvite crystal gradually became smaller with increasing pH. It is obvious that at pH7 large size crystals were more easily built up than at higher pH levels. Matynia et al. (2006) has reported that an increase of pH from 8 to 11 decreased 5.5-fold of the mean crystal size of struvite formed in synthetic solutions. This effect could be related to the supersaturation of struvite, which drops with increasing pH as simulated by PHREEQC (Table 24). At high supersaturation struvite nucleation outweighs crystal growth, therefore a high portion of struvite appeared as crystal with small size or even undetectable nuclei, which was also an explanation for the difference in struvite mass found between pH8 and pH10 (Table 25). Moreover, based on the report of Le Corre et al. (2009), the size of struvite crystals may be restricted by electrostatic repulsion. Individual struvite particles usually present a negative zeta-potential, which limits agglomeration of particles. As pH increases its zeta-potential becomes further negative, and the corresponding particle size decreases as a consequence.

Throughout all of particle size distribution, only a pH of 7 yielded double peaks at 50μm and 90μm, indicating a possible change in precipitation kinetics during struvite crystallization, due to a variation of the surface charge or surface influence from the increased amounts of $H_2PO_4^-$ at pH 7 (Ronteltap et al., 2010). Besides, the particle size distribution at pH8 was similar to pH9, meaning that the pH was not a key factor for struvite precipitation within pH range of 8-9. But once pH was adjusted to 10, the leading diameter decreased to 20μm, taking up ca. 3.8% of total volume. This substantial shift may be a result of the formation of some phosphates like $Mg_3PO_4 \cdot 22H_2O$, which usually precipitates at pH>9 and is stable than struvite, thereby interfere with struvite formation (Le Corre et al., 2009).

Table 25 also presents that at pH7 occurred a low struvite production and a low P-removal efficiency. Therefore, taking into account particle size and P-removal efficiency as well as need of pH adjustment, pH8 would be preferable for struvite precipitation in the brackish filtrate from the studied CSTRs, which were usually operated at around pH8.

Table 24 Component availability and struvite SI at tested pH levels

pH	Con. (M) before mixing			Con. (M) after mixing from PHREEQC			Struvite SI
	NH_4^+	Mg^{2+}	PO_4^{3-}	NH_4^+	Mg^{2+}	PO_4^{3-}	
7	1.83E-01	1.11E-02	8.17E-03	1.12E-01	[illegible]	[illegible]	1.33
8	1.83E-01	1.11E-02	8.17E-03	1.09E-01	2.62E-03	4.60E-08	2.38
9	1.83E-01	1.11E-02	8.17E-03	8.58E-02	2.04E-03	3.20E-07	3.01
10	1.83E-01	1.11E-02	8.17E-03	2.66E-02	1.27E-03	[illegible]	[illegible]

Table 25 Struvite production, P-removal and average diameter at tested pH levels

pH	TSS (g/L)	TSS/P (g/gPO_4 added)	P-removal	Avg. D (μm)
7	0.71	2.80	67.91%	42
8	1.19	4.74	99.22%	22
9	1.11	4.44	99.68%	19
10	1.22	4.94	99.74%	18

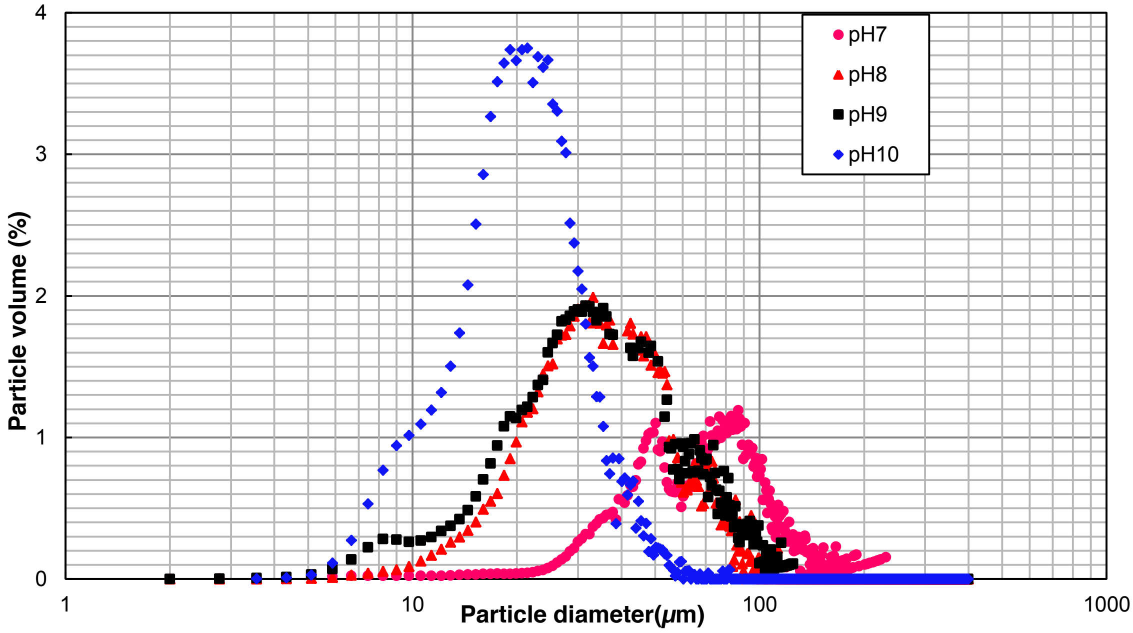

Figure 24 Particle size distribution of struvite at different pH levels

6 Conclusions

6.1 Performances of three CSTRs

- **Performances of three reactors**
  Three mesophilic CSTRs presented stable conditions at each last phase of the entire operation period. R1 and R2 was operated at OLR of 0.93-3.14kg COD/(m^3·d), while R3 was operated at OLRs of 1.86-4.42kg COD/(m^3·d). All specific methane yields at steady state in three CSTRs were various within the range of 0.156-0.256LCH_4/g COD added. The COD and VS removal efficiencies were 40-83% and 45-85%, respectively. These results were much better than those reported by Gebauer (2004), where saline sludge was anaerobically treated with inoculum adapted to low salinity in mesophilic CSTR.

- **Effects of OLR on reactor**
  Increasing OLR improved specific methane yield in mesophilic CSTR fed with saline sludge. The specific methane yield at steady state was promoted 10-14% when doubling the corresponding OLRs (0.93/1.03kgCOD/(m^3·d)).

- **Effects of mixing regime on reactor**
  Compared to biogas recirculation, the specific methane yield at steady state was improved 7% by using an impeller during anaerobic digestion of saline sludge in mesophilic CSTRs.

6.2 Variable impacts on BMP

- **Addition of compatible solutes**
  Compared to the control group, the methane potential of saline sludge was enhanced 5% at the present of 0.5g/L trehalose, and the hydrolysis rate was improved 16% at the present of 0.5g/L betaine.

- **Addition of Inorganic coagulants**
  Addition of PAS at 2.4gAl^{3+}/L was more inhibitory to methane potential of saline sludge than $FeCl_3$ at 6.0gFe^{3+}/L, since methane potential decreased 18% and 5% in PAS group and $FeCl_3$ group respectively, compared to the control group. The hydrolysis rate was improved 43% by addition of $FeCl_3$ at 6.0gFe^{3+}/L.

6.3 Variable impacts on PA

- **Addition of compatible solutes**
  PA deactivation was a function of time. Compared to the control group, 16% decrease in acid PA deactivation appeared in trehalose group and combination group, indicating that 1mM or 0.5mM trehalose enhanced acid PA in saline sludge during the digesting period of 0.25h-72h.

- **Shear forces from biogas recirculation**
  The extent of PA deactivation decreased with increasing shear stress from biogas recirculation, indicating that high levels of shear stresses stimulated acid and alkaline PA in saline sludge during the digesting period of 0.25h-12h.

6.4 Variation of particle size distribution of struvite

- **At different Mg/P molar ratios in the synthetic brackish liquid**

The dominant size of struvite crystal was closed to 30μm at all tested ratios except ratio of 0.91 and 1.09. The average size of struvite crystal varied slightly within the range of tested ratio. The optimal ratio would be around one in the synthetic brackish solution in order to produce much struvite with large sizes and obtain a high P-removal efficiency at the same time.

- **At different Mg/P molar ratios in the filtrate of R2**
  At the similar Mg/P molar ratio, the dominant and average size of struvite crystals were smaller in the filtrate of R2 than those in the synthetic brackish solution, and the P-removal efficiency was significantly lower in the filtrate.

- **At different temperatures in the synthetic brackish liquid**
  The dominant size of struvite crystal increased with increasing temperatures. The average size of struvite crystal varied slightly within the range of tested temperatures. 25°C or 35°C is recommendable for operation to achieve a high P-removal efficiency and produce much struvite crystals with large sizes.

- **At different pH levels in the synthetic brackish liquid**
  As pH increased both dominant and average size of struvite crystal decreased in the synthetic brackish liquid. pH8 would be preferable for struvite precipitation in the filtrate of saline digestate in order to harvest much struvite crystals with large size and obtain a high P-removal efficiency.

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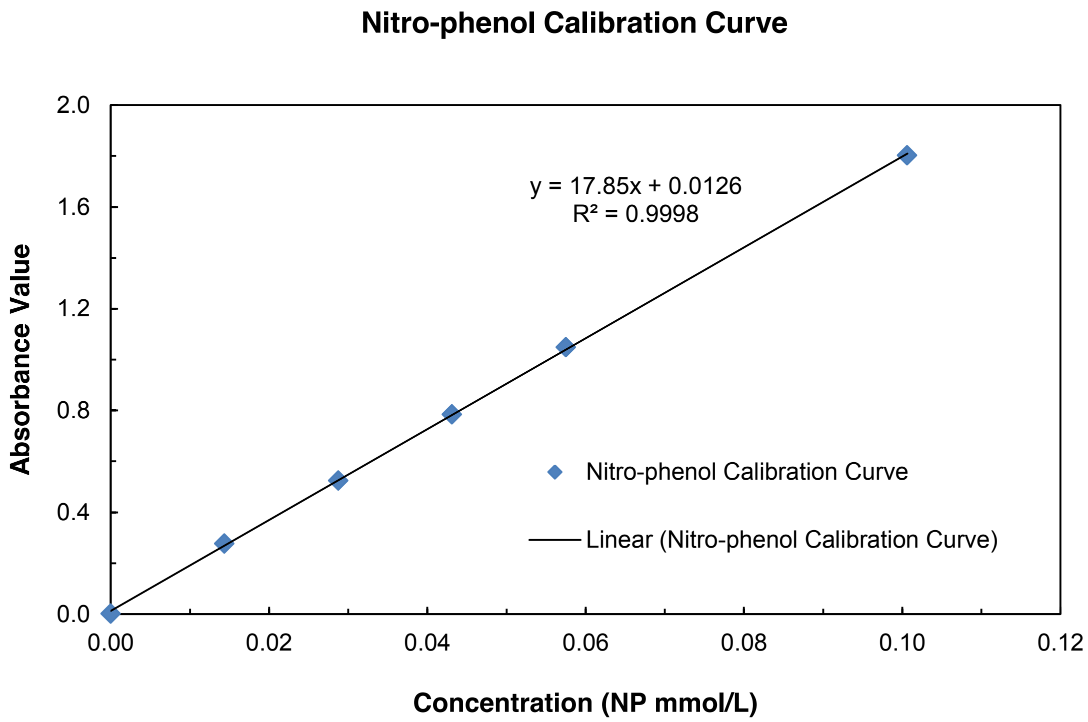
Appendix 1


Nitro-phenol Calibration Curve
y = 17.85x + 0.0126
R² = 0.9998
Nitro-phenol Calibration Curve
Linear (Nitro-phenol Calibration Curve)
Absorbance Value
Concentration (NP mmol/L)
2.0
1.6
1.2
0.8
0.4
0.0
0.00
0.02
0.04
0.06
0.08
0.10
0.12

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