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MASTER'S THESIS

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Abstract

Recirculating Aquaculture System (RAS) is a technology in which water is reused after different biological and mechanical treatment steps in fish farming. RAS produces a nitrate rich water effluent containing approximately 70-100 mg NO_3/l, and a typical plant will have to offload between 50-100 kg NO_3-N/d which needs to be denitrified before release to the marine environment to avoid eutrophication. Denitrification is a heterogenic process whereby reduced substrates (primarily organic, but also some reduced inorganic salts, like H_2S and Fe^{2+}, may serve as electron donors) are oxidized anoxically by reduction of NO_3 and NO_2 to N_2. Organic substrates may come from external sources (easily biodegradable substrates like acetate or methanol) or from internal, like the collected fish waste sludge containing feces and feed pellet residuals. Fish waste sludge is mainly particulate slowly biodegradable, and hydrolysis is necessary for use as C-source for denitrification. Fish sludge has been considered waste in the fish farming industries. Therefore, it is free, and applying it to run RAS is a resource recovery process. The kinetics (reaction rate) of denitrification using fish sludge is dependent on the chemical oxygen demand (COD) level; slowly biodegradable CODs (sbCODs) should be converted to readily biodegradable CODs (rbCODs) to provide the nitrate uptake process.

Raw fish sludge was step-fed once or twice a day to a batch reactor containing substrate adapted activated sludge loaded with an initial nitrate concentration of 360 mg/l. Fish sludge characterization wet analysis was done on three different sludge batches and were compared. Two fermentation tests at 12 and 20 ℃ were done on fish sludge to investigate the effect of fermentation on biodegradability of fish sludge. Biomass specific nitrate uptake rates (NUR) were measured by an ion selective electrode, and substrate degradability and was estimated. NUR was also estimated using an equivalent initial COD concentration of acetate, and maximum NUR rates using fish sludge and fermented fish sludge were evaluated relative to the acetate driven denitrification rate. Fish sludge COD were split into three biodegradable fractions (easily biodegradable, slowly biodegradable, and slowly biodegradable particulate) based on NUR profiles, and their corresponding COD estimated using typical denitrifying yield factors.

The observed acetate specific denitrification rate was 3.64 mg NO_3-N/g VSS. h while the fish sludge rates were estimated to 1.2, 0.9 and 0.2 mg NO_3-N/g VSS. h for the easily, slowly, and particulate degradable COD fractions respectively. Additionally, the effect of fermentation during anaerobic storages (over seven days) on sludge characteristics and volatile fatty acid production was investigated and the specific denitrification rate of settled and supernatant fermented sludge for easily degradable CODs was 3 and 2.2 mg NO_3-N/g VSS. h.

We conclude that direct use of fish sludge for denitrification of RAS effluents is possible, but design and operation would have to allow for the relative slow kinetics of the process, hypothetically limited by hydrolysis of slowly biodegradable dissolved and particulate COD fractions, which could be accelerated through fermentation.

Keywords: RAS, denitrification, NUR, fish sludge, biodegradable CODs, fermentation.

1. Introduction

1.1. Land based aquaculture

Aquaculture has a significant role for assuring food production, providing the world's population with high-quality protein, creating more jobs, and improving economic development (Béné et al., 2016). Traditional massive aquaculture methods, including outdoor pond systems, rely too much on natural land and area; moreover, they are subjected to many diseases and pollutants that is out of control which make them unsuitable for the sustainable progress of aquaculture (Turcios & Papenbrock, 2014). On the other hand, environmental control, standards, and regulations on wastewater discharge to the natural water environment necessitated more and more investigation and management in the aquaculture and fish farming industries (Davidson et al., 2014). This is demanded to reduce effluent discharge and water inlets to achieve the minimum adverse environmental effects (Ng et al., 2018). That’s why land-based recirculating aquaculture systems (RAS) are receiving more attention worldwide d (Ng et al., 2018).

The strict regulations of wastewater discharge in natural water systems increase the importance of wastewater management in aquaculture and fish farming industries (Gichana et al., 2018). The condition of production systems in RAS is much more controlled than other aquaculture processes, which is a benefit for marketing (Azaria & van Rijn, 2018). In addition, fewer environmental impacts have been observed using this potential new technology (Yogev & Gross, 2019). It was studied that about 10% of operation and investment costs could be reduced using RAS systems, including denitrifying reactors (Martins et al., 2010).

The land-based (indoor) RAS technology aquaculture and fish farming production examined to be sustainable because of utilizing small areas and space, higher yield production, being expandable, less potential diseases, and less environmental impacts (Qi, Zhu, et al., 2020). Also, the water and energy conservation rates are essentially much higher in RAS compared to traditional aquaculture systems, as water is being reused and recycled up to 90–99% in RAS technology (Gichana et al., 2018).

1.2. Nitrogen pollution, removal, and denitrification

However, toxic components such ammonia, nitrate, nitrite and particulate matters will build up in RAS as a result of feeding and excretion of fishes (H. Li et al., 2023). Aquaculture wastes are produced mainly by unconsumed feed, consumed, and expelled as ammonia through the gills or feces (Meriac et al., 2014). (Cripps & Bergheim, 2000) claims that any components or nutrients that are not eliminated during harvesting and are not kept as fish biomass can be considered waste. Only one-third of the nutrients in fish feed are digested, absorbed, and used in metabolic processes; the remainder is expelled as non-fecal or fecal wastes into the environment (Meriac et al., 2014). (Meriac et al., 2014) argue that fecal loss, consisting of undigested feed nutrients, is one of aquaculture's primary sources of solid waste. The fecal feces consists of suspended particles and dissolved nutrients with phosphorus and nitrogen (Losordo & Westers, 1994). Most non-fecal

losses are the metabolites of feed nutrients that fish consume but do not retain as biomass. Ammonia and urea, two nitrogen forms, make up most of the non-fecal loss's excretion (Meriac et al., 2014). (Montanhini Neto & Ostrensky, 2015) they are calculated that 1040.63 kg of organic matter, containing 44.95 kg of nitrogen and 14.26 kg of phosphorus, are produced, and released into the environment per ton of tilapia produced.

Nitrate, nitrite, and ammonia are the three primary nitrogen pollutants. Ionic ammonia (NH_4^+) and non-ionic ammonia (NH_3) are among them, and the ratio of the two forms in water is influenced by temperature and pH. Since NH_3 is soluble in lipid and rapidly crosses cell membranes, it is generally more hazardous than NH_4^+ and causes immediate damage to aquatic organisms by increasing the ammonia concentration in tissues (J.-C. Chen et al., 1990). Even in small amounts, nitrite and ammonia are toxic to aquatic life (Van Rijn et al., 2006). Despite some research indicating that nitrate is either non-toxic or weakly hazardous to aquatic life, nitrate accumulates quickly in a high-density RAS, where its concentration may even rise to 200 mg/L or higher (Poulsen et al., 2018). Nitrate removal in RAS should not be avoided because high nitrate concentrations can result in hypoxia, disturbing fish's ability to regulate their osmotic pressure, creating hormonal disorders, and damage to their gills and liver (Yu et al., 2021). Table 1 summarizes the RAS wastewater total nitrogen (TN) and total solid (TS) composition for different fish farming industries reported by (Van Rijn et al., 2006).

TABLE 1, DIFFERENT FISH SPECIES WASTE PRODUCTION IN RAS

Fish species	TN	TS
	kg per ton of fish production	
Rainbow trout	41-71	148-338
Brown trouta	49.2	438
Lake trouta	65.3	564
Barramundi	21.8-101.7	29-302.3
Gilthead seabream	102.9	447.5
Tilapia	72.4	520-650
Tilapia	48-72.7	192-268.8
Atlantic salmon	32	224

The environment receiving the waste and nitrogen pollutants could become eutrophicated and lose oxygen (Iwama, 1991). To avoid eutrophication of the receiving natural water systems, ensure the long-term sustainability of aquaculture, and maintain the integrity of the receiving environment, it is crucial to manage the discharge of aquaculture wastes appropriately (Bureau & Hua, 2010).

Water is continuously circulated and flowing by pumps in RAS. The nitrification process happens in RAS biofilters with nitrate as the final product. Nitrogen gas (N_2) is a safe form of nitrogen resulting from the biological nitrate removal of RAS effluent. The process is called denitrification, and the produced N_2 gas can quickly be released into the atmosphere (de Melo Filho et al., 2020). Moreover, the continuous water renewal in RAS also controls the extra NO_3–N concentration.

Nevertheless, nitrate accumulation prevention by denitrification is observed to be more efficient than standard water renewal system, which needs a sizeable hydraulic load of new water, about 11–59%. In an anoxic denitrification reaction, nitrate is used as an electron acceptor, and the organic carbon source acts as an electron donor (Díaz et al., 2012).

Several researchers have illustrated the practicality of denitrification reactors in marine and freshwater RAS systems. Using organic carbon matters sourced from fish sludge (fish feces and uneaten fish feed) for denitrification of freshwater RAS system has been investigated in fish farming (Shnel et al., 2002). These wastes should not accumulate in culture systems as their decomposition can result in oxygen deprivation and ammonia toxicity (L. Cao et al., 2007).

1.3. C-source, internal or fermentation

The efficiency of biological nutrient removal processes is solidly affected by the concentration levels of short-chain fatty acids (SCFA) and readily biodegradable CODs (rbCODs). The essential factor in the process has been illustrated to be readily biodegradable CODs concentration (Brinch et al., 1994). In the shortage of it, an external source of carbon is required to obtain a steady and efficient nutrient removal process (Police et al., 1993). Slowly biodegradable (particulate) organic matter hydrolysis rate is the bottleneck in nutrient removal and organic carbon cycling in most wastewater treatment plants (Morgenroth et al., 2002). The goal of sludge hydrolysis is to provide an internal carbon source increasing the rbCODs and SCFA portion. The complex organic matter is turned into simpler compounds in anaerobic fermentation (Canziani et al., 1995).

So, it is required to treat the RAS effluent and decrease the nutrient (nitrate) levels to prevent eutrophication and keep down the toxicity level. This study checks if fish sludge (directly or after fermentation) can be used as a substrate for nitrogen removal of RAS effluent through the denitrification process. Moreover, using fish sludge as a substrate is super beneficial to decrease the disposal and biological waste treatment fees and minimize the costs of buying external substrates like acetate or methanol. This is also important for the green shift for any RAS fish farming as Norwegian policy restrict further expansion of the aquaculture industry in open sea cages. Hence, growth and probably also restrictions of sea location use will require land-based systems. In addition, experience from Tytlandsvik show significantly better production performance in land-based RAS plants in terms of increasing production rate, reducing production time, lower mortality, better control on feeding system, less diseases and parasite problems, and no escape of fish.

2. Background

2.1. Land based fish farming process and unit operations

Land-based Recirculating aquaculture systems where water is (partially) reused after mechanical and biological treatment in an effort to reduce water and energy consumption and the release of

nutrients into the environment is an efficient solution for fish farming industries. Reduced consumption of water, more options for nutrient recycling and waste management, enhanced disease management, and better control over biological pollution are all benefits of RAS (Zohar et al., 2005). RASs are still improving and there are two trends to look out: technical advancements inside the recirculation loop, and nutrient recycling (Martins et al., 2010).

Oxygen, pH, carbon dioxide, alkalinity, and metals (such as aluminum) are the primary water quality indicators in RAS. These variables are important to control inlet water flow. The initial limiting factor for the required water flow is thought to be oxygen (Fivelstad et al., 2004). The water will be low in oxygen and high in suspended particles, ammonia and nitrogen, phosphorus, and carbon dioxide after it has been passed through the rearing tanks. Prior to reuse in the fish tanks, the treatment loop's components are selected and created to improve the water quality. They include biological (aerobic and anaerobic biofiltration) and physico-chemical (mechanical filtration, liquid-gas exchange, pH regulation, and disinfection) processes. The quality of added inlet water must be carefully regulated, and the wastes are typically treated in a separate supplementary side loop. As mentioned, a recirculation system involves a variety of treatment unit operations that recover the water making it ready for the continuous cultivation of the desired product. The need for a given unit operation varies depending on elements like the amount of water reused and the objective water quality requirements, so all the previously mentioned unit operation cannot be found in every RASs. Additionally, there are local variations in companies' preferences for technologies which are generally influenced by economics (J.-P. Blancheton et al., 2007).

However, for fish pre-growing and on-growing level, every recirculation system follows the same core design, stated by several authors (J. P. Blancheton, 2000; E. Eding & Kamstra, 2002). First, a mechanical filter with a range of 40 to 100 µm in mesh size is used to remove particles from the fish tanks' outlet flow. The water is then directed to a biofilter where ammonia is converted to nitrogen through the nitrification process, which can occur in either a trickling filter or a submerged filter or both. Gas transfer equipment is commonly placed after biofiltration to remove extra carbon dioxide and provide oxygen. For efficient removal of carbon dioxide, which is produced by fish, a customized vented packed column or a ventilated trickling biofilter, which may also be utilized to cool the water when necessary is employed. By using a high-pressure compressor (often a cone), the oxygen (which is required for fish respiration) can be directly delivered into the inlet water flow to the tanks (E. H. Eding et al., 2006).

Typically, different systems vary in at least two key aspects, both of which are connected to the biofilter, the primary part of the treatment system. Every type of biofilter performs nitrification and particulate carbon transformation. Water aeration, degassing (carbon dioxide removal), and optional cooling are all combined in a single unit using trickling bio filters and air-stirred moving beds. For aeration, CO_2 degassing, and cooling, a separate column with ventilation is needed in systems with submerged filters. Due to the self-cleaning feature of trickling filters and the continuous operation of water treatment procedures without breaks caused by back flushing, the labor required is also decreased. The volume of a submerged biofilter gets smaller (by a factor of

10) but, depending on the type, it can also require backwashing. For instance, a submerged moving bed biofilter might not require backwashing, whereas static bed biofilters often require it 2 to 4 times every month (J.-P. Blancheton et al., 2007).

An example of process and involved unit operations in RAS is running at Tytlandsvik salmon production company having five mechanical drum filters with the mesh size of 40 – 60 µm, eight aerated biofilters, CO_2 stripping compartment and Ozone treatment unit for disinfection, and oxidation. Figure 1 shows the simplified process diagram of Tytlandsvik (Tytlandsvik Aqua AS).

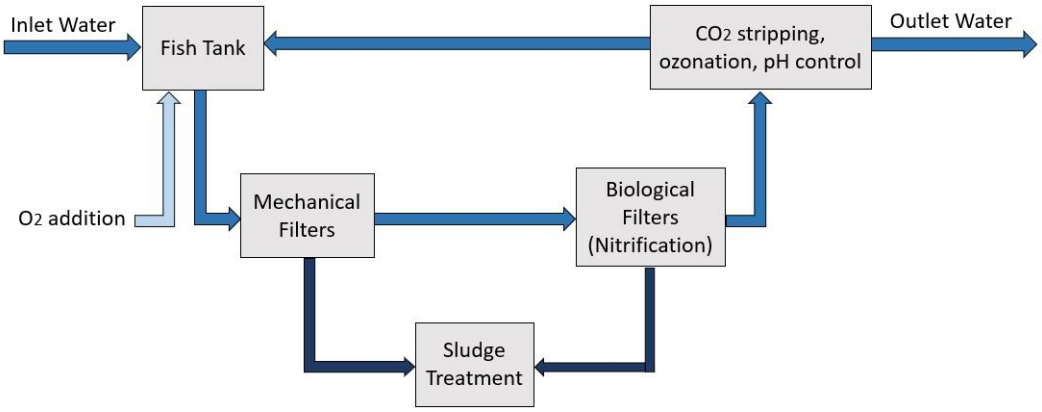

FIGURE 1, TYTLANDSVIK PRODUCTION UNIT

2.2. Nitrogen dynamics in RAS systems

As mentioned, the most involving risk to aquaculture habitats is currently nitrogen pollution. Ammonia and nitrite are in particular dangerous to aquatic life. In the RAS, biofiltration mostly eliminates the nitrogen contaminants. Ammonia and nitrite are eliminated through the nitrification reaction, when aerobic condition is present, and the appropriate functional bacteria and archaea are active. Nitrification reaction is often separated into two phases, ammonia is initially converted to nitrite under the presence of ammonia oxidizing archaea or ammonia oxidizing bacteria (Ebeling & Timmons, 2010):

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H_2 + H_2O \qquad \text{(Eq.1)}$$

and nitrate is produced by further nitrite oxidizing under the presence of nitrite oxidizing bacteria:

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \qquad \text{(Eq. 2)}$$

Additionally, RAS contains complete ammonia oxidizers like *Nitrospira sp.*, which have the necessary genetic components for combined ammonia and nitrite oxidation ($NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$) (Preena et al., 2021):

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \qquad \text{(Eq. 3)}$$

Also, several anaerobic anammox (ammonium oxidizing) bacteria have the ability to oxidize ammonia to nitrite and then turn it into nitrogen gas (N_2) (Ruiz et al., 2020):

$$NH_4^+ + NO_2^- \rightarrow N_2(g) + 2H_2O \qquad \text{(Eq. 4)}$$

A few fishes in aquaculture, like the Nile tilapia (*Oreochromis niloticus*), can resist high nitrate concentrations (500 mg/L), but not all fish can (Monsees et al., 2017). For instance, NO_3 levels more than 50 mg/L can cause endocrine disorders in juvenile turbot (*Scophthalmus maximus*) as well as tissue damage to the liver and gills (Yu et al., 2021). It is advised to grow *Oplegnathus punctatus* in RAS with nitrate levels less than 165 mg/L (Yang et al., 2019). As a result, although nitrate is less harmful than nitrite and ammonia, proper controlling techniques must be employed to ensure that all nitrogenous contaminants are eliminated to from a steady aquaculture system. In addition, other different treatment methods has been applied in recent years to remove nitrogen pollutants from RAS like electrochemical catalysis, trickling filters, rotating biological contactors, fluidized sand biofilters, moving bed biofilm reactors, fixed bed biofilm reactors, floating bead filters, microbead biofilters, and constructed wetlands (H. Li et al., 2023).

2.3. Denitrification

Denitrification is a process that converts nitrate to nitrogen gas using organic carbon under anaerobic microbial conditions. Gaseous nitrous oxide, nitrite, and nitrate are reduced through the denitrification process (Pungrasmi et al., 2013). Heterotrophic bacteria, autotrophic facultative aerobic bacteria, along with certain fungi that are often found in the environment all participate in the microbial process of biological denitrification, which removes nitrate from RAS. Denitrification is performed step-by-step, nitrate gradually converted into nitrite, nitric oxide (NO), nitrous oxide (N_2O), and finally nitrogen (N_2) under the effect of denitrifying bacteria (Ebeling & Timmons, 2010).

$$NO_3^- + 2e^- + 2H^+ \rightarrow NO_2^- + H_2O \qquad \text{(Eq. 5)}$$

$$NO_2^- + e^- + 2H^+ \rightarrow NO + H_2O \qquad \text{(Eq. 6)}$$

$$2NO + 2e^- + 2H^+ \rightarrow N_2O + H_2O \qquad \text{(Eq. 7)}$$

$$N_2O + 2e^- + 2H^+ \rightarrow N_2 + H_2O \qquad \text{(Eq. 8)}$$

And the overall stoichiometry for heterotrophic denitrification (glucose as substrate) follows:

$$5C_6H_{12}O_6 + 24NO_3^- + 24H^+ \rightarrow 30CO_2 + 12N_2 + 42H_2O \qquad \text{(Eq. 9)}$$

When organic carbon components are present, nitrate and nitrite are employed as terminal electron acceptors in denitrification (Gutierrez-Wing et al., 2012). Each nitrate ion loses one atom to become a nitrite ion when oxygen is absent, making nitrate the terminal electron acceptor. Although denitrification can be either heterotrophic or autotrophic, most denitrification systems

are heterotrophic, using organic carbon sources to drive facultative anaerobic bacteria (Van Rijn et al., 2006).

Since denitrifiers are more prevalent in the natural environment and grow more quickly than nitrifiers, denitrification may be a way to overcome the restrictions of nitrification. Additionally, because aeration is not required, the procedure lowers energy costs. Water usage is also significantly decreased because water exchange is minimal (Gichana et al., 2018).

Lots of bacterial organisms can grow without needing oxygen by converting certain types of nitrogen compounds into gas forms. The majority of bacteria that engage in denitrification belong to various genera such as *Achromobacter, Acinetobacter, Aeromonas, Gallionella, Halobacterium, Halomona, Hyphomicrobium, Janthinobacterium, Neisseria, Paracoccus* (previously known as *Micrococcus*), *Propionibacterium, Pseudomonas, Rhizobium, Rhodobacter* (previously known as *Rhodopseudomonas*), *Thiobacillus, Thiosphaera, Vibrio*, and *Xanthomonas* (Matěj\uu et al., 1992).

2.3.1. Competition

Some bacteria from the family *Paracoccus, Thiobacillus, Thiosphaera*, and others, can carry out denitrification autotrophically consuming hydrogen or different reduced sulfur compounds, like S^0, S^{2-}, $S_2O_3^{2-}$, $S_4O_2^{2-}$, or SO_3^{2-} as their energy source. Also, Ferrous iron can be used by bacteria from the families *Ferrobacillus, Gallionella, Leptothrix*, and *Sphaerotillus* as a source of energy for autotrophic denitrification. Carbon dioxide or bicarbonate is utilized as a source of carbon for microbial cell formation when autotrophic growth conditions are present. In lack of an organic carbon source, *Paracoccus denitrificans* can denitrify utilizing hydrogen. Reduced sulfur compounds and ferrous iron are the energy sources used by *Thiobacillus denitrificans* and *Ferrobacillus ferrooxidans*, respectively. If a source of organic carbon is available, *Paracoccus denitrificans* and *T. denitrificans* can develop heterotrophically. The exclusively *autotrophic F. ferrooxidans* use carbon dioxide as its carbon source (Burghate & Ingole, 2014).

2.3.2. Microbiology

When conditions become favorable for denitrification, the enzymes related to this process are synthesized. The production of denitrifying enzymes is usually tightly controlled and regulated. In general, denitrifying enzymes are responsive and can be induced under certain conditions. The synthesis of these enzymes takes place in anaerobic conditions, although denitrification can still happen even in the presence of oxygen. In some examples, the induction of enzymes may even necessitate low levels of oxygen (P. G. Lee et al., 2000).

The action of the transfer of electrons from an electron donor (organic matter) to an electron acceptor (nitrate) is known as nitrate respiration. A protons gradient is then created across the bacterial cell membrane as a result of this redox reaction. ATP synthase then transforms this gradient of protons into energy in the form of ATP (J. Chen & Strous, 2013). Initially, the electrons from the main source of electrons generate co-enzymes, such as NADH, or other possible electron

donors, like succinate, which deliver the electrons to the respiratory cycle. Then, three various kinds of electron transporters move electrons across the respiratory chain: the coenzyme Q, the cytochrome bc complex, and the cytochrome c. They can interact with various reductases (J. Chen & Strous, 2013; van Spanning et al., 2007).

The nitrate reductase (Nar) is the first reductase. Bacterial Nar complexes come in three different varieties. The molybdoenzyme NarGHI, having active site towards the cytoplasm, is a membrane complex. This complex, a NO_3/NO_2 antiporter that takes NO_3 and releases NO_2, is typically next to the narK membrane protein. Although the periplasmic reductase Nap reduces nitrate, it is unable to maintain the proton gradient. Assimilatory nitrate reduction is carried out by the final nitrate reductase (Nas), a periplasmic complex that is completely distinct from other reductase systems (Richardson et al., 2007).

Nirite reductase (Nir) then reduces the nitrite that is generated during the nitrate reduction. The heme-based cd-nitrite reductase and the copper-based nitrite reductase are the two periplasmic kinds of enzymes that were previously described (Rinaldo & Cutruzzolà, 2007). The membrane nitric oxide reductase (Nor), an enzyme of the heme-copper oxidases class that is also able to reduce oxygen, transforms nitric oxide (the product of nitrite reduction). Nitrous oxide reductase does the last step. The enzyme is periplasmic and has double Cu cores (J. Chen & Strous, 2013; de Vries & Pouvreau, 2007).

Based on detailed investigations of the enzyme systems in specific bacterial species, it can be inferred that the factors influencing the induction and repression of these enzymes are not the same for all denitrifying bacteria. Denitrifying bacteria have genetic diversity and metabolic adaptability as a collective group, which accounts for the observed variation in factors controlling enzyme induction and repression. Several research has been done to investigate the impact of oxygen concentration, pH, temperature, electron donor, as well as nitrate and intermediate concentrations on the denitrification performance of specific bacterial species. In contrast, there is limited knowledge regarding the regulatory interdependence of the involved reductase (Burghate & Ingole, 2014).

2.3.3. Temperature

The hydrolysis of endogenous carbon components and the activity of denitrifying bacteria is affected by temperature (Canziani et al., 1999). The Arrhenius equation describes the relationship between denitrification rate and temperature between the minimum (0 ℃) and optimal (around 40 ℃) values; this equation's coefficients are affected by the carbon source and the pH level. It was discovered that the temperature coefficient relied on the kind of carbon source only between 10 to 20 degrees Celsius. There was no clear pattern observed in the higher temperature range that they studied (Elefsiniotis & Li, 2006).

Denitrifying bacteria prefer temperatures between 20 and 40 ℃; below 15 ℃, bacterial activity decreases and nearly stops at temperatures below 5 ℃ (J. Wang & Chu, 2016). (J. Wang & Chu,

2016) noticed that a 5% drop in temperature resulted in a 50% reduction in nitrate uptake. As a result, the uptake rate of nitrate depends on temperature and rises as the temperature rises. For every 10 ℃ change in temperature, denitrification rates rise by a factor of 2, similar to other physiological processes (Warneke et al., 2011).

2.3.4. pH and Alkalinity

If the environment is not buffered, microbial denitrification is always followed by the formation of OH ions, which change the pH of the environment (Albina et al., 2019). Alkalinity is formed during the denitrification process along with an increase in pH; 3.57 mg of alkalinity is produced approximately for one milligram of nitrate reduction (Pungrasmi et al., 2013). However, the ideal denitrification rates occur between pH levels 6.7 and 7.5 and the ideal pH range for most types of neutrophilic denitrifying bacteria is between 7.5 and 9.5. Denitrification is limited below pH values of 6.0 and above pH values of 8.0 (S. Cao et al., 2013). In denitrification systems, increased pH can inhibit nitrate and nitrite reduction, whereas lowered pH entirely prevents denitrification since denitrifiers cannot denitrify in low pH environments. In addition to how the enzymes are affected by the acidic pH, the nitrous acid (HNO_2) formation from nitrite can decrease the bacterial activity as nitrous acid is able to pass through bacterial membranes (Marais et al., 1988). Due to the effect of pH on the way every enzyme function, there can be an unbalance in the kinetics of denitrification's reduction. Thus, buildup of intermediates like nitrite is a result of alkaline pH. Even alkaliphilic bacteria can only survive a maximum pH of about 11.5 to 12 (Sorokin, 2005).

2.3.5. Dissolved oxygen

Using facultative anaerobes, the anaerobic microbial process of denitrification converts nitrate to dinitrogen gas (Van Rijn et al., 2006). Therefore, denitrification is feasible in fresh and marine environments at dissolved oxygen (DO), typically 0.2 mg L^{-1} or less (Van Rijn et al., 2006). Because oxygen is a more effective electron acceptor than nitrate, high dissolved oxygen concentrations may impede the process by inhibiting enzymes or directly competing with them. (Xu et al., 2009) Nitrate removal dropped from 85% to 50% at DO amounts higher than 4.0 mg L^{-1}.

2.3.6. Carbon supply

One of the essential substrates that affect the denitrification process is organic carbon. The process of denitrification needs a carbon to nitrogen ratio of 3-6 g COD/g NO_3-N. The rate of denitrification is typically determined by dividing the difference between influent and effluent nitrate concentrations by hydraulic retention (Van Rijn et al., 2006). When there is a carbon shortage, exogenous organic carbon sources like methanol, ethanol, acetic acid, acetate, and glucose are usually added to ordinary wastewater treatment plants, even though they are expensive (Arbiv & van Rijn, 1995). Table 2 contains the equations that show the stoichiometric amounts of different organic carbon substrates needed for nitrate dissimilation (Burghate & Ingole, 2014).

TABLE 2, HETEROTROPHIC DENITRIFICATION STOICHIOMETRIC WITH DIFFERENT CARBONACEOUS SUBSTRATES

Substrate	Stoichiometric Equation
Ethanol	$5C_2H_5OH + 12NO_3^- \rightarrow 10HCO_3^- + 2OH^- + 9H_2O + 6N_2$
Acetic Acid	$5CH_3COOH + 8NO_3^- \rightarrow 8HCO_3^- + 2CO_2 + 6H_2O + N_2$
Cellulose	$5(C_6H_{10}O_5)_n + 24NO_3^- \rightarrow 12nN_2 + 6nCO_2 + 13nH_2O + 24nHCO_3^-$
Glucose	$C_6H_{12}O_6 + 2.3H^+ + 2.8NO_3^- + 0.5NH_4^+ \rightarrow 0.5C_5H_7NO_2 + 1.4N_2 + 3.5CO_2 + 6.4H_2O$

Utilizing these carbon sources causes the formation and buildup of organic acids, like acetic acid, which damages fish physiology and microorganisms (P. G. Lee et al., 2000). (Stief, 2001) observed accumulation of nitrite during denitrification while utilizing glucose as the carbon source. Less unstable organic substances cause considerable nitrite accumulation, or specific forms of carbon can drive out actual denitrifiers and encourage the growth of facultative organisms that only reduce nitrate to nitrite. A variety of alternatives have been investigated as carbon sources; for example, (Soares et al., 2000) used cotton wool as the only source of carbon to reduce nitrate in well water with high concentrations.

2.4. Particulate C-sources: Hydrolysis and fermentation

Fecal waste has been effectively used in various studies as an internal supply of carbon for denitrification (Gelfand et al., 2003). However, the organic matter in feces is in the form of particles and is not readily usable by microbes. But, fermentation and hydrolysis can transform the compounds into volatile fatty acids (VFAs) (S.-I. Lee et al., 1995). Furthermore, by hydrolysis and fermentation, total ammonia nitrogen and phosphorus can be solubilized by requiring further treatment of the two components (Conroy & Couturier, 2010). When fish waste is used as a carbon source, organic loading can be reduced (Van Rijn et al., 2006).

One of the most critical factors in anaerobic digestion reactions is the biodegradation of particulate organic substrates (Gao et al., 2016). Generally, bacterial cells cannot take the particles up directly, so extracellular depolymerization of particles is needed for enough size reduction for transport into the membrane of bacterial cells. The cellular uptake size limit is commonly considered 0.6-1 kDa. The controlling mechanisms in forming sub polymeric intermediate and depolymerization are depolymerisation and hydrolytic (White et al., 1995). Substrate availability is mainly affected by colonization and increased porosity of particles, especially for dispersed biomass, while particle breakup significantly impacts flocculated biomass (Ravndal & Kommedal, 2017).

Different methods are introduced for sludge reduction, including ultrasonic mechanical disintegration, ozonation, advanced oxidation processes, thermal treatment, freeze-thawing, acidic or alkali chemical treatment, and enzymatic biological hydrolysis treatment. However, biological hydrolysis using aerobic or anaerobic digesters is considered more efficient and cost effective in wastewater treatment plants (Ayol et al., 2008). The size reduction rate of bigger particulate organic matters does not change so much with increasing the rate of organic load and decreasing

the hydraulic retention time, which shows that if soluble organics accumulate in the digestate, the rate limiting step is not the disintegration of bigger particulate organics. This indicates that the speed of the hydrolysis process is limited by the enzymatic hydrolysis of soluble organics step (Gao et al., 2016). It is claimed that biodegradability enhancement is more effective in optimizing the digestion process than the size reduction of the particles (Gao et al., 2016).

Various indicating techniques are introduced to measure the amount of sludge disintegration, including COD solubilization, degree of disintegration (DD), and efficiency of biogas production (Foladori et al., 2010; Jin et al., 2015; Negral et al., 2015). Although total COD (tCOD) is not a precise method for monitoring the biodegradation of organic compounds, chemical oxygen demand is a standard indicator in wastewater treatment processes (Ciaciuch et al., 2017).

2.4.1. Sludge pre-treatment

In a wastewater treatment system, the mechanism and rate of hydrolysis and degradation are determined by the particle size and content. In the activated sludge models, "slowly biodegradable organic matter" is tangentially related to particle size (Henze et al., 2000). However, it can be expected that most of the slowly biodegradable organic matter lies between 103 amu and 100 mm (Morgenroth et al., 2002).

Research on sludge minimization approaches has recently increased to address sludge-related issues, lower investment, and operating costs, and improve the effectiveness of following treatment and final disposal processes (Ayol et al., 2008). Pretreatment of organic particle matters alters the typical size and structure of solids in a waste (Hobson & Wheatley, 1993).

Applying sewage sludge pre-treatment before anaerobic digestion (AD) is more frequent to enhance hydrolysis. The disintegration techniques that are most frequently used are: ozonation (G. Zhang et al., 2009), sonication (P. Zhang et al., 2007), microwave pre-treatment (Appels et al., 2013), thermal disintegration (Ferrer et al., 2008; Pilli et al., 2015) and acidic and alkaline hydrolysis (Y. Zhang et al., 2012).

The breakdown of the sludge flock structure and the cell walls of the microorganisms in the activated sludge represent the actions of the different disintegration techniques. As a result, the microbial cell content is dispersed into the sludge liquid, making it easier for microbes to degrade (Eskicioglu et al., 2006).

Protozoa can initially take up micrometer range particles and degrade them intracellularly through phagocytosis (Alberts et al., 1994). As a result, most wastewater particles do not have to undergo extracellular hydrolysis before protozoa can digest them. Since most protozoa are aerobic, anoxic and anaerobic environments would significantly inhibit their activity (Morgenroth et al., 2002).

Various techniques, from size reduction to cell disintegration, have been tested to improve anaerobic biodegradation (Palmowski & Müller, 2003). One method for speeding the digestive process is particle size reduction to enhance the effective specific surface. According to

(Kayhanian & Hardy, 1994), the feedstock particle size had a negative relationship with the methane production rate. (Wen et al., 2004) demonstrated that after 96 hours of treating animal dung, reducing the particle size from 840–590 to 590–350 µm increased glucose output by 29%; however, further reducing the particle size had no impact.

2.4.2. Hydrolysis

The term "hydrolysis" describes breaking down organic substrate matter into smaller compounds that can be ingested and degraded by bacteria. There are two distinct types of hydrolysis: (a) hydrolysis of primary substrate, which refers to the breakdown of organic substrate found in the initial wastewater, and (b) hydrolysis of secondary substrate, which corresponds to the breakdown of substrate generated by the bacteria (for instance, the hydrolysis of products from internal storage, compounds produced by bacteria through regular metabolism, or particles generated when bacteria decay) (Bryers & Mason, 1987; Van Loosdrecht & Henze, 1999).

The transformation of slowly biodegradable organic matter into rapidly biodegradable organic matter, which can act as an essential source of carbon for denitrification or biological phosphorus removal, is determined by hydrolysis rates, which are considered when designing reactors in a nutrient removal treatment plant. The capacity of nutrient removal plants is directly impacted by the organic particle fraction and related hydrolysis rates (Morgenroth et al., 2002).

The precise definition of hydrolysis is breaking a polymer into small parts caused by the presence of water (Brock & Madigan, 1991). The procedure of hydrolysis in wastewater treatment plants enumerates all methods that provide a slowly biodegradable substrate accessible for bacterial growth (Gujer et al., 1999).

2.4.2.1.Hydrolysis modeling and mechanisms

Fragmentation, hydration, and free extracellular enzymatic activity may all work together to cause particle cracking (Ravndal & Kommedal, 2017). For particle hydrolysis, many mechanisms and modeling techniques have been presented (Morgenroth et al., 2002; Vavilin et al., 2008). The solid waste particles in a model for anaerobic digestion put out by (Vavilin et al., 1996) are supposed to be colonized by hydrolytic bacteria, which then create hydrolytic enzymes. Particulate organic matter hydrolysis is as diverse as the various organisms and particles engaged. Particles are made up of many organic substances, and higher organisms and a variety of microorganisms are involved in the process (Morgenroth et al., 2002).

Two distinct depolymerization processes mediated by various enzyme types can be distinguished as exo- and endo-enzymes. Endo-enzymes generally operate on internal polymer bonds far from the terminal monomers, whereas exo-enzymes selectively act on a specific bonding upstream of the end. Consequently, various endo/exo couple activities are typically involved in polymer degradation (Morgenroth et al., 2002). The Enzyme Handbook (Schomburg & Stephan, 1997) states that there are 197 extracellular enzymes known. Eleven lyases and about 145 hydrolytic enzymes are present.

(Hobson & Wheatley, 1993) recommended that a reload of sludge digesters for particle animal waste must be discontinuous, with a brief feeding interval. The model had the assumption that some of the feed would be swept away with the overflow. Sometimes, the degradation of suspended solids is estimated using the Monod equation (Lin, 1991).

The amount of accessible particle surface area will determine whether particulate degradation occurs by colonialization or flocs adsorption. Degradation is dependent on particle morphology as well as particle–biomass contact. The shrinking particle model is one of the two suggested models (SPM) (W. T. M. Sanders et al., 2000); furthermore, the particle breakup model (PBM) (Dimock & Morgenroth, 2006). According to the SPM, particles slowly shrink as they degrade, resulting in a reduction in surface area. When particles degrade in the PBM, they break apart, increasing the surface area that is accessible. As a result, the PBM's ratio of surface area to volume is a scale variable (Ravndal & Kommedal, 2017).

In both activated sludge models, the hydrolysis process is defined as an enzymatic degradation process (Henze et al., 2000) and an anaerobic digestion model (Batstone et al., 2002). Electron acceptors do not affect the kinetics of hydrolytic enzymes (Goel et al., 1998), as a result, hydrolysis research done under aerobic conditions has relevance under anaerobic conditions and conversely. However, because of the connection between cellular yield and electron acceptor conditions, the concentration of the hydrolytic enzyme may fluctuate (anaerobic condition provides lower concentration) (Kommedal, 2003). (Negri et al., 1992) suggested a hydrolytic stage mixed model (heterogeneous and homogeneous) for solid waste anaerobic digestion in the plug-flow digester.

The hydrolytic microbial cells and the discharged enzymes come into touch with the particle substrates throughout hydrolysis. Hydrolysis kinetics should be described in terms of two primary phases. In the first stage, the hydrolytic bacteria cover the surface of the particles during the colonization phase. To make the monomers that can be used by both the hydrolytic bacteria and other bacteria, bacteria on or around the solid surface generate enzymes (Zavarzin, 1986). The young cells separate and enter the liquid phase before attempting to adhere to a different place on a solid surface. In the second phase, when bacteria cover all the available surfaces, the surface will degrade at a consistent depth per unit of time (Vavilin et al., 1996).

2.4.2.2.Disintegration, solubilization, and enzymatic hydrolysis

In most practical situations reported in the literature, the notions of solubilization, disintegration, and enzymatic hydrolysis are typically conveyed by the essential kinetic term of hydrolysis (Batstone et al., 2002). The breakdown of composite material results in forming particle carbohydrates, proteins, and lipids together with particulate and soluble inert material. Microorganisms take advantage of the soluble products and create the necessary hydrolytic enzymes due to the enzymatic breakdown of particulate carbohydrates, proteins, and lipids, which leads to the formation of monosaccharides, amino acids, long chain fatty acids, and glycerol (Vavilin et al., 2008).

The hydrolytic enzymes, including glucosidases, lipases, and proteases, significantly depend on the hydrolysis of complex organic compounds in biodegradable particulate organic matter degradation. Earlier studies on the kinetics of hydrolysis on the anaerobic degradation of particulate organics have noted that the EPS retains the extracellular hydrolytic enzymes as a network (Vavilin et al., 1996).

The anaerobic digestion (AD) process, frequently used to stabilize sewage sludge, causes complex organic components to degrade due to microbial activity. Microorganisms release extracellular enzymes, which dissolve in solution and hydrolyze suspensions and dissolved particles. This process continues through several steps till the monomers that bacteria can absorb are formed. Because biological growth is typically slower than the hydrolysis of organic molecules, the hydrolysis step is viewed as a limiting AD process from the perspective of reaction rate (Ciaciuch et al., 2017).

When degrading complex organic structures into biodegradable particulate organic matter, hydrolytic enzymes such as lipases, glucosidases, and proteases play a crucial role. Extracellular polymeric substances (EPS) play an essential part in this degradation process by regulating extracellular hydrolytic enzymes (Ayol et al., 2008). Enrichment of active enzymatic systems may result in floc disintegration during aerobic or anaerobic digestion, which logically causes more EPS breakdown in the sludge (Ayol et al., 2008).

Because of the prokaryotic cell wall, most bacteria cannot degrade particulate and aqueous polymers by phagocytosis. Therefore, extracellular depolymerization precedes cellular uptake, followed by metabolization (Chrost, 1991). Some eukaryotes, such as fungi and yeast, also produce depolymerization enzymes. Extracellular hydrolases and lyases typically carry out depolymerization. Lyase creates an unsaturated and protonated end, whereas hydrolytic cleavage is characterized by adding hydroxyl and proton (water molecule) (Morgenroth et al., 2002).

Extracellular enzymes (hydrolases) perform the hydrolysis of organic polymers. The differences in the rate of hydrolysis of the particulate carbohydrates, proteins, and lipids are due to the concurrent enzymatic reactions with cellulases, proteinases, and lipases, correspondingly (Stryer, 1988). Hydrolysis products, volatile fatty acids (acetate, butyrate, propionate, lactate, etc.), and hydrogen are produced during the breakdown of monosaccharides, amino acids, and long-chain fatty acids. These compounds serve as precursors for methane formation (Vavilin et al., 2008).

2.4.2.3.Hydrolysis in denitrification

The quantity and rates of hydrolysis processes frequently restrict nutrient removal (denitrification and phosphorus removal), and particle organic matter might be relevant for selecting particular bacterial populations (Frigon et al., 2002). The eventual fate of particulate organic matter in wastewater was less essential as long as wastewater treatment plants did not need denitrification or biological phosphorus removal (Morgenroth et al., 2002).

Nitrate (NO_3^--N) is a frequent hazardous contaminant that can result in severe water eutrophication, human brain damage, and even death (Qi, Taherzadeh, et al., 2020). Worryingly, due to the rapid economic growth, various businesses, including food processing, metal finishing, and aquaculture, are generating NO_3^--N-containing wastewater at an alarmingly fast rate (Ghafari et al., 2008). The most recommended NO_3^--N treatment method is biological denitrification, which converts NO_3^--N to harmless nitrogen gas due to its excellent removal performance and process stability (Liu et al., 2018).

It is generally recognized that compared to complex matter, readily biodegradable organic matter (RBOM) offers higher NO_2^--N formation. In recent years, RBOM has been a popular carbon source for partial denitrification (PD). Examples of this ethanol (Du et al., 2017), acetate (Du et al., 2019; W. Li et al., 2018), glucose (Qin et al., 2017), and glycerol (Le et al., 2019). Nevertheless, slowly degradable organic matter (SBOM) typically makes up 30–85% of the influent carbon source in various types of wastewater (De Kreuk et al., 2010; Goel et al., 1998), and it has not been recognized as being suited for an effective PD (S. Cao et al., 2017; Shi et al., 2019). The main obstacles to executing a cost-effective PD are the lack of RBOM and SBOM unavailability. Recently, it was proposed to use the hydrolytic acidification method to provide the regular SBOM carbon source for the PD process (Shi et al., 2020b).

2.4.2.4.First-order kinetics of organic matter hydrolysis

First-order kinetics has typically been used to model hydrolysis. First-order kinetics for difficult substrates should be adjusted for highly resistant material. It has been demonstrated that models that link the growth of hydrolytic bacteria to hydrolysis perform well when there is a high or fluctuating organic loading (Vavilin et al., 2008). Identifying substrate fractions' biodegradation rates is one of the most critical developments in the conceptual comprehension and modeling of activated sludge systems (Orhon et al., 1999). The most straightforward sort of substrate degradation kinetics, first-order kinetics (equation 1), has been effectively employed to describe hydrolysis (Orhon et al., 1999).

$$dX_s/dt = -K_h X_s \qquad \text{(Eq. 10)}$$

As stated by (Eastman & Ferguson, 1981), the empirical expression of the first-order hydrolysis function captures the synergistic effects of numerous processes. Hydrolysis is slower in large particles with a low surface-to-volume ratio than in small particles. Suspended degradable particles are converted into dissolved organic substrates by hydrolytic enzymes generated by biomass (Vavilin et al., 1996). When the surface of the particulate substrate acts as the rate limiting factor and bioavailability or biodegradability phenomena do not intervene, first-order kinetics can be used (W. T. Sanders et al., 2003).

2.4.2.5.First-order kinetics of carbohydrate, lipid, and protein degradation

Particles must degrade through various stages before uptake (Ravndal & Kommedal, 2017). Complex organic particles (proteins, polysaccharides, lipids) make up the majority of the substrates with a slow biodegradation rate (XS) (Ciaciuch et al., 2017). The appropriate electron acceptor uptake rate (OUR, NUR) is required to observe the destiny of slowly biodegradable COD in particular wastewater (Ekama et al., 1986).

The rate coefficient value for various substrates that can be found throughout the literature is compiled in Table 3. A wide variety of first-order rate coefficient values can be observed for complex and basic organic materials, such as carbohydrates, lipids, and proteins. Different experimental settings, various hydrolytic ratios of biomass to the substrate, and the combined effect of disintegration and hydrolysis can all describe this wide range of results (Vavilin et al., 2008).

TABLE 3, KINETIC COEFFICIENT OF THE FIRST ORDER RATE OF HYDROLYSIS

Substrate	K_h (day $^{-1}$)	T (°C)	Reference
Carbohydrates	0.025-0.2	55	(Christ et al., 2000)
Proteins	0.015-0.075	55	(Christ et al., 2000)
Lipids	0.005-0.010	55	(Christ et al., 2000)
Lipids	0.63	25	(Masse et al., 2002)
Cellulose	0.066	35	(Liebetrau et al., 2004)
Cattle manure	0.13	55	(Vavilin et al., 2008)
Proteins (gelatine)	0.65	55	(Flotats et al., 2006)
Municipal solid waste	0.1	15	(Bolzonella et al., 2005)
Primary sludge	0.4-1.2	35	(O'ROURKE, 1968)
Primary sludge	0.99	35	(Ristow et al., 2006)
Secondary sludge	0.17-0.6	35	(Ghosh, 1981)

2.4.2.6.Temperature dependence of first-order hydrolysis rate

(Veeken & Hamelers, 1999) some solid organic waste components with first-order hydrolysis rates were evaluated for temperature dependency, and the Arrhenius equation was used to determine the average activation energy (64 ± 14 kJ mol^{-1}).

(Pu et al., 2019) observed that raising the temperature accelerated hydrolysis by increasing the activity of the hydrolytic enzyme. By selectively increasing the Lactobacillus (90.6% of relative abundance), a mesophilic temperature (37 °C) proved beneficial for forming organic acids, especially lactic acid. While limiting the rate of acidogenesis (18.9%), thermophilic temperature (55 °C) could cause a buildup of carbohydrates in the fermented mixture. Macromolecular and particle organic components can be used as slowly biodegradable carbon sources, while organic

acids in the food waste fermented slurry operate as easily biodegradable in the denitrification processes.

2.4.2.7.Bacterial Groups

(Shi et al., 2020a) reported that the *Thauera* (7.1%), *Paludibacter* (11.2%), *Propioniciclava* (11.9%), and *Dechloromonas* (15.2%) were the predominant groups in the seeding sludge. These findings were in agreement with earlier research that examined the metagenomic of the identical sludge sample (Shi et al., 2020b); In that attempt, it was discovered that *Dechloromonas* and *Thauera* not only produced NO_2^--N, but also contained complete and plentiful gene sequences for SBOM glycolysis and acetate fermentation. *Paludibacter* and *Propioniciclava* may also contribute to SBOM metabolism as typical fermentative genera (C. Chen et al., 2019) in this instance, *Dechloromonas* and *Thauera* are also present.

The other genera for denitrification and SBOM metabolism are shown in Table 4.

TABLE 4, DOMINANT GENERA ENRICHED IN THE CULTURED SLUDGE

Genera	Roles	References
Dechloromonas	denitrification; SBOM metabolism	(Shi et al., 2020b)
Thauera	denitrification; SBOM metabolism	(Shi et al., 2020b)
norank_f_norank_o_SJA-15	SBOM metabolism	(Y. Lu et al., 2013)
norank_f_Calditrichaceae	Denitrification	(L. Wang et al., 2019)
Blvii28_wastewater-sludge_group	SBOM metabolism	(Su et al., 2014)
norank_f_Anaerolineaceae	SBOM metabolism	(Narihiro et al., 2012)
norank_f_Spirochaetaceae	SBOM metabolism	(L. Wang et al., 2019)
norank_f_Bacteroidetes_vadinHA17	Denitrification	(Feng et al., 2016)

2.5. Systems for denitrification

Different systems have been reported for denitrification plants which can be divided into two major groups: (i) fixed film process like anaerobic (anoxic) filters, rotating anaerobic disc filters, expanded bed (upflow) granular media, and anaerobic fluidized bed system, (ii) suspended growth processes like separate sludge system and combined sludge system with anoxic zones (Burghate & Ingole, 2014).

Because the amount of produced sludge in biofilm denitrification processes is minimal, biofilm technologies like moving bed biofilm reactors and fluidized sand biofilters are often used in RAS denitrification processes. Sludge processes, which are often applied to remove nitrogen in RAS, have strong flocculation and denitrification performance. Single-sludge, fed batch reactors, up-flow sludge bed reactors, up-flow anaerobic sludge bed reactors, etc. are some of the commonly used sludge processes (H. Li et al., 2023).

Wood chip bioreactors have gained increased attention in recent years, considering their capacity to eliminate nitrate in RAS. The majority of them consist of a groove filled with carbonaceous material, such as wood chips, from which nitrate was removed from effluent water by denitrification. A woody medium contains more diversified communities of microbes than a non-woody one, which improves the conditions required for denitrification and creates a strong microbial ecosystem, making it more suitable for nitrate removal (Lepine et al., 2018).

(J. Lu et al., 2020) developed a new reactor by combining MBBR and the simultaneous partial nitrification, anammox, and denitrification biofilm. Anammox bacteria (*Planctomycetales*), ammonia oxidizing bacteria (*Nitrosomonadaceae*), nitrite oxidizing bacteria (*Nitrospira* and *Devosia*), and denitrifying bacteria (*Pseudoxanthomonas*, *Acinetobacter* and *Pseudomonas*) cohabit in this combined biofilm reactor to produce N_2 from ammonia, nitrite, and nitrate.

Electrochemical methods are also reported to be effective at removing nitrogen pollution from RAS. The benefits of electrochemical technology are quick start-up, simple operation, less sludge formation, and high treatment efficiency. Electrochemical technology enable the transformation and decomposition and of pollutants by a sequence of physico-chemical reactions under the effect of an applied electric field (Mook et al., 2012). They have special advantages for marine RAS operations due to the high conductivity of seawater, which may decrease consumption of operating energy (Yang et al., 2019). Additionally, a variety of hazardous microbial organisms in wastewater can be turned inactive by generated oxidants through electrochemical reactions (free chlorine, hydrogen peroxide, and hydroxyl radicals) (Ding et al., 2017).

2.6. Objective:

In this project, using fish sludge as substrate for nitrate removal of RAS effluent have been tested. The tests are being done in a sequencing batch reactor (SBR) to check the kinetics (reaction rate) of denitrification using fish sludge as carbon source. The nitrate concentration change in the reactor will be measured using electrochemical electrode directly to compare with typical municipal wastewater rates; and a combination of chemical and biological characterization analysis will be followed on the sludge.

The analysis will illustrate what the sludge contains in terms of biodegradable CODs and macro molecules in order to treat RAS effluent and role in denitrification reactions. It is required to have an overview of the disintegration and hydrolysis mechanisms of particulate organic substrates considering shear forces, temperature, hydration, enzymes, stoichiometry, kinetics, etc. Overall, the direct use of fish waste sludge as a carbon source for denitrification of RAS process water effluents and use of pre-fermented fish sludge for the same denitrification process have been investigated. The result will determine if internally generated fish waste sludge can be used as C-source for denitrification of RAS effluents and if fermentation of raw sludge can increase bioavailability of carbon.

The main objective of the project can be summarized in three steps: (i) estimating nitrate uptake rates (NUR) on specified substrates by ion selective electrode (ISE), (ii) characterizing the fish sludge, (iii) estimating fermentation rates and characterising fermentation products.

3. Materials and Methods

3.1. Theory of ISE, NO_3-electrodes

Electrochemical sensing techniques such as voltametric, impedimetric, and potentiometric methods have drawn significant interest in the last two decades because of their superior selectively, sensitively, accuracy, and simple configuration (Alahi et al., 2018). The most widely applied electrochemical technique, potentiometry, basically measures a cell's generating potential under near equilibrium conditions (Frant & Ross Jr, 1970).

In a variety of applications, especially environmental monitoring, clinical chemistry, and industries, ion selective electrodes (ISEs) with solvent-polymeric membranes are frequently utilized as ion activity sensors. The primary focus of the theory of the ISE mechanism is on ion-exchange activities at the aqueous solution and membrane interface (Ivanova & Mikhelson, 2018; Morf, 1981). The ISE potential for totally dissociated membranes can be described using advanced ISE models that account for both the low and high limits of their responses (Jasielec et al., 2010, 2015).

Nitrate (NO_3) content in groundwater and wastewater must be accurately and continuously measured over a period of time in order to provide actual information about treatment effectiveness and pollutant change over time. Several nitrate monitoring systems, such as chromatographic, biometric, colorimetric, and electrochemical sensors, have been established (Fan et al., 2020). Ion selective electrodes have become critical elements in laboratory analyzers because of their capacity to offer precise, accurate, and affordable measurements of these essential analytes on relatively small volumes in a range of sample varieties in a very short period of time (Dimeski et al., 2010).

3.1.1. Instrumentation and chemicals

Setting up the instrumentation had been done carefully following steps in the user manual provided by the manufacturer. The catalog information about the parameter meter (figure 2) and Nitrate ion selective electrode is provided in table 5.

TABLE 5, INSTRUMENTS CATALOG INFORMATION

	Model
ISE meter	Orion™ Versa Star Pro™ pH/ISE/Conductivity/Dissolved Oxygen Multiparameter Benchtop Meter, Thermo Scientific™
NO_3 ISE	Orion™ Nitrate ionplus™ Sure-Flow™ Plastic Membrane Combination ISE, Cat. No. 9707BNWP

Nitrate ionic strength adjuster (ISA) solution is used in all samples in order to eliminate measurement errors caused by of some ions which are electrode interferences (Cl^-, PO_4^{3+}, Ac^-, I^-, NO_2^-, …). Adding ISA leads to a stable background ionic strength for samples and standards. After considering possible ions concentration in the filling media of denitrifying reactor and calculation of the ionic strength (equation 11), it was assessed to add 0.75 ml of ISA solution per 30 ml of samples before ISE measurement.

$$ionic\ strenght = {}^{1}/_{2} \sum(C_i Z_i^2) \qquad \text{(Eq. 11)}$$

Where C_i and Z_i are the concentration and charge of ions respectively. The ISA solution for NO_3^- electrode was prepared using Ammonium Sulfate and the concentration was calculated 2 mol/l solution of $(NH_4)_2SO_4$.

Aiming to enhance electrode life and function it is required to change the electrode outer chamber filling solution regularly. Orion™ Optimum Result™ F Cat. No. 900046 fresh filling solution was being used for daily electrode maintenance.

Nitrate standard and sample solutions were prepared utilizing EMPLURA™ Sodium nitrate crystals Cat. No. 1.06535.1000 and diluted with tap and sea water with known portion to adjust the ionic strength.

FIGURE 2, NO3-ISE MEASUREMENT DEVICE

3.1.2. NO_3-electrode calibration

Simple definition of calibration is adjusting the cell constant. The calibration of Nitrate ISE was done precisely according to manufacturer's user guide based on direct calibration technique. Four standard solutions were prepared with different concentrations considering the actual Nitrate concentration in the reactor. Dilution of the standard solutions was done in accord with the standard serial dilution method ($C_1V_1=C_2V_2$).

The four standard solution points were perfectly in the linear region of the calibration curve and the lowest concentration was chosen to be out of non-linear region. The standard solution having the lowest concentration was measured first and calibration procedure was continued increasingly to the highest concentrated standard solution at the end. The slope of the linear part of calibration curve was -55.6 mV which is within the nominal range (-54 to -60 mV) at ambient temperature.

The ionic strength of all standard solutions was adjusted using investigated amount of sea water and ISA solution (table 6).

TABLE 6, CALIBRATION STANDARD SOLUTION

Nitrate stock solution concentration	Dilution media	ISA amount in 30 ml standard solution	Standard solution concentration (mg/l NO_3-N)
7.2 g/l NO_3-N	6% seawater + 94% tap water	1.5 ml	7.2
			36
			120
			360

3.1.3. ISE validation using photometric method

The validation of NO_3-ISE method was done against spectroquant using the appropriate NO_3-N kit. Six actual samples from the reactor in addition to three random prepared samples were tested with both ISE and spectroquant methods (table 7). The samples were diluted with the ratio of 3.75:96.25 using deionized (D.I.) water so that they were in the range of used NO_3-N kits (0.5 – 18 mg/l NO_3-N). Spectroquant™ Nitrate Cell Test kits from Merck were utilized in validation experiments.

TABLE 7, COMPARISON OF ISE AND SPECTROQUANT METHODS

Samples	ISE (mg/l NO_3-N)	Spectroquant (mg/l NO_3-N)	Difference (%)
1st reactor	396	373.3	5.90
2nd reactor	384	418.7	8.65
3rd reactor	362	405.3	11.39
4th reactor	254	282.7	10.70
5th reactor	208	237.3	13.16
6th reactor	157.6	189.3	18.328
1st random	81.4	74.7	8.58
2nd random	136	145.3	6.61
3rd random	266	264	0.75
	Average		**9.34%**

The percentage difference between two methods is calculated by dividing the absolute value of the difference between two concentrations by the average of those two values of each sample multiplying by 100. The difference is due to both random and systematic errors such as potential errors in calibration or instrumentation errors due to membrane aging. Moreover, as the range of NO_3-N kits was lower than the concentration of NO_3-N in the samples (3.75/100), dilution errors had an impact as well. Overall, the average percentage difference is 9.34%.

The data of both methods are sorted in order from the smallest concentration to the largest and plotted to show the correlation between the two methods. Figure 2 shows the measurements of ISE tests (reference) on X axis and spectroquant tests on Y axis.

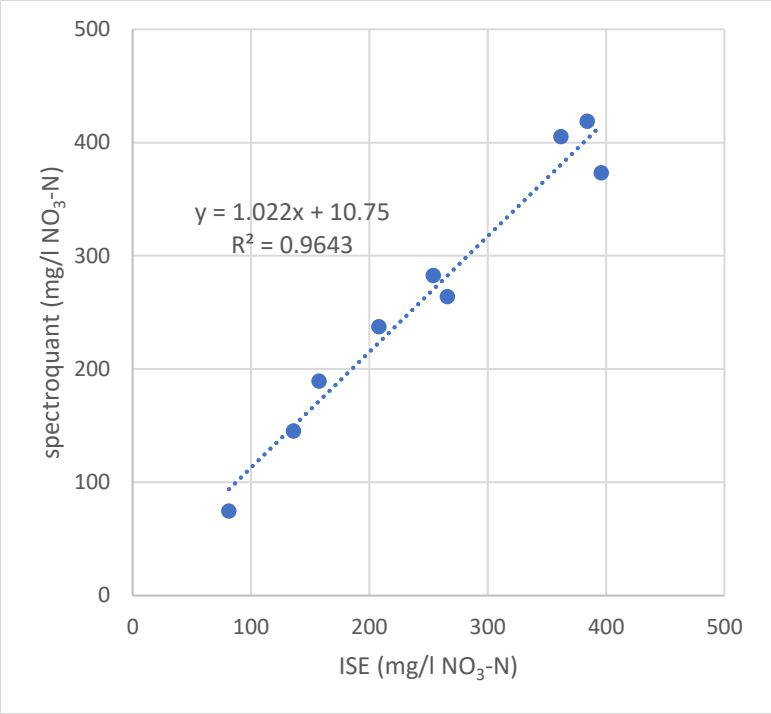

FIGURE 3, CORRELATION OF ISE AND SPECTROQUANT METHODS

The R-squared value of the trendline is 0.9643 which shows an acceptable correlation between those two methods; also, the slope of the trendline is almost close to one which shows the X and Y values are similar enough.

Figure 3 illustrates the comparison between tested methods having the concentrations on vertical axis while horizontal axis indicates the order of data from lowest concentration to highest. The figure provides an illustration of how the measurements using ISE and spectroquant methods react to different solutions with different concentrations comparing to each other. The data points of table 3 are being analyzed in this step as well.

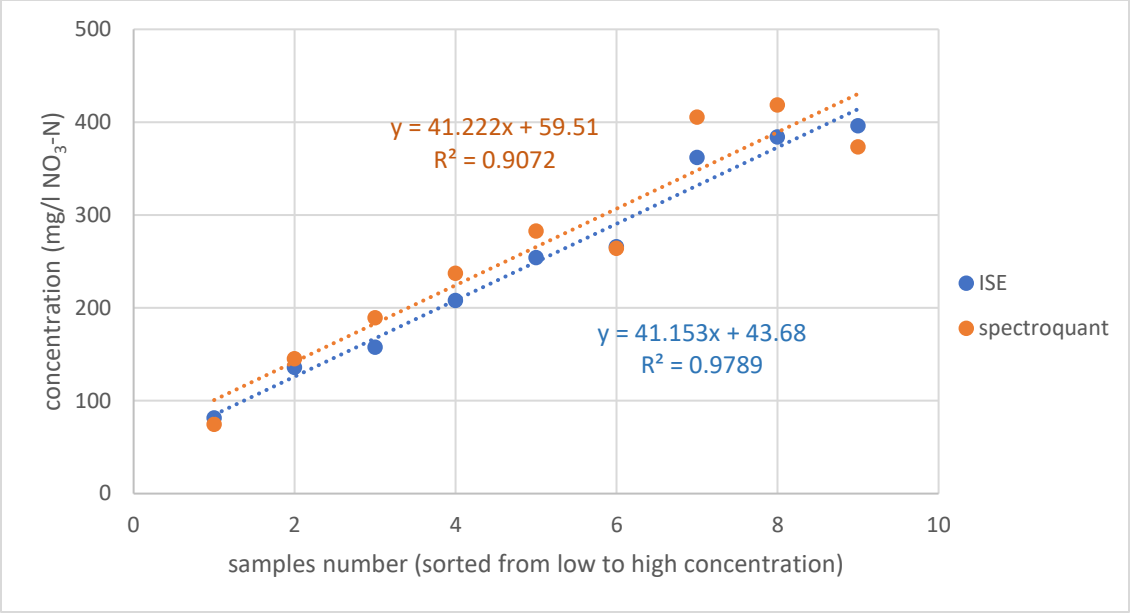

FIGURE 4, COMPARISON BETWEEN ISE AND SPECTROQUANT METHODS

The trendline of the measured concentrations related to each method is plotted and the comparison between the slopes shows that the rise in the measurements follows almost the same trend in both ISE and spectroquant methods.

Based on the data obtained from spectroquant method it can be concluded that the measurements by ISE method are validated and further experiments will be suitable for NUR tests using current instrumentation and chemicals.

3.1.4. Methodological variances

Methodological variances of ISE are being determined in this section by adding a known amount of prepared Nitrate spike solution to the samples. The concentration of spike solution was 950 mg/l NO_3-N, and the dilution media used for spike contained 6% of seawater and 94% of tap water to have the same ionic strength as the samples. Also 1.5 ml of ISA solution was added per 30 ml of spike solution. In these experiments, 1 ml of spike solution was added to 30 ml of sample which theoretically should add 29.23 mg/l of NO_3-N to the samples' concentration.

At the first experiment set, six samples (S1 to S6) were taken from the reactor at the same time and NO_3-N concentration was measured in them using ISE. Then the spike solution was added to each sample and a second measurement was done. Table 8 represents the concentration data of the first experiment set. The concentration difference between samples and samples plus spike solution is calculated as well as average, variance, standard deviation (s.d.), error, and relative error (C.V.).

TABLE 8, VARIANCE OF ISE METHOD BY ADDING SPIKE SOLUTION TO SAMPLE

Samples	NO_3-N (mg/l)	NO_3-N_{sample} + NO_3-N_{spike} (mg/l)	Difference
S1	394	448	54
S2	392	448	56
S3	390	440	50
S4	392	444	52
S5	396	446	50
S6	398	450	52
Average	393.7	446	52.33
Variance	8.67	12.8	5.47
s.d.	2.94	3.58	2.34
error	1.20	1.46	0.96
C.V.	0.30%	0.33%	1.83%
Measurement	**394 ± 1**	**446 ± 2**	**52 ± 1**

The values for average, variance, and standard deviation are calculated based on the standard statistics equations; error is derived from standard deviation divided to square root of measurement repetition number (six) and relative error is the division of error to average value.

The impact of spike solution on sample concentration is presented to be 52 ± 1 mg/l NO_3-N which does not hold the theorical value (29.23 mg/l of NO_3-N) in its range; it is due to experimental errors like dilution and weighting during spike solutions preparing or additional amount of ISA. For these tests, the big difference between theoretical value and measured value of spike solution impact is probably due to wrong reported concentration of spike solution. However, ISE measurement can be considered as a valid method for our case as the aim of the study is to analysis the NUR and the difference of nitrate concentration illustrated with ISE has high accuracy.

By the way, based on statistical rules, this the presented concentration difference is just 68.2% confident. If the confidence of the measurement rises to 90%, the error is multiplied to 2.01 and the newly presented measured difference is 52 ± 2 mg/l NO_3-N.

In the second experiment set, six different samples were drawn from the reactor in different time with different concentrations and NO_3-N level was tested in each sample with ISE method. After that 1 ml of the spike solution was added to 30 ml of samples and NO_3-N test was repeated by ISE. Table 9 shows the data comparing the difference between Nitrate concentration before and after adding spike solution.

Average, variance, standard deviation, error, and relative error are calculated for this experiment set as well and the difference of concentrations related to spike solution is represented 56 ± 3 mg/l NO_3-N.

TABLE 9, NITRATE CONCENTRATION BEFORE AND AFTER ADDING SPIKE SOLUTION

Samples	NO_3-N (mg/l)	NO_3-N_{sample} + NO_3-N_{spike} (mg/l)	Difference (mg/l NO_3-N)
R1	394	448	54
R2	384	442	58
R3	362	406	44
R4	254	313	59
R5	208	268	60
R6	158	218	60
Average			55.83
Variance			38.57
s.d.			6.21
error			2.54
C.V.			4.55%
The difference of measurements			**56 ± 3**

3.1.5. Interfere of NO_2-N and ISA

In order to check the interference of nitrite concentration on ISE measurement of nitrate concentration the following experiment had been done; seven identical nitrate solution samples were prepared with the same nominal concentration of 500 mg/l NO_3-N but different nitrite concentrations of 0, 3.3, 16.7, 33.3, 66.7, 133.3, and 333.3 mg/l NO_2-N. The samples were made using $NaNO_3$, KNO_2 and a dilution media consist of 6% seawater plus 94% tap water. Also 1.5 ml of ISA solution was added to each 30 ml of samples in advance to ISE measurement. Table 10 shows the result of nitrite interference on nitrate ISE measurement.

TABLE 10, INTERFERENCE OF NITRITE ON NITRATE ISE MEASUREMENT

Sample number	Nominal concentration of nitrate and nitrite	Nitrate concentration using ISE (mg/l NO_3-N)
1	500 mg/l NO_3-N + 0 mg/l NO_2-N	537
2	500 mg/l NO_3-N + 3.3 mg/l NO_2-N	537
3	500 mg/l NO_3-N + 16.7 mg/l NO_2-N	534
4	500 mg/l NO_3-N + 33.3 mg/l NO_2-N	537
5	500 mg/l NO_3-N + 66.7 mg/l NO_2-N	540
6	500 mg/l NO_3-N + 133.3 mg/l NO_2-N	558
7	500 mg/l NO_3-N + 333.3 mg/l NO_2-N	603

The result shows that nitrite concentration up to 66 mg/l does not affect the ISE measurement for NUR test. However, to prevent the potential higher nitrite concentrations effect on the measurements, it was decided to dilute samples 1:2 using tap water which is discussed in the next sections. Accordingly, the volume of added ISA solution to each sample was modified to 0.75 ml

following equation 11 and testing three different added volumes of ISA solution to three identical diluted (1:2) nitrate solutions with nominal concentration of 100 mg/l NO_3-N (Table 11).

TABLE 11, REQUIRED AMOUNT OF ISA SOLUTION

Sample number	Nominal concentration (mg/l NO_3-N)	Added volume of ISA (ml)	ISE measured concentration (mg/l NO_3-N)
1	100	0.75	107
2	100	1	108
3	100	1.25	112

3.2. Reactor operation

A SBR operated 2000 ml laboratory scale anoxic bioreactor (figure 5) will be used as source reactor for specific (substrate specific) denitrification batch tests and operated on a 12-hour cycle inside an incubator at 12 ℃. The cycle consists of a (i) filling stage, (ii) reaction interval, (iii) sludge wasting, (iv) settling phase and (v) decanting stage. Media containing 50 ml of substrate (fresh/fermented fish sludge or 40 mg/l acetate solution), 120 ml seawater, 10 ml buffer solution and 100 ml of a 43.8 g/l $NaNO_3$ solution diluted to 1200 ml with tap water (980 ml) was pumped in during the filling stage and left to denitrify for 11.5 hours. At the end of the reaction phase, 100 ml of mixed liquor suspended solids (MLSS) was drawn from the reactor by a 100 ml plastic syringe. Following sludge wasting, the reactor mixer was turned off and the MLSS left to settle for 30 min. Thereafter, 1100 ml of the clarified liquid was decanted by peristaltic pumping from the 800 ml mark (depth). This terminated the 12-hour SBR cycle and a new was initiated by filling. The inoculated biomass sludge consists of initial fish sludge which was operated in the reactor through 12-hours cycles for 3 to 4 weeks to be adapted and stabilized in steady state condition for denitrification. Table 12 shows the components added to the reactor and corresponding concentrations per cycle.

TABLE 12, FILLING MEDIA CHEMICALS AND CONCENTRATIONS

	Substrate	Nitrate	Buffer	Seawater
Volume (ml)	50	100	10	120
Concentration of solution	55 g/l NaAc	43.8 g/l $NaNO_3$	0.1 M	
Concentration in reactor (2 liter)	1000 mg/l COD	360 mg/l NO_3-N		6%

Different substrate solutions like sodium acetate (NaAc), acetic acid (HAc), glucose and yeast extract were also used separately or mixed with different ratios to adjust pH or the concentration of biomass (MLSS) in the reactor (with same COD concentration: 1 g/l); for example, glucose and yeast extract were used to increase biomass concentration and it was observed that using glucose

eventually decrease the pH of reactor as it is too fermentative but sodium acetate increased the pH of reactor media.

FIGURE 5, ANOXIC BIOREACTOR

3.3. NUR test

Each series of NUR test had three main steps; first, and endogenous cycle with no substrate to make sure all the CODs are consumed in the reactor. Second, a cycle with an external substrate (50 or 15 ml of acetate) to gain the maximum NUR, and finally the fish sludge (50 ml) cycle as the main step. Each cycle has usually been sampled for four to six days with higher frequency of sampling in the first day and a couple of samples on the final days. Nitrite, ammonium, VSS and TSS of reactor media were also measured in addition to pH and nitrate measurement for better analysis of reactor behaviour. Spectroquant™, Merck, nitrite and ammonium cell test kits with proper concentration range were utilized for the measurements.

The sampling procedure of reactor for NUR test followed Standard Methods 4500D and samples volume was chosen based on the reactor volume, duration of each test, ionic strength of samples and the probe size of instruments (pH and NO_3 meter) and is performed as steps bellow. First, 15 ml of mixed liquid was drawn from the reactor by syringe and transferred to a graduated vial for centrifuging at 4000 G for 2 minutes to separate the solid phase for different mixed solid fractions. Second, 10 ml of supernatant was drawn into a small beaker using right pipette for pH measurements. Next, the 10 ml of supernatant was dilute 1:2 (30 ml diluted solution) using tap water and 0.75 ml of ISA was added to the sample for NO_3-ISE measurement. Each sampling was done duplicated to compute systematic error.

3.4. Fermentation setup

For the fermentation test, three 500 ml baffled conical flask were filled with the same fresh fish sludge and were sparged with N_2 gas for 10 minutes. The opening of the flasks was sealed with a sponge cap with a tube passing the cap centre for daily sampling. The tubes were kept close by clamp to make sure that there is no oxygen leakage into the flasks. All the fermenting reactors were placed on a shaker inside an incubator at 12 ℃. Two other flasks with the same specification were set for fermentation as well at ambient temperature (20 ℃) in order to result comparison. The test was running for seven days, and the final fermented sludge was stored at 3 ℃ to be used as substrate for NUR tests, both settled and supernatant portions of sludge were tested separately in NUR experiment.

The sampling was done every day in duplicate from each reactor in a way that 15 ml of media was drawn by a syringe and transferred to graduated vial; 2 ml of the sample was homogenised and used for tCOD measurement. Then, the samples were centrifuged at 4000 G for 2 minutes and the solid pellets were used for TSS and VSS measurement; 2 ml of the clear phase was filtered and diluted 1:1 with D. I. water for sCOD measurement. Next, 5 ml of clear phase was diluted 1:5 using D.I. water and the 30 ml solution was titrated to obtain pH and VFA amount. 40 mM hydrochloric acid (HCl) was being utilized for the pH drop in titration. Finally, 1 ml of the filtered sample was filtered again for ion chromatography (I. C.) analysis to obtain different fractions of acids (acetic, propionic, and butyric acids).

3.5. TS, TSS, VS, VSS, pH, COD, BOD, VFA

The characterization procedure of sludge and reactor media was done according to Standard Methods for the Examination of water and wastewater by the American Water and Wastewater Association (AWWA). To determine total solid (TS, solid fraction which remains after evaporation) and total volatile solid (VS, organic fraction of total solid) the homogenized sample with known exact volume was transferred to the evaporation porcelain dish and placed in an oven to evaporate over a night at 100 ℃; the dried solid residue was weighted using analytical balance to calculate TS. Right after, the dish was placed in muffle oven for combustion at 550 ℃ for a couple of hours; the weight of burnt residue was used for VS calculation.

Total suspended solids (TSS) and volatile suspended solids (VSS) were determined after the homogenised wasted sludge/fish sludge sample was weighed (known volume) and centrifuged at 4000 G for 2 minutes in order to pellet washing; the solid washing was done three times for each sample using 15 ml tap water added into centrifuge vial and mixed thoroughly before centrifugation; the washing liquid was decanted without losing the solids in each step. The solid pellet was then transferred quantitatively to a porcelain dish and evaporated, combusted, and weighted using analytical balance (SM 2540D and 2540E). The experiments to identify the different solid fraction was done in duplicate and sometimes triplicated as well as COD measurement tests.

Total and dissolved COD values were gained using Spectroquant™ COD Cell Test kits from Merck according to Merck manual. The homogenised samples were transferred to the specified COD vials using pipette and were digested in thermo-reactor for 2 hours at 148 ℃. For sCOD, the clear phase after centrifugation was diluted (usually 1:1 with D. I. water), filtered using 0.45 µm Whatman® syringe filters and then pipetted to the cell (SM 5220D).

Total and dissolved BOD tests were done following Manometric method using OxiTop BOD bottle (510 ml flask), OxiTop head and NaOH pellets in the alkaline CO_2 trap. Samples were diluted 1:39 for tBOD and 1:11 for sBOD with tap water in order to decrease the COD to the range of 500 mg/l. Then, 200 ml of diluted sample was transferred to the BOD bottle and a couple of D. I. water drops were added to the two NaOH pellets and the bottle was fastened with cap; all the BOD were running for 12 days, and each sample was tested in triplicate.

VFA analysis was done according to 4-point (6.7, 5.9, 5.2 and 4.3) titration and conductivity measurements on centrifuged diluted (1:5) samples (Moosbrugger, 1993). Different acid fractions were obtained using I. C.; the samples were filtered again through 0.2 µm GHP Acrodisc® syringe filters and diluted 1:99 for I. C. analysis. Table 13 mentions the instrumentation used for the tests.

TABLE 13, INSTRUMENTS LIST

Test	Instrument
pH	Thermo Scientific™ Orion VersaStar Pro™ pH Meter
Titration	TitroLine® 5000
Spectroquant	Spectroquant® Prove 300, Merck
BOD	WTW, OxiTop® OC 110
Conductivity	VWR® phenomenal® CO 3100L, Conductivity/TDS/°C Meter
Ion Chromatography	Dionex™ ICS 5000

4. Results

4.1. Sludge characterization

The summary of the three latest fish sludge batches which were used in different NUR tests is shown in table 14. TSS and VSS have various amounts changing between approximately 6 to 15 g/l. Total COD of the three sludge batches (S1, S2 and S3) are 18.81, 7.13 and 31.09 g/l respectively, however, sCOD has the value of approximately 3 g/l for all those three.

TABLE 14, SLUDGE CHARACTERIZATION (WET ANALYSIS)

Sludge number	S1 (8/February)	S2 (30/March)	S3 (5/May)
	Average (g/l)		
TS	11.55 ± 0.04	14.0 ± 0.3	
VS	8.87 ± 0.06	10.1 ± 0.3	
TSS	6 ± 1	13 ± 1	15.5 ± 0.1
VSS	5 ± 1	11 ± 1	13.1 ± 0.3
tCOD	19 ± 4	7.1 ± 0.8	31 ± 1
sCOD	3.7 ± 0.3	2.63 ± 0.09	3.9 ± 0.3
$tBOD_{12}$	13.7 ± 0.5	12.2 ± 0.7	
$sBOD_{12}$		1.21 ± 0.01	

The difference between characteristics of sludge number one, two and three is because the first sludge was frozen for a while and the analysis was done after defrosting, but the analysis was done on fresh sludge number two and three. Also, as different sludge batches came from different time periods, they have different properties due to change of fish density, change of feeding ingredients and change of fish metabolism in the pools.

Table 15 illustrates the computed ratio of VSS/TSS, sCOD/tCOD, sBOD/tBOD and tCOD/VSS. The VSS/TSS ratio has almost the same amount for all the sludge series (0.85-0.9), but other fractions differ in a wider range.

TABLE 15, VSS, TSS, BOD AND COD RATIOS

	S1	S2	S3
VSS/TSS	0.9	0.85	0.85
sCOD/tCOD	0.19	0.37	0.13
tCOD/VSS	3.45	0.65	2.37
sBOD/tBOD		0.1	

Different computed ratios might be due to sampling errors and analysis, especially those related to COD concentration as small samples for COD tests are very sensitive to sludge homogenization (a single tiny particle can make a big difference in the result).

The trend of BOD tests has been prepared in figures 6 showing the comparison between tBOD of first sludge, tBOD of second sludge and sBOD of second sludge respectively; each curve represents the average value of the three parallel BOD bottles. Total BODs for the first and second sludge batches follow the same rising trend, faster rate at the first two days and eventually slowing down after the fourth day. The maximum difference between the tBOD of sludge one and two is 11.6%.

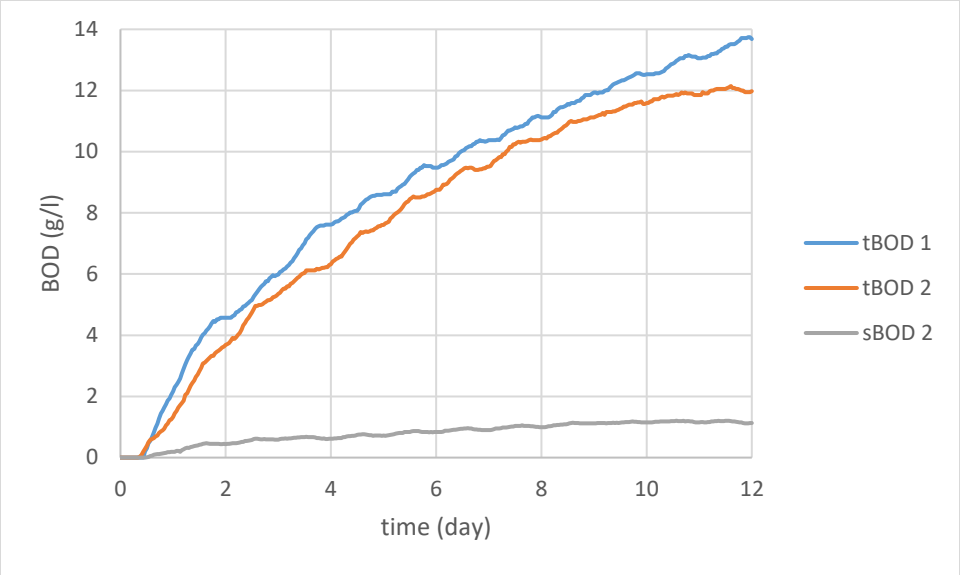

FIGURE 6, COMPARISON AND TREND OF BOD TESTS

4.2. Fermentation test

Figure 7-11 demonstrate the change of TSS, VSS, tCOD, sCOD and pH of sludge number three after seven days of fermentation in order. As mentioned, the fermentation test was done in three parallel reactors at 12 ℃ and each figure holds the data of all reactors (R1, R2 and R3) for further comparison.

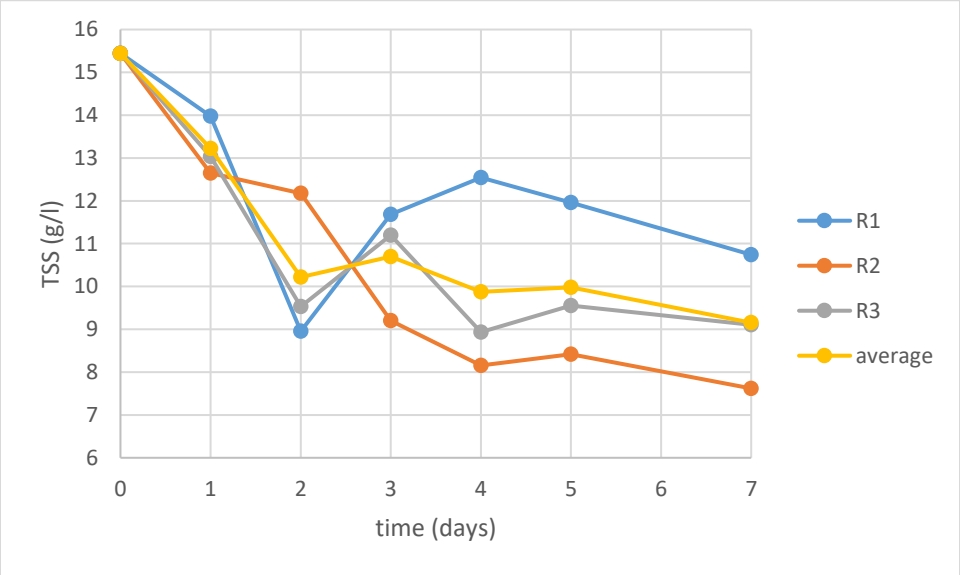

FIGURE 7, TSS CHANGE IN FERMENTATION AT 12 ℃

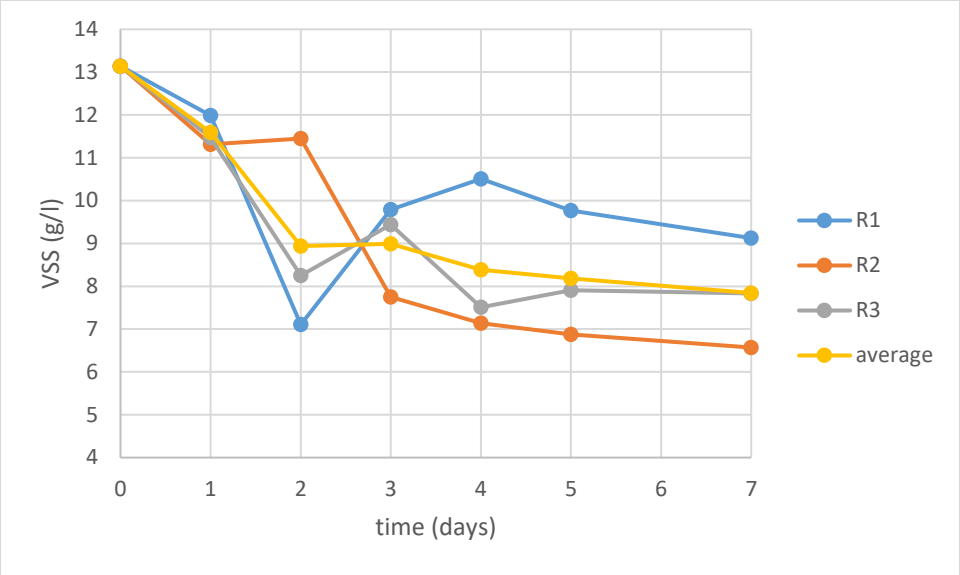

FIGURE 8, VSS CHANGE IN FERMENTATION AT 12 ℃

The TSS and VSS have similar behaviour and they have been decreased in all the reactors in average to around 9 and 8 g/l respectively. There are a couple of odd data in VSS and TSS measurement because of experimental errors mostly related to non-homogeneous samples, as the mixing of the reactors was done by shaker and there could be uneven distribution of solids in the reactors.

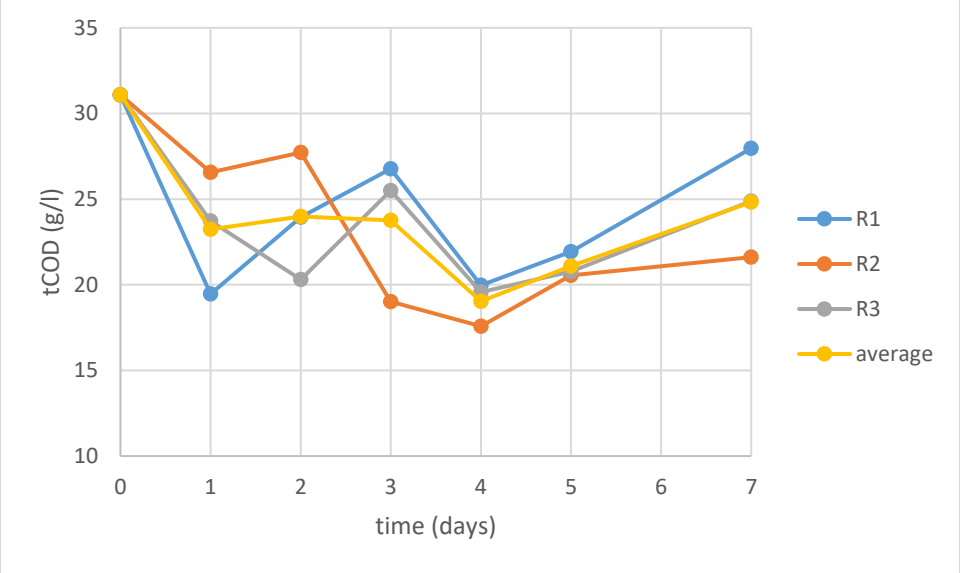

FIGURE 9, TCOD CHANGE IN FERMENTATION AT 12 ℃

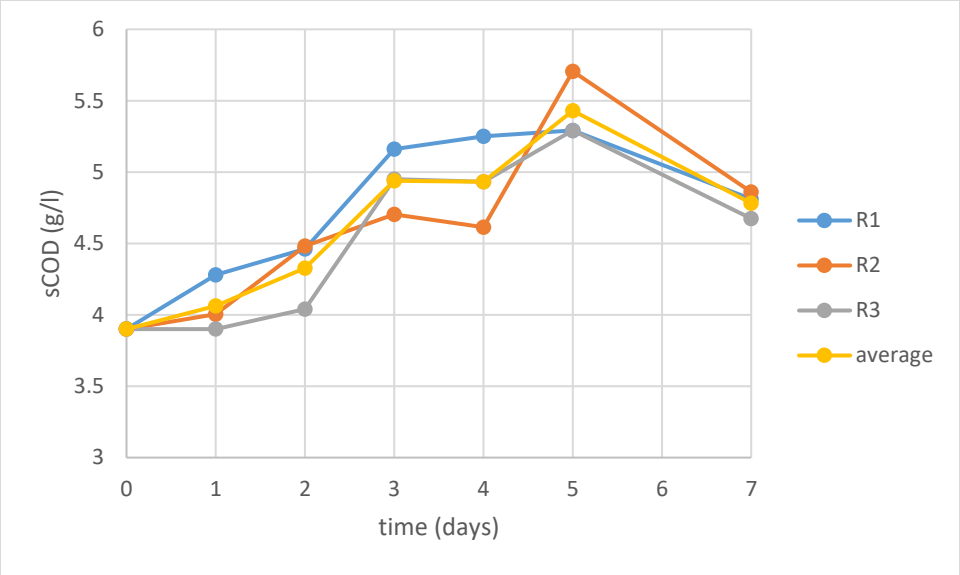

FIGURE 10, SCOD CHANGE IN FERMENTATION AT 12 °C

Total COD has a descending trend and had been lowered to 24.83 after seven days of fermentation (about 20% decreased), in contrast to sCOD which has been ascending to 4.78 from 3.9 g/l.

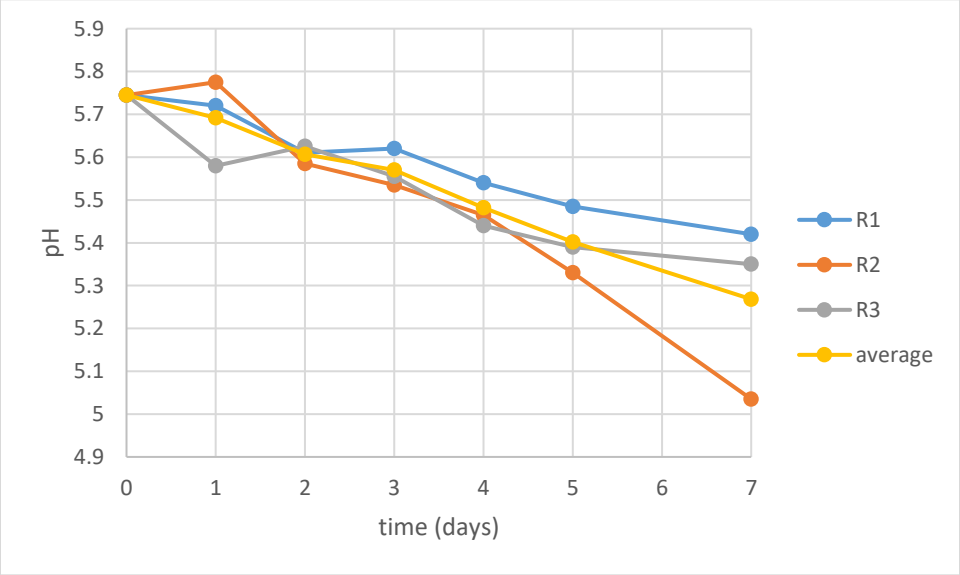

FIGURE 11, PH CHANGE IN FERMENTATION AT 12°C

pH average has been decreased almost linearly during the fermentation test as expected from 5.75 to 5.27.

Nitrate and ammonium concentrations had also been measured at the beginning and end of fermentation tests at 12 °C. The initial nitrate concentration of the fish sludge was 56 ± 2 mg/l NO_3-N which fell done to zero after the fermentation. On the other hand, ammonium concentration was very low (0.4 mg/l NH_4-N) at the start of the test but rose to 55 ± 2 mg/l NH_4-N after seven days.

As mentioned, the fermentation test had also been done at 20 ℃ in two parallel reactors on the same sludge to be compared with fermentation at 12 ℃, table 16 shows the comparison of average total and suspended solids, CODs, and pH between 12 and 20 degrees Celsius.

TABLE 16, COMPARISON BETWEEN 12 ℃ AND 20 ℃ FERMENTATION DATA

	TSS	VSS	tCOD	sCOD	pH
			Average (g/l)		
initial	15.5 ± 0.1	13.1 ± 0.3	31 ± 1	3.9 ± 0.3	5.7 ± 0.1
12 ℃	9.2 ± 0.6	7.8 ± 0.5	25 ± 2	4.78 ± 0.06	5.27 ± 0.08
20 ℃	12.6 ± 0.8	10.4 ± 0.6	29.9 ± 0.3	8.6 ± 0.2	4.70 ± 0.05

The data difference between the two temperatures is because the reactors at 12 ℃ were being sampled daily but reactors at 20 ℃ were sampled just once at the seventh day. But both of them show the decline of solid fraction values as well as tCOD and pH, in opposition to sCOD.

Figure 12 shows the production of total volatile (short chain) fatty acids in the seven-days fermentation tests for the three reactors which is followed by different acids fractions separately in figures 13, 14 and 15 respectively for acetic acid (HAc), propionic acid (HPro) and butyric acid (HBu).

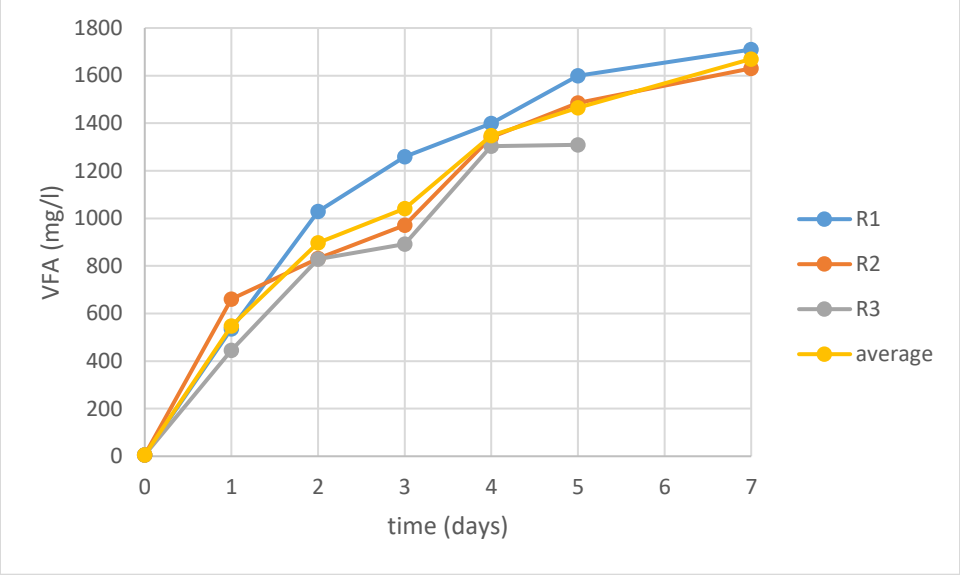

FIGURE 12, SHORT CHAIN FATTY ACIDS PRODUCTION IN FERMENTATION AT 12 ℃

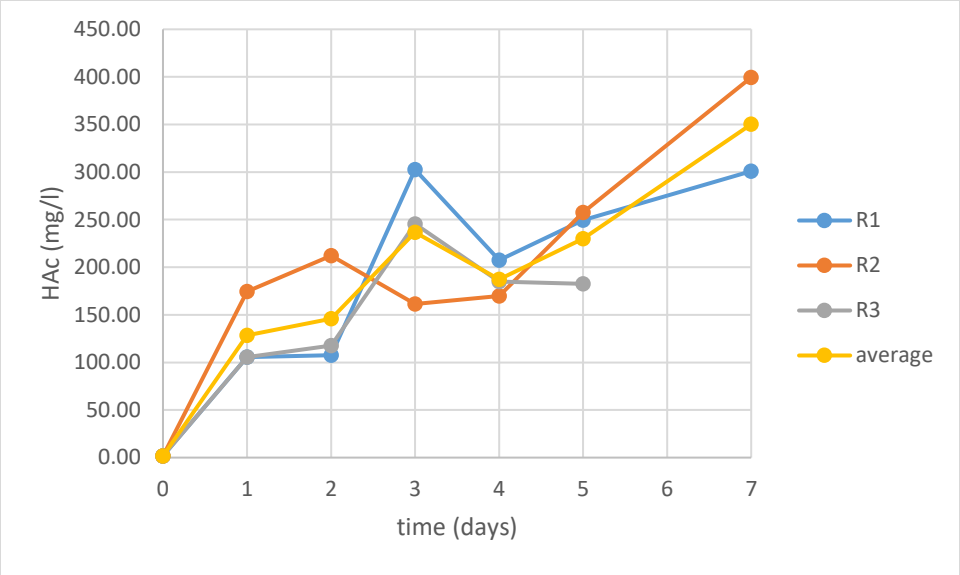

FIGURE 13, ACETIC ACID PRODUCTION IN FERMENTATION AT 12 ℃

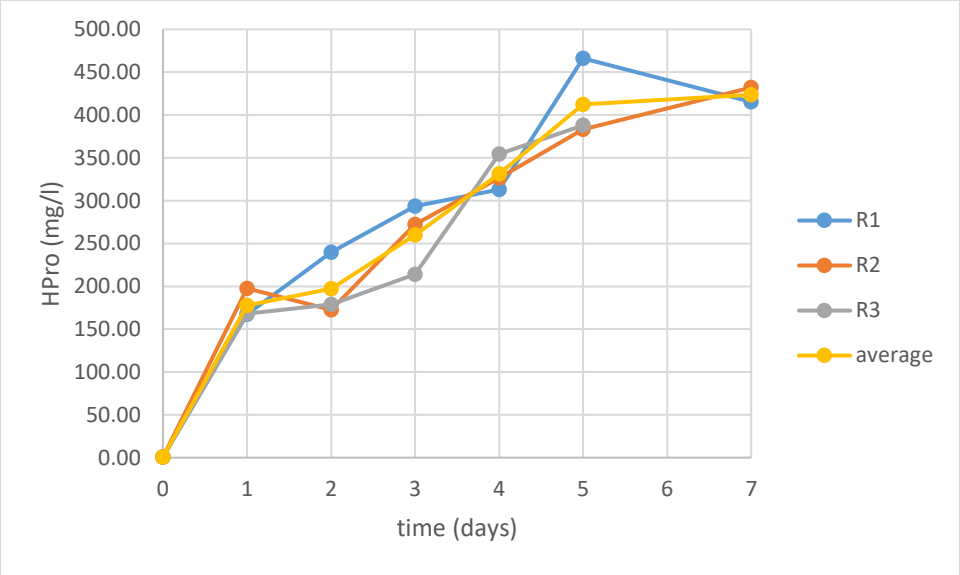

FIGURE 14, PROPIONIC ACID PRODUCTION IN FERMENTATION AT 12 ℃

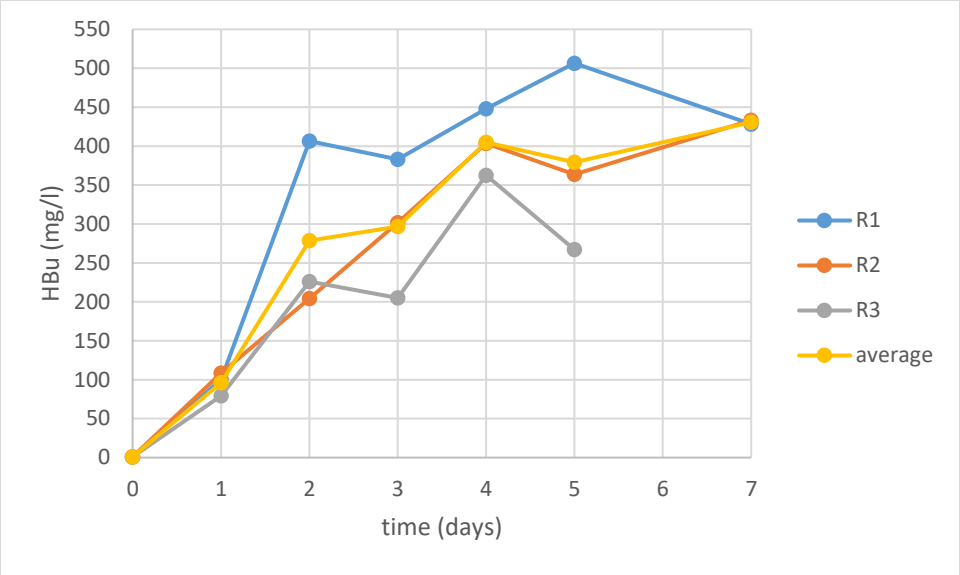

FIGURE 15, BUTYRIC ACID PRODUCTION IN FERMENTATION AT 12 ℃

Total short chain fatty acid produced after seven days of fermentation is 1670 ± 180 mg/l VFA; the ranking of largest portion is butyric acid, propionic acid, and acetic acid.

A comparison between acid production between 12 and 20 degrees Celsius is presented in table 17. As the table shows, the rate of short chain acids production at 20 ℃ is almost double the rates at 12 ℃. Several studies have shown the effect of temperature on fermentation and short chain acid production (Federico et al., 2022).

TABLE 17, COMPARISON BETWEEN 12 ℃ AND 20 ℃ FERMENTATION ACID PRODUCTION

	VFA	HAc	HPro	HBu
	Average (mg/l)			
12 ℃	1670 ± 180	350 ± 39	424 ± 71	431 ± 48
20 ℃	3322 ± 69	829 ± 45	821 ± 54	882 ± 30

Also, the fermentation rate is computed based on the VFA production in three-time intervals over seven days (days 1-2, 2-5 and 5-7) for the reactors at 12 ℃ and overall fermentation rate for both the reactors at 12 and 20 degrees Celsius, table 18 shows the fast, medium, and slow fermentation rates at 12 ℃ and the overall fermentation rates.

TABLE 18, FERMENTATION RATE BASED ON VFA PRODUCTION

	Fermentation rate (mg VFA/l. day)
Days 1-2 (fast fermentation, 12 ℃)	446 ± 33
Days 2-5 (medium fermentation, 12 ℃)	189 ± 17
Days 5-7 (slow fermentation, 12 ℃)	64 ± 7
Days 1-7 (overall, 12 ℃)	245 ± 8
Days 1-7 (overall, 20 ℃)	474 ± 10

4.3. NUR tests

In this chapter, NUR results have been reported for various substrates as well as pH and nitrite concentration change during denitrification. The substrates used in these tests are acetate (high and low concentration), fish sludge and fermented fish sludge (both supernatant and settled parts).

Figure 16 shows NUR result of different tests with acetate as substrate (50 and 15 ml) and figures 17 and 18 show the pH change and nitrite concentration behavior of those tests.

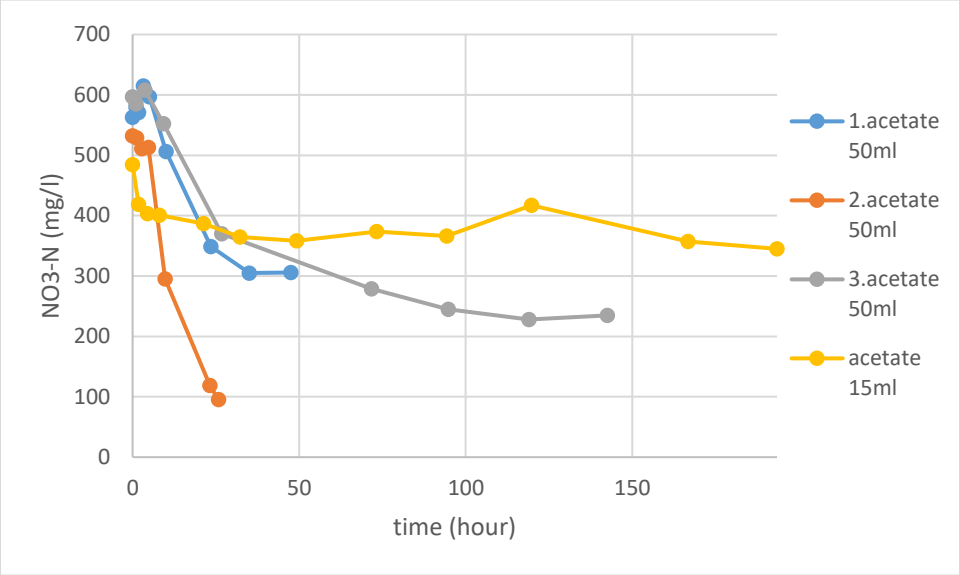

FIGURE 16, NITRATE CONCENTRATION CHANGE WITH ACETATE AS SUBSTRATE

Having VSS of reactor media, the specific denitrification rate (r_{DN}) for acetate (50 ml) is computed to be 3.64 ± 0.07 mg NO_3-N/g VSS. h (Eq. 12); first and second acetate NUR data have been used and acetate is considered as easily biodegradable COD (rbCOD).

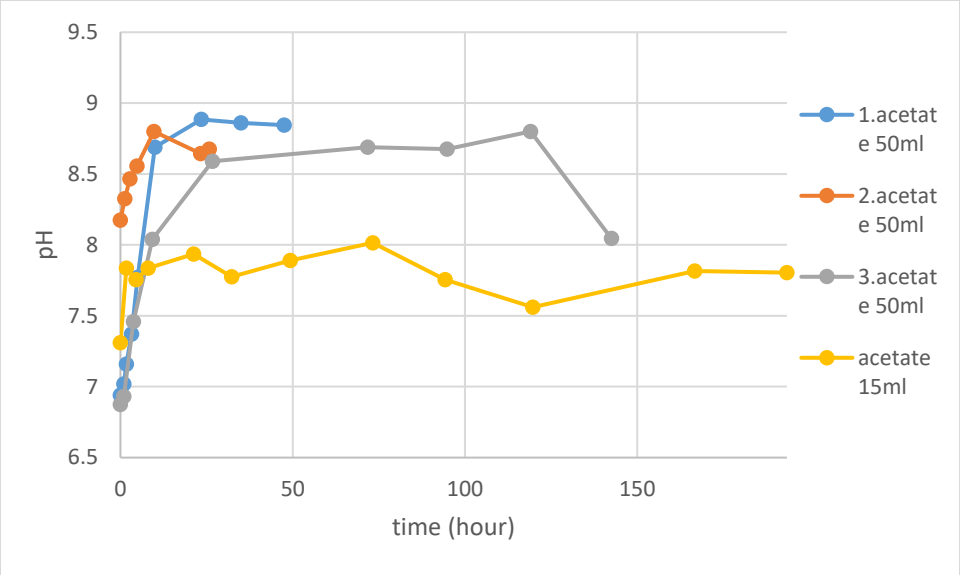

FIGURE 17, PH CHANGE WITH ACETATE AS SUBSTRATE

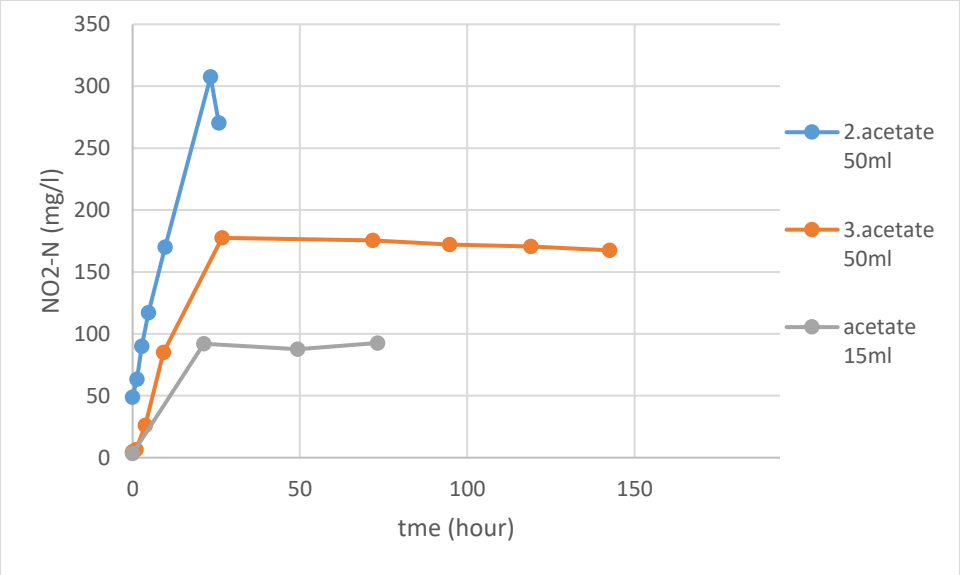

FIGURE 18, NITRITE CONCENTRATION CHANGE WITH ACETATE AS SUBSTRATE

It is observed that pH tends to increase up to 9 for high concentration of acetate and up to 8 for the lower concentration; also, there is nitrite accumulation up to 300 mg/l when acetate is used as substrate with high concentration (50 ml) and up to 100 mg/l when using acetate with low concentration (15 ml); the reason is that some denitrifier bacterial groups cannot reduce nitrite and specially at high rate of denitrification nitrite accumulates (Suri et al., 2021).

Figures 19, 20 and 21 show the nitrate, pH and nitrite change of reactor respectively when fish sludge was used as substrate.

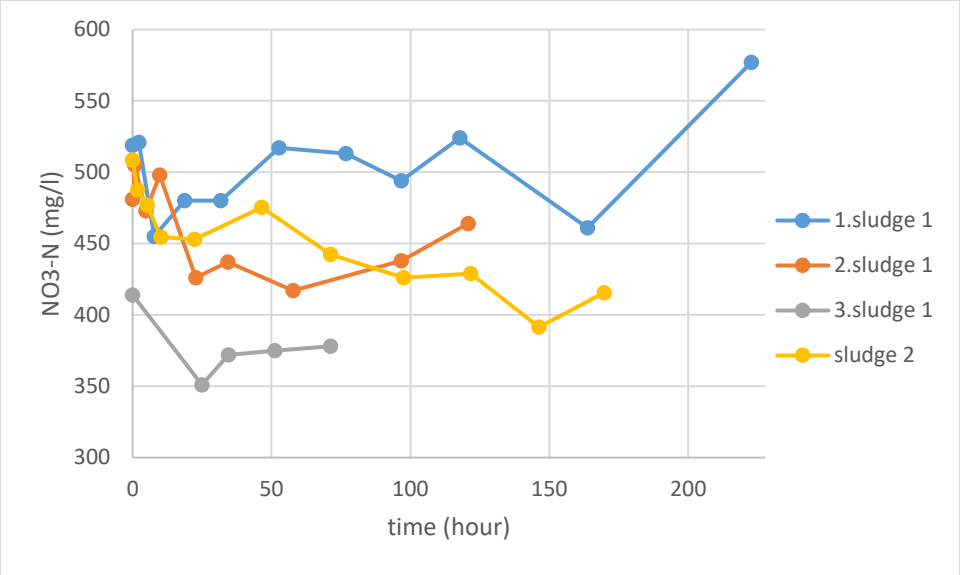

FIGURE 19, NITRATE CONCENTRATION CHANGE WITH SLUDGE AS SUBSTRATE

First and second fish sludge batches were used in these tests and nitrate concentration is highly fluctuating. As NUR test using fish sludge were done right after NUR tests with acetate, the accumulated nitrite in the reactor affects the denitrification rate and caused fluctuation of nitrate concentration over time, it can be proved looking at figure 19 which shows consumption of nitrite in those NUR tests.

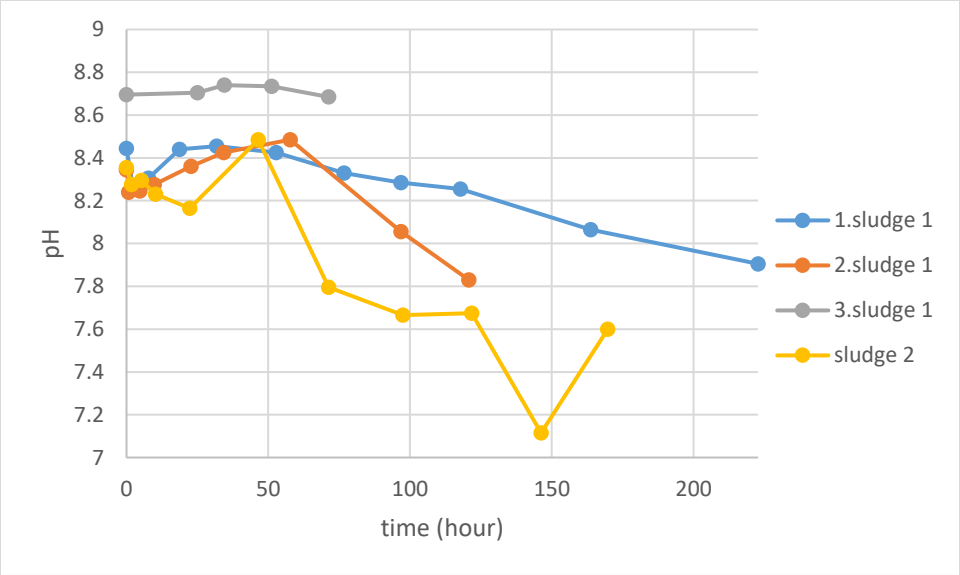

FIGURE 20, PH CHANGE WITH SLUDGE AS SUBSTRATE

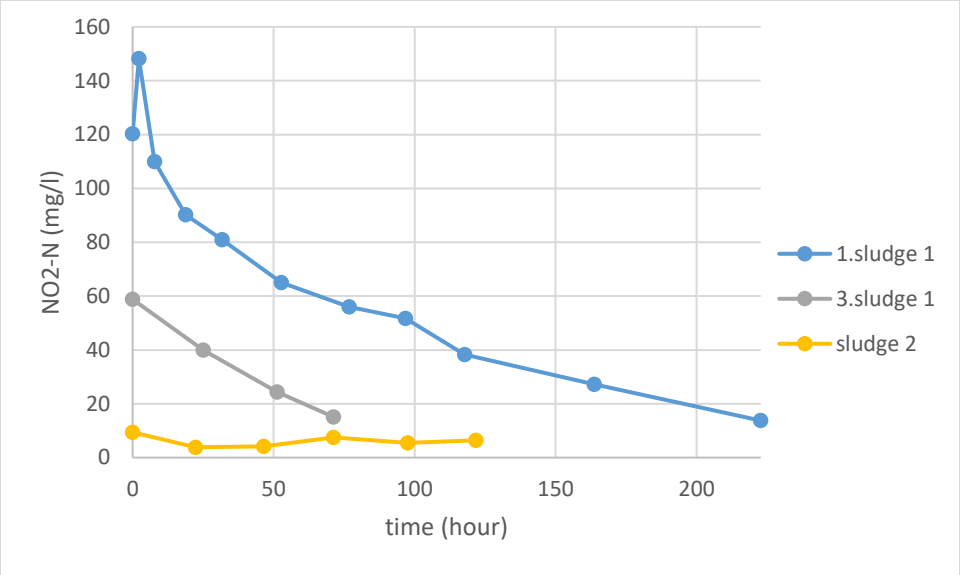

FIGURE 21, NITRITE CONCENTRATION CHANGE WITH SLUDGE AS SUBSTRATE

As NUR tests using fish sludge were always done right after NUR tests with acetate, pH and nitrite values are at high level in the beginning of tests. As the tests continue, pH tends to be regulated to approximately 7.7 and nitrite concentration also decreased to about 10 mg/l in all the tests while fish sludge was used as substrate.

Figure 22 illustrate the nitrate concentration change during NUR test using fish sludge and fermented fish sludge. The third sludge was used in this series and as mentioned, both settled (solid) and supernatant (clear) parts of fermented fish sludge been tested.

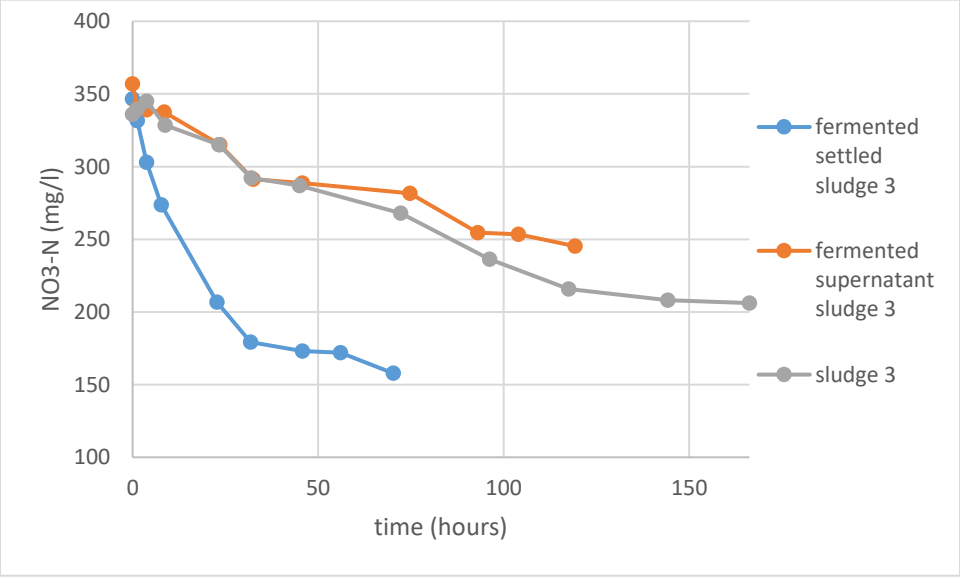

FIGURE 22, NITRATE CONCENTRATION CHANGE WITH FERMENTED SLUDGE/SLUDGE AS SUBSTRATE

An example of VSS and TSS values of reactor media which is used for specific denitrification rate calculation is shown in figure 23, the values are related to three different cycles with acetate, first fish sludge and second fish sludge as substrate.

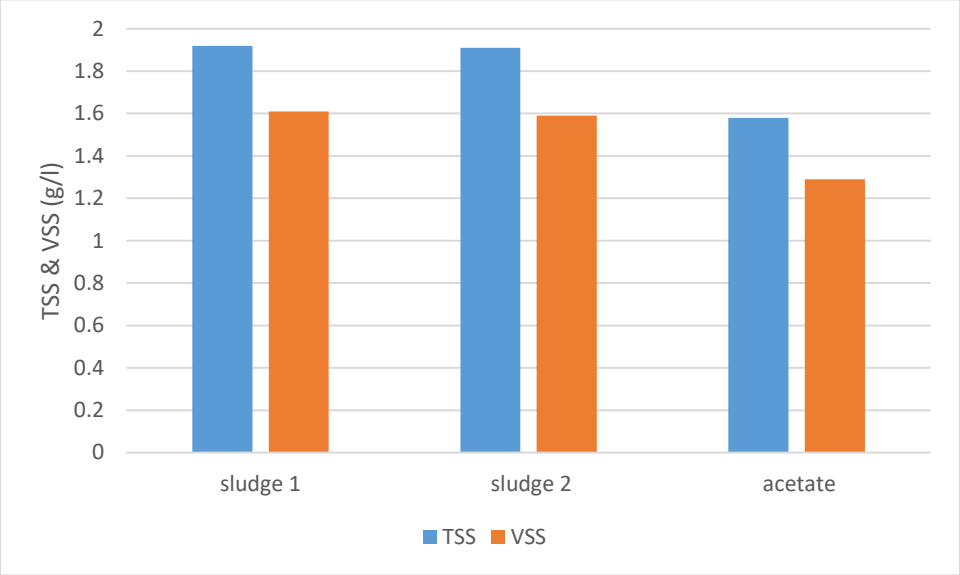

FIGURE 23,TSS AND VSS OF REACTOR MEDIA

Three different r_{DN} for NUR tests using fish sludge, fermented settled fish sludge, and fermented supernatant fish sludge can be assumed based on different COD fractions: readily biodegradable (rbCOD), slowly biodegradable ($sbCOD_S$) and slowly biodegradable particulate ($sbCOD_X$) fractions. Table 19 holds the calculated different specific denitrification rate values for sludge and fermented sludges according to VSS of reactor media and NUR for all COD fractions. The standard error for r_{DN} is comparatively high due to the uncertainty of VSS measurement and its impact on r_{DN} calculation.

TABLE 19, SPECIFIC DENITRIFICATION RATE FOR FERMENTED SLUDGE/SLUDGE

substrate	COD fraction	specific denitrification rate (mg NO_3-N/g VSS. h)
Fermented settled sludge	rbCOD	3.0 ± 0.5
	$sbCOD_S$	1.2 ± 0.2
	$sbCOD_X$	0.18 ± 0.03
Fermented supernatant sludge	rbCOD	2.2 ± 0.7
	$sbCOD_S$	0.9 ± 0.3
	$sbCOD_X$	0.3 ± 0.1
Fish sludge	rbCOD	1.9 ± 0.6
	$sbCOD_S$	0.9 ± 0.3
	$sbCOD_X$	0.20 ± 0.06

Figures 24 and 25 hold pH and nitrite concentration behaviour in NUR tests utilizing fish sludge, fermented settled fish sludge, and fermented supernatant fish sludge as substrate.

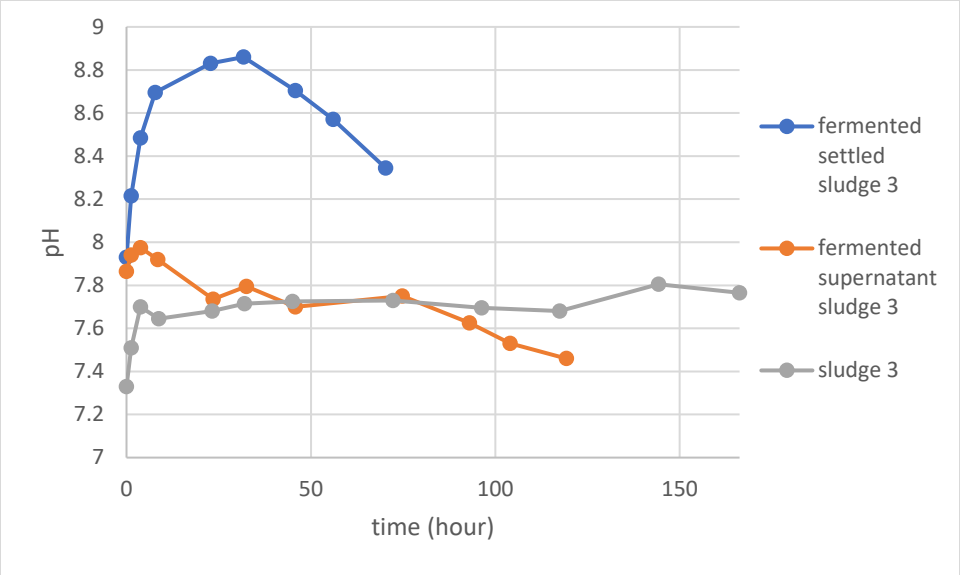

FIGURE 24, PH CHANGE WITH FERMENTED SLUDGE/SLUDGE AS SUBSTRATE

Sludge and supernatant fermented sludge increased the pH of reactor media initially when the denitrification rate is high, but eventually the pH is regulated to approximately 7.7, almost the same the same behavior observed in figure 18. However, settled fermented sludge sharply rose the pH up to 8.9 at the beginning of cycle which is due to higher denitrification rate just like what acetate did as substrate (higher sCOD values according to table 16). Surprisingly pH starts to decrease in the middle of cycle period which might be due to presence and activity of fermentative microorganisms in the reactor (coming from settled fermented sludge substrate).

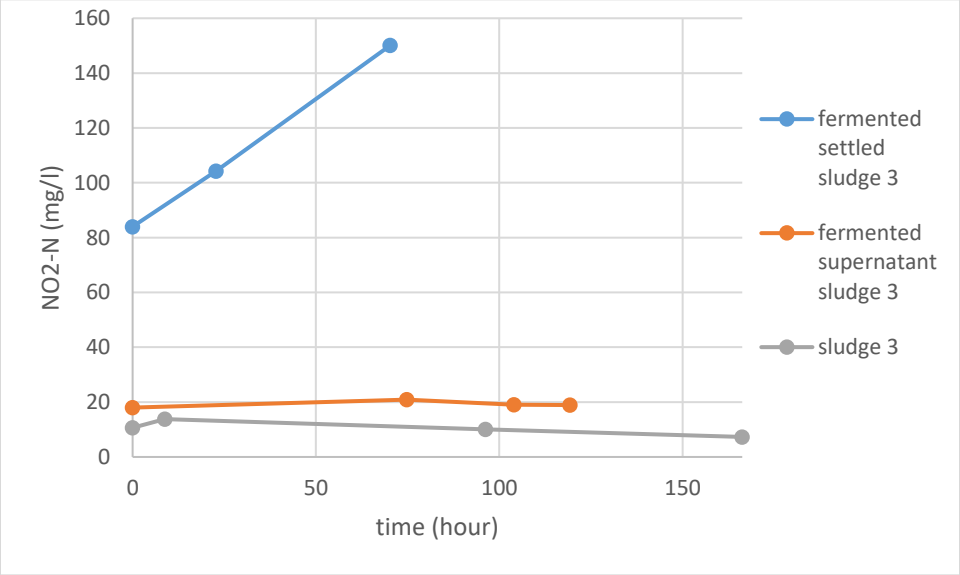

FIGURE 25, NITRITE CONCENTRATION CHANGE WITH FERMENTED SLUDGE/SLUDGE AS SUBSTRATE

Here again sludge and fermented supernatant sludge almost have the same trend and the concentration of nitrite remains almost stable at a low range (10-20 mg/l) just like figure 19. On

the other hand, utilizing fermented settled sludge as substrate led to accumulation of nitrite in the reactor similar to acetate (figure 16) up to 150 mg/l.

The summary of specific denitrification rate and biodegradability percentage of each substrate is reported in table 20. COD consumption rate for denitrification is assumed to be 5 g COD/g NO_3-N (Van Rijn et al., 2006).

TABLE 20, SPECIFIC DENITRIFICATION RATE AND BIODEGRADABILITY OF EACH SUBSTRATE

Substrate	COD fraction	r_{DN} (mg NO_3-N/g VSS. h)	Initial COD (g/l)	Biodegradable fractions (%)	Total biodegradability (%)
Acetate	rbCOD	3.64 ± 0.07	1	129	129
Fish sludge	rbCOD	1.9 ± 0.6	0.78	19	74
	$sbCOD_S$	0.9 ± 0.3		49	
	$sbCOD_X$	0.20 ± 0.06		6	
Settled fermented sludge	rbCOD	3.0 ± 0.5	0.63	58	150
	$sbCOD_S$	1.2 ± 0.2		75	
	$sbCOD_X$	0.18 ± 0.03		17	

The table proves the positive effect of fermentation on biodegradability of fish sludge and improving denitrification rate (S.-I. Lee et al., 1995). Although the initial COD of fermented fish sludge fed to reactor is lower than fish sludge, higher denitrification rate and higher biodegradability percentage of all different COD fractions is observed.

4.4. Experimental uncertainty

The uncertainty of the different obtained result can be analysed based on the calculated standard error foe each experiment. For example, pH and BOD measurement data show an accepted level of reliability (less than 10% difference). On the other hand, VSS, TSS, CODs, and VFA data are more uncertain in comparison. The error in VFA measuring analysis is comparatively higher as is it dependent on other experimental steps like dilution, titration, and conductivity measurement. Also, for TSS, VSS, sCOD and tCOD values, the experimental tests are highly affected by the homogeneity of samples which is impacted by laboratory equipment like mixer, shaker, centrifuge, filters, and homogeniser (grinder). They even have higher impact for COD analysis which a tiny particle can cause a big difference in the result.

The uncertainty of ISE measurement has been already discussed and NUR tests based on ISE method is considered to be reliable as the validity tests using spectroquant method showed 9.34% difference in average. Moreover, denitrification rate calculation is based on the difference between nitrate concentrations and the calculated relative error (C.V.) for several NO_3-ISE measurement is less than 1%.

5. Discussion

5.1. Fish sludge characterization

According to table 14, fish sludge characteristics, specially VSS, TSS and tCOD, are very depended on the fish production season and they can vary a lot in different time periods of year. The characterization of a typical high strength wastewater is shown in table 21 (Metcalf et al., 1991).

TABLE 21, WASTEWATER CHARACTERIZATION

	TS	VS	TSS	VSS	tCOD	$tBOD_5$
Concentration (g/l)	1.612	0.445	0.389	0.304	1.016	0.4

Obviously, the wastewater is much diluter than fish sludge in terms of solid fractions and oxygen demand, but the ratio of VSS/TSS and tCOD/VSS is in the same range for both fish sludge and wastewater sludge (table 22).

TABLE 22, COMPARISON OF FISH SLUDGE AND WASTEWATER SLUDGE

	Fish sludge (average)	Wastewater sludge
VSS/TSS	0.87 ± 0.02	0.78
tCOD/VSS	2.2 ± 0.8	3.34

The comparison of VSS/TSS ratio indicates that wastewater sludge has slightly more portion of non-volatile suspended solids than fish sludge which is reasonable as wastewater sludge carries more amount of sediments.

tCOD/VSS shows that wastewater sludge contains broader variety of biodegradable organic matters than fish sludge due to the difference of their origin; fish sludge is mainly consisting of fish faeces and fish food leftovers, but wastewater sludge could have household, agriculture, and even industrial source. Moreover, as fishes already digested most of the nutrients of their food, wastewater sludge probably has higher levels of various nutrients as well.

5.2. Denitrification rates

Specific denitrification rate (g NO_3-N/kg VSS. h) was determined using equation 12.

$$r_{DN} = {}^{dNO_3^-}/_{X_{MLVSS}.dt} \qquad \text{(Eq. 12)}$$

The denitrification rate in other studies using easily biodegradable substrates (acetate, glucose, etc) is reported 30 mg NO_3-N/g VSS. h (Henze et al., 2008; Turk & Mavinic, 1987) which is almost ten times higher than the rates calculated in this study. The big difference could be due to two main

reasons, firstly the low operating temperature which is an important factor in denitrification rate and hydrolysis, secondly the biomass sludge which was adapted from fish sludge and probably not enriched enough in perspective of microorganisms. The fermentation rates with other COD fractions have been reported to be 9, 4, 2.9 mg NO_3-N/g VSS. h respectively for rbCOD, $sbCOD_S$ and $sbCOD_X$.

5.3. Fermentation

As discussed in chapter two, the complex organic matter is turned into simpler compounds in anaerobic fermentation and fermentation of raw sludge can increase bioavailability of organic carbon; according to figure 5 and 6 particulates organic matters and solid fractions amounts will fall off and convert to simple organics which are ready for denitrification process. The other proof is growing the sCOD values after fermentation as figure 8 shows. Moreover, fermentation and hydrolysis can transform the compounds into volatile fatty acids which is shown in detail by the data of figures 10-13.

Denitrification rate and biodegradability of fermented fish sludge as substrate shown to be higher than fresh fish sludge as substrate (table 18 and 19) in both readily biodegradable and slowly biodegradable fractions. However, the denitrification rate of slowly biodegradable particulate fractions had slightly decreased while using fermented fish sludge, the reason is the decline of TSS and VSS values during fermentation.

So, fermentation of fish waste is necessary in order to use fish sludge as a C-source to achieve higher rates of denitrification and considering the results (figures 8 and 10), optimum duration of fermentation can be 5 days at 12 ℃. Fermentation rate is calculated using VFA production over seven days (overall) and in three different intervals. Table 18 proves the temperature effect on the fermentation rate by showing that fermentation rate at 20 ℃ is almost double the rate at 12 ℃. Also, the highest fermentation rate is in the first two days, and it slows down eventually which shows the impact of organic matter availability and pH of media.

5.4. NO_2 accumulation

Nitrite has been shown to accumulate during the denitrification, most likely as a result of a slower nitrite reduction rate than the nitrate reduction rate, the reason could be microbial communities with facultative anaerobes can only reduce nitrate to nitrite. At high NO_3 levels, acetate, settled fermented sludge and high pH, accumulation of NO_2 is observed and it is apparently due to conversion kinetics (Wilderer et al., 1987).

E. coli (and probably a range of bacteria) are not able to reduce NO_2. So, both kinetically and by community composition, NO_2 will accumulate as an intermediate especially at high denitrification rates, which is the case for the acetate and settled fermented sludge stimulated denitrification batch test (Suri et al., 2021).

5.5. Future work

For the further investigation on denitrification using fish sludge as substrate, it would be beneficial to use actual biomass sludge from the related industry to be able to obtain more reliable/accurate data. Also, fish sludge fermentation could be done at higher temperature to achieve higher fermentation rate and more percentage of VFAs and rbCODs. Doing Nutrient analysis (fatts, proteins, minerals, etc) on the fish sludge and fermented fish sludge is also helpful to have a comprehensive overview of sludge characterisation and nutrient level. Future studies also could assess fish sludge's exact disintegration and hydrolysis procedure for the denitrification process.

The other suggestion is to estimate denitrification rate using OxiTop respirometry, denitrified N_2 partial pressure can be measured at regular intervals by OxiTop screw cap heads. Performing OxiTop method in parallel with nitrate ISE measurement provides useful information about the denitrification rate in different time intervals.

6. Conclusion

Different hydrolysis and disintegration mechanisms of particulate organic material in anaerobic digestion and nutrient removal systems are summarized considering the effect of temperature, kinetics, enzymes, etc; and a variety of research illustrated that the hydrolysis step is the bottleneck of denitrification. The hydrolysis process follows first-order kinetics, but there might be inaccuracy in describing complex and specific substrates.

Validation of the NO_3-ISE method against spectroquant method using appropriate NO_3-N kit is checked and shows promising result based on the trend of concentration increase. The direct measurement of nitrate concentration change in SBR reactor using ISE is practical and reliable to calculate denitrification rate as the relative error for NO_3-ISE measurement is less than 1%.

Different solid fractions, chemical and biological characterization of fish sludge have been analysed and tested, the ratio of sCOD to tCOD of fish sludge is in the range of 13-37% and the ratio of VSS to TSS of sludge is in the range of 85-90%. Looking at the estimated denitrification rates, fish sludge contains 19% readily biodegradable, 49% slowly biodegradable, and 6% slowly particulate biodegradable CODs, the rest 26% can be assumed as inert compounds. Although there is a big difference between fish sludge and wastewater sludge wet analysis results, the two ratios of VSS/TSS and tCOD/VSS are in the same span.

The production of readily biodegradable CODs via fermentation could be competitive with the price of external substrates (acetate or methanol) for denitrification processes, also acetate is not suitable external substrate as it increases pH and nitrite accumulation of reactor. The characteristic of fermented sludge is reported, and fermentation rate have been estimated using VFA production at 12 ℃ and optimum duration of fish sludge fermentation process could be assumed 5 days. Fermentation improves the denitrification rate by increasing bioavailability of carbon and as it is

not comparatively an expensive unit operation, it is highly recommended for related industries considering the fact that higher temperature enhances the fermentation rate.

NUR is calculated and normalized based on VSS of reactor media, the rate shows that nitrate removal of RAS effluent is possible using internally generated fish sludge as C-source but NUR rate for sludge as substrate is low at 12 ℃ comparing to typical municipal wastewater rates due to hydrolysis of COD fractions; also, biodegradable fraction of sludge is calculated based on COD consumption and COD added.

Appendix

Figure 26: The NUR test done at 20 ℃ using acetate, fish sludge and no carbon source (endogenous).

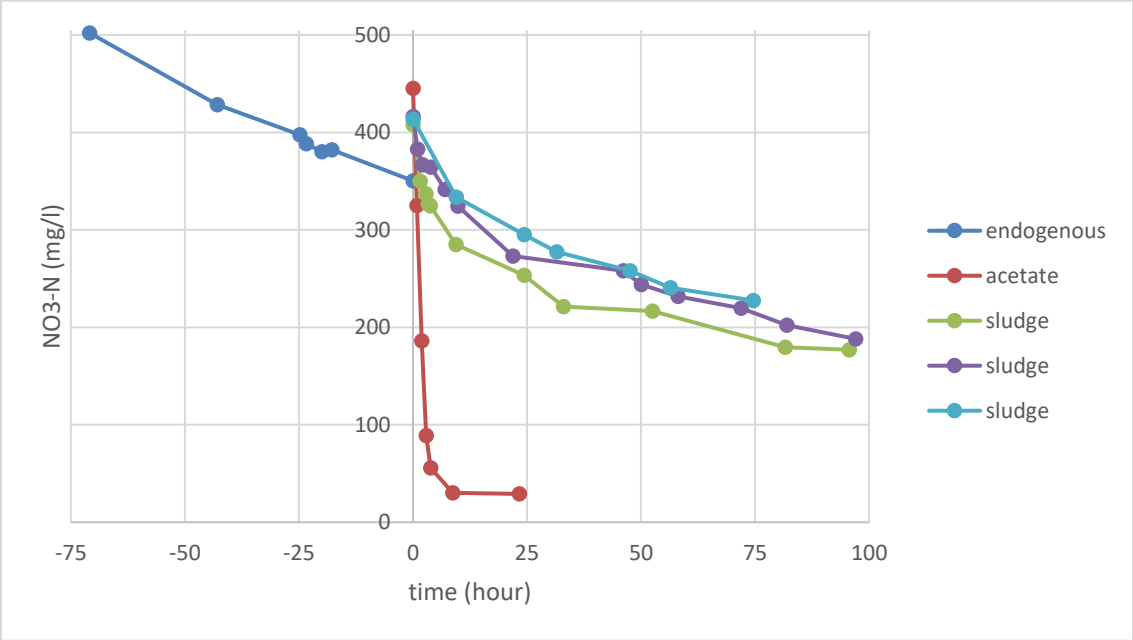

FIGURE 26, NUR TEST AT 20 ℃

Figure 27: Nitrate concentration change in reactor at 20 ℃, measured during daily cycles.

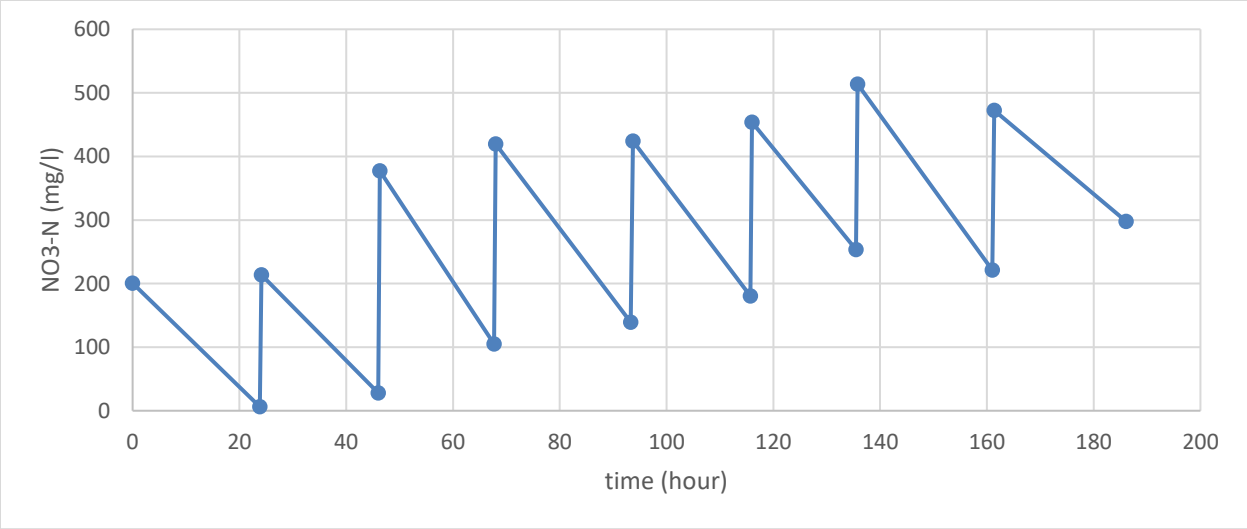

FIGURE 27, DAILY NUR AT 20 ℃

Figure 28: Trial OxiTop test using fish sludge and blank samples, the data of blank samples has been deducted from fish sludge samples (the average data of three parallel reactors).

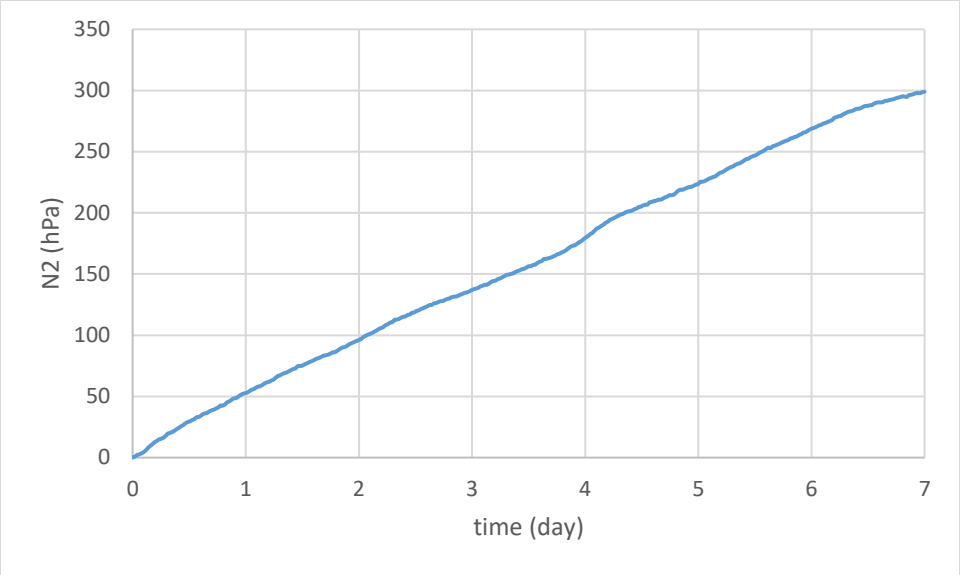

FIGURE 28, NITROGEN PRODUCTION PRESSURE

Figure 29: two days fermentation test of fish sludge at 20 ℃ in three parallel reactors.

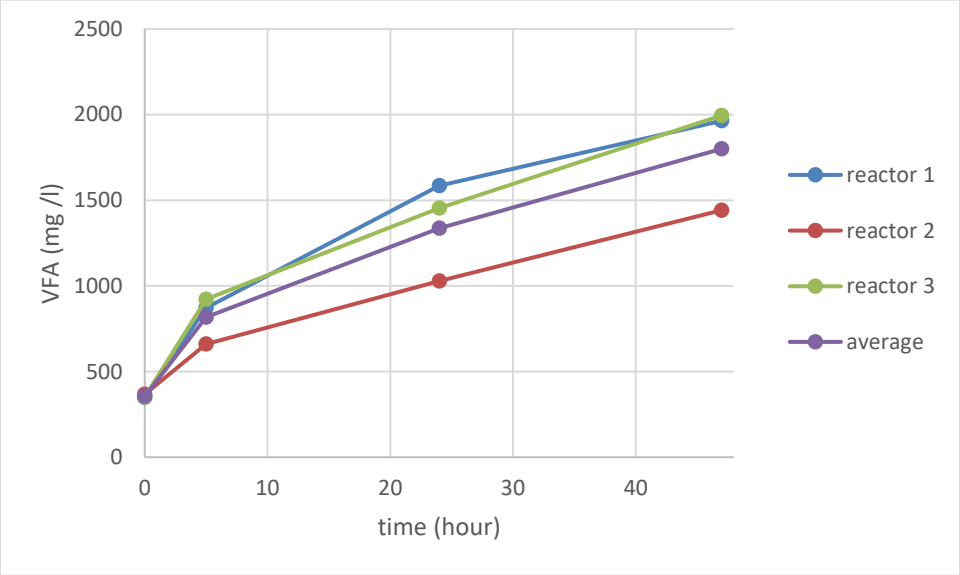

FIGURE 29, TWO DAYS FISH SLUDGE FERMENTATION AT 20 ℃

Table 23: fish sludge characterisation, 11/Feb/2022.

TABLE 23, FISH SLUDGE CHARACTERISTICS

VSS	tCOD (g/l)	sCOD
4.6 ± 0.4	18 ± 2	8.26 ± 0.06

References

Alahi, M. E. E., Mukhopadhyay, S. C., & Burkitt, L. (2018). Imprinted polymer coated impedimetric nitrate sensor for real-time water quality monitoring. *Sensors and Actuators B: Chemical*, *259*, 753–761.

Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., & Watson, J. D. (1994). Molecular biology of the cell, Garland Publishing Inc. *New York*.

Albina, P., Durban, N., Bertron, A., Albrecht, A., Robinet, J.-C., & Erable, B. (2019). Influence of hydrogen electron donor, alkaline pH, and high nitrate concentrations on microbial denitrification: A review. *International Journal of Molecular Sciences*, *20*(20), 5163.

Appels, L., Houtmeyers, S., Degrève, J., Van Impe, J., & Dewil, R. (2013). Influence of microwave pre-treatment on sludge solubilization and pilot scale semi-continuous anaerobic digestion. *Bioresource Technology*, *128*, 598–603.

Arbiv, R., & van Rijn, J. (1995). Performance of a treatment system for inorganic nitrogen removal in intensive aquaculture systems. *Aquacultural Engineering*, *14*(2), 189–203.

Ayol, A., Filibeli, A., Sir, D., & Kuzyaka, E. (2008). Aerobic and anaerobic bioprocessing of activated sludge: Floc disintegration by enzymes. *Journal of Environmental Science and Health Part A*, *43*(13), 1528–1535.

Azaria, S., & van Rijn, J. (2018). Off-flavor compounds in recirculating aquaculture systems (RAS): Production and removal processes. *Aquacultural Engineering*, *83*, 57–64.

Batstone, D. J., Keller, J., Angelidaki, I., Kalyuzhnyi, S. V., Pavlostathis, S. G., Rozzi, A., Sanders, W. T. M., Siegrist, H., & Vavilin, V. A. (2002). The IWA Anaerobic Digestion Model No 1 (ADM1). *Water Science and Technology*, *45*(10), 65–73. https://doi.org/10.2166/wst.2002.0292

Béné, C., Arthur, R., Norbury, H., Allison, E. H., Beveridge, M., Bush, S., Campling, L., Leschen, W., Little, D., Squires, D., Thilsted, S. H., Troell, M., & Williams, M. (2016). Contribution of Fisheries and Aquaculture to Food Security and Poverty Reduction: Assessing the Current Evidence. *World Development*, *79*, 177–196. https://doi.org/10.1016/j.worlddev.2015.11.007

Blancheton, J. P. (2000). Developments in recirculation systems for Mediterranean fish species. *Aquacultural Engineering*, *22*(1–2), 17–31.

Blancheton, J.-P., Piedrahita, R., Eding, E. H., Lemarie, G., Bergheim, A., Fivelstad, S., & Roque D'Orbcastel, E. (2007). Intensification of landbased aquaculture production in single pass and reuse systems. *Aquacultural Engineering and Environment*, 21–47.

Bolzonella, D., Fatone, F., Pavan, P., & Cecchi, F. (2005). Anaerobic fermentation of organic municipal solid wastes for the production of soluble organic compounds. *Industrial & Engineering Chemistry Research*, *44*(10), 3412–3418.

Brinch, P. P., Rindel, K., & Kalb, K. (1994). Upgrading to nutrient removal by means of internal carbon from sludge hydrolysis. *Water Science and Technology*, *29*(12), 31.

Brock, T. D., & Madigan, M. T. (1991). Biology of Microorganisms. Prentice-Hall. *New Jersey*, *19862*.

Bryers, J. D., & Mason, C. A. (1987). Biopolymer particulate turnover in biological waste treatment systems: A review. *Bioprocess Engineering*, *2*(3), 95–109.

Bureau, D. P., & Hua, K. (2010). Towards effective nutritional management of waste outputs in aquaculture, with particular reference to salmonid aquaculture operations. *Aquaculture Research*, *41*(5), 777–792.

Burghate, S. P., & Ingole, N. W. (2014). Biological denitrification—A review. *Journal of Environmental Science, Computer Science and Engineering & Technology*, *14*, 009–028.

Canziani, R., Pollice, A., & Ragazzi, M. (1995). Feasibility of using primary-sludge mesophilic hydrolysis for biological removal of nitrogen and phosphorus from wastewater. *Bioresource Technology*, *54*(3), 255–260.

Canziani, R., Vismara, R., Basilico, D., & Zinni, L. (1999). Nitrogen removal in fixed-bed submerged biofilters without backwashing. *Water Science and Technology*, *40*(4–5), 145–152.

Cao, L., Wang, W., Yang, Y., Yang, C., Yuan, Z., Xiong, S., & Diana, J. (2007). Environmental impact of aquaculture and countermeasures to aquaculture pollution in China. *Environmental Science and Pollution Research-International*, *14*(7), 452–462.

Cao, S., Du, R., Li, B., Wang, S., Ren, N., & Peng, Y. (2017). Nitrite production from partial-denitrification process fed with low carbon/nitrogen (C/N) domestic wastewater: Performance, kinetics and microbial community. *Chemical Engineering Journal*, *326*, 1186–1196.

Cao, S., Wang, S., Peng, Y., Wu, C., Du, R., Gong, L., & Ma, B. (2013). Achieving partial denitrification with sludge fermentation liquid as carbon source: The effect of seeding sludge. *Bioresource Technology*, *149*, 570–574.

Chen, C., Ming, J., Yoza, B. A., Liang, J., Li, Q. X., Guo, H., Liu, Z., Deng, J., & Wang, Q. (2019). Characterization of aerobic granular sludge used for the treatment of petroleum wastewater. *Bioresource Technology*, *271*, 353–359.

Chen, J., & Strous, M. (2013). Denitrification and aerobic respiration, hybrid electron transport chains and co-evolution. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1827*(2), 136–144.

Chen, J.-C., Liu, P.-C., & Lei, S.-C. (1990). Toxicities of ammonia and nitrite to Penaeus monodon adolescents. *Aquaculture*, *89*(2), 127–137.

Christ, O., Wilderer, P. A., Angerhöfer, R., & Faulstich, M. (2000). Mathematical modeling of the hydrolysis of anaerobic processes. *Water Science and Technology*, *41*(3), 61–65.

Chrost, R. J. (1991). Environmental control of the synthesis and activity of aquatic microbial ectoenzymes. In *Microbial enzymes in aquatic environments* (pp. 29–59). Springer.

Ciaciuch, A., Gaca, J., & Lelewer, K. (2017). Effect of the two-stage thermal disintegration and anaerobic digestion of sewage sludge on the COD fractions. *Polish Journal of Chemical Technology*, *19*(3).

Conroy, J., & Couturier, M. (2010). Dissolution of minerals during hydrolysis of fish waste solids. *Aquaculture*, *298*(3–4), 220–225.

Cripps, S. J., & Bergheim, A. (2000). Solids management and removal for intensive land-based aquaculture production systems. *Aquacultural Engineering*, *22*(1–2), 33–56.

Davidson, J., Good, C., Welsh, C., & Summerfelt, S. T. (2014). Comparing the effects of high vs. Low nitrate on the health, performance, and welfare of juvenile rainbow trout Oncorhynchus mykiss within water recirculating aquaculture systems. *Aquacultural Engineering*, *59*, 30–40.

De Kreuk, M. K., Kishida, N., Tsuneda, S., & Van Loosdrecht, M. C. M. (2010). Behavior of polymeric substrates in an aerobic granular sludge system. *Water Research*, *44*(20), 5929–5938.

de Melo Filho, M. E. S., Owatari, M. S., Mouriño, J. L. P., Lapa, K. R., & Soares, H. M. (2020). Application of nitrification and denitrification processes in a direct water reuse system for pacific white shrimp farmed in biofloc system. *Aquacultural Engineering*, *88*, 102043.

de Vries, S. S., & Pouvreau, L. A. (2007). Nitric oxide reductase: Structural variations and catalytic mechanism. In *Biology of the nitrogen cycle* (pp. 57–66). Elsevier.

Díaz, V., Ibáñez, R., Gómez, P., Urtiaga, A. M., & Ortiz, I. (2012). Kinetics of nitrogen compounds in a commercial marine Recirculating Aquaculture System. *Aquacultural Engineering*, *50*, 20–27.

Dimeski, G., Badrick, T., & St John, A. (2010). Ion selective electrodes (ISEs) and interferences—A review. *Clinica Chimica Acta*, *411*(5–6), 309–317.

Dimock, R., & Morgenroth, E. (2006). The influence of particle size on microbial hydrolysis of protein particles in activated sludge. *Water Research*, *40*(10), 2064–2074.

Ding, J., Zhao, Q.-L., Jiang, J.-Q., Wei, L.-L., Wang, K., Zhang, Y.-S., Hou, W.-Z., & Yu, H. (2017). Electrochemical disinfection and removal of ammonia nitrogen for the reclamation of wastewater treatment plant effluent. *Environmental Science and Pollution Research*, *24*, 5152–5158.

Du, R., Cao, S., Li, B., Niu, M., Wang, S., & Peng, Y. (2017). Performance and microbial community analysis of a novel DEAMOX based on partial-denitrification and anammox treating ammonia and nitrate wastewaters. *Water Research*, *108*, 46–56.

Du, R., Cao, S., Peng, Y., Zhang, H., & Wang, S. (2019). Combined Partial Denitrification (PD)-Anammox: A method for high nitrate wastewater treatment. *Environment International*, *126*, 707–716.

Eastman, J. A., & Ferguson, J. F. (1981). Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. *Journal (Water Pollution Control Federation)*, 352–366.

Ebeling, J. M., & Timmons, M. B. (2010). *Recirculating aquaculture*. Cayuga Aqua Ventures Ithaca, NY, USA.

Eding, E. H., Kamstra, A., Verreth, J. A. J., Huisman, E. A., & Klapwijk, A. (2006). Design and operation of nitrifying trickling filters in recirculating aquaculture: A review. *Aquacultural Engineering*, *34*(3), 234–260.

Eding, E., & Kamstra, A. (2002). Netherlands farms tune recirculation systems to production of varied species. *Global Aquaculture Advocate*, *5*(3), 52–55.

Ekama, G. A., Dold, P. L., & Marais, G. v R. (1986). Procedures for determining influent COD fractions and the maximum specific growth rate of heterotrophs in activated sludge systems. *Water Science and Technology*, *18*(6), 91–114.

Elefsiniotis, P., & Li, D. (2006). The effect of temperature and carbon source on denitrification using volatile fatty acids. *Biochemical Engineering Journal*, *28*(2), 148–155.

Eskicioglu, C., Kennedy, K. J., & Droste, R. L. (2006). Characterization of soluble organic matter of waste activated sludge before and after thermal pretreatment. *Water Research*, *40*(20), 3725–3736.

Fan, Y., Huang, Y., Linthicum, W., Liu, F., Beringhs, A. O., Dang, Y., Xu, Z., Chang, S.-Y., Ling, J., & Huey, B. D. (2020). Toward Long-Term Accurate and Continuous Monitoring of Nitrate in Wastewater Using Poly (tetrafluoroethylene)(PTFE)–Solid-State Ion-Selective Electrodes (S-ISEs). *ACS Sensors*, *5*(10), 3182–3193.

Federico, B., Giuseppe, S., Valentino, F., Gottardo, M., Villano, M., Matos, M., Silva, F., Reis, M. A., Mata-Alvarez, J., & Astals, S. (2022). New insights in food waste, sewage sludge and green waste anaerobic fermentation for short-chain volatile fatty acids production: A review. *Journal of Environmental Chemical Engineering*, 108319.

Feng, Y., Li, X., Yu, Y., Qi, J., Jia, X., Wang, J., & Li, X. (2016). Production of unburned calcium silicon filter material (UCSFM) from oyster shell and its performance investigation in an A/O integrated biological aerated filter reactor (A/O-BAF). *Rsc Advances*, *6*(88), 85324–85332.

Ferrer, I., Ponsá, S., Vázquez, F., & Font, X. (2008). Increasing biogas production by thermal (70 C) sludge pre-treatment prior to thermophilic anaerobic digestion. *Biochemical Engineering Journal*, *42*(2), 186–192.

Fivelstad, S., Bergheim, A., Hølland, P. M., & Fjermedal, A. B. (2004). Water flow requirements in the intensive production of Atlantic salmon (Salmo salar L.) parr–smolt at two salinity levels. *Aquaculture*, *231*(1–4), 263–277.

Flotats, X., Palatsi, J., Ahring, B. K., & Angelidaki, I. (2006). Identifiability study of the proteins degradation model, based on ADM1, using simultaneous batch experiments. *Water Science and Technology*, *54*(4), 31–39.

Foladori, P., Andreottola, G., & Ziglio, G. (2010). *Sludge reduction technologies in wastewater treatment plants*. IWA publishing.

Frant, M.-S., & Ross Jr, J. W. (1970). Potassium ion specific electrode with high selectivity for potassium over sodium. *Science*, *167*(3920), 987–988.

Frigon, D., Oerther, D. B., Morgenroth, E., & Raskin, L. (2002). Oligonucleotide probe hybridization and modeling results suggest that populations consuming readily degradable substrate have high cellular RNA levels. *Water Science and Technology*, *45*(6), 115–126.

Gao, X., Liu, X., & Wang, W. (2016). Biodegradation of particulate organics and its enhancement during anaerobic co-digestion of municipal biowaste and waste activated sludge. *Renewable Energy*, *96*, 1086–1092. https://doi.org/10.1016/j.renene.2016.01.019

Gelfand, I., Barak, Y., Even-Chen, Z., Cytryn, E., Van Rijn, J., Krom, M. D., & Neori, A. (2003). A novel zero discharge intensive seawater recirculating system for the culture of marine fish. *Journal of the World Aquaculture Society*, *34*(3), 344–358.

Ghafari, S., Hasan, M., & Aroua, M. K. (2008). Bio-electrochemical removal of nitrate from water and wastewater—A review. *Bioresource Technology*, *99*(10), 3965–3974.

Ghosh, S. (1981). Kinetics of acid-phase fermentation in anaerobic digestion. *Biotechnol. Bioeng. Symp.;(United States)*, *11*.

Gichana, Z. M., Liti, D., Waidbacher, H., Zollitsch, W., Drexler, S., & Waikibia, J. (2018). Waste management in recirculating aquaculture system through bacteria dissimilation and plant assimilation. *Aquaculture International*, *26*(6), 1541–1572.

Goel, R., Mino, T., Satoh, H., & Matsuo, T. (1998). Enzyme activities under anaerobic and aerobic conditions in activated sludge sequencing batch reactor. *Water Research*, *32*(7), 2081–2088.

Gujer, W., Henze, M., Mino, T., & Van Loosdrecht, M. (1999). Activated sludge model No. 3. *Water Science and Technology*, *39*(1), 183–193.

Gutierrez-Wing, M. T., Malone, R. F., & Rusch, K. A. (2012). Evaluation of polyhydroxybutyrate as a carbon source for recirculating aquaculture water denitrification. *Aquacultural Engineering*, *51*, 36–43.

Henze, M., Gujer, W., Mino, T., & van Loosdrecht, M. C. (2000). *Activated sludge models ASM1, ASM2, ASM2d and ASM3*. IWA publishing.

Henze, M., van Loosdrecht, M. C., Ekama, G. A., & Brdjanovic, D. (2008). *Biological wastewater treatment*. IWA publishing.

Hobson, P. N., & Wheatley, A. D. (1993). *Anaerobic digestion: Modern theory and practice*.

Ivanova, A., & Mikhelson, K. (2018). Electrochemical properties of nitrate-selective electrodes: The dependence of resistance on the solution concentration. *Sensors*, *18*(7), 2062.

Iwama, G. K. (1991). Interactions between aquaculture and the environment. *Critical Reviews in Environmental Science and Technology*, *21*(2), 177–216.

Jasielec, J. J., Sokalski, T., Filipek, R., & Lewenstam, A. (2010). Comparison of different approaches to the description of the detection limit of ion-selective electrodes. *Electrochimica Acta*, *55*(22), 6836–6848.

Jasielec, J. J., Sokalski, T., Filipek, R., & Lewenstam, A. (2015). Neutral-Carrier Ion-Selective Electrodes Assessed by the Nernst–Planck–Poisson Model. *Analytical Chemistry*, *87*(17), 8665–8672.

Jin, L., Zhang, G., & Zheng, X. (2015). Effects of different sludge disintegration methods on sludge moisture distribution and dewatering performance. *Journal of Environmental Sciences*, *28*, 22–28.

Kayhanian, M., & Hardy, S. (1994). The impact of four design parameters on the performance of a high-solids anaerobic digestion of municipal solid waste for fuel gas production. *Environmental Technology*, *15*(6), 557–567.

Kommedal, R. (2003). *Degradation of polymeric and particulate organic carbon in biofilms*.

Le, T., Peng, B., Su, C., Massoudieh, A., Torrents, A., Al-Omari, A., Murthy, S., Wett, B., Chandran, K., & DeBarbadillo, C. (2019). Impact of carbon source and COD/N on the concurrent operation of partial denitrification and anammox. *Water Environment Research*, *91*(3), 185–197.

Lee, P. G., Lea, R. N., Dohmann, E., Prebilsky, W., Turk, P. E., Ying, H., & Whitson, J. L. (2000). Denitrification in aquaculture systems: An example of a fuzzy logic control problem. *Aquacultural Engineering*, *23*(1–3), 37–59.

Lee, S.-I., Koopman, B., Park, S.-K., & Cadee, K. (1995). Effect of fermented wastes on denitrification in activated sludge. *Water Environment Research*, *67*(7), 1119–1122.

Lepine, C., Christianson, L., Davidson, J., & Summerfelt, S. (2018). Woodchip bioreactors as treatment for recirculating aquaculture systems' wastewater: A cost assessment of nitrogen removal. *Aquacultural Engineering*, *83*, 85–92.

Li, H., Cui, Z., Cui, H., Bai, Y., Yin, Z., & Qu, K. (2023). Hazardous substances and their removal in recirculating aquaculture systems: A review. *Aquaculture*, *569*, 739399. https://doi.org/10.1016/j.aquaculture.2023.739399

Li, W., Li, H., Liu, Y., Zheng, P., & Shapleigh, J. P. (2018). Salinity-aided selection of progressive onset denitrifiers as a means of providing nitrite for anammox. *Environmental Science & Technology*, *52*(18), 10665–10672.

Liebetrau, J., Kraft, E., & Bidlingmaier, W. (2004). The influence of the hydrolysis rate of co-substrates on process behaviour, w: Guiot, SG. *Proceedings of the Tenth World Congress on Anaerobic. Canadian Association on Water Quality, Montreal*, *12961300*.

Lin, C. Y. (1991). Anaerobic digestion of landfill leachate. *Water Sa*, *17*(4), 301–306.

Liu, Y.-J., Gu, J., & Liu, Y. (2018). Energy self-sufficient biological municipal wastewater reclamation: Present status, challenges and solutions forward. *Bioresource Technology*, *269*, 513–519.

Losordo, T. M., & Westers, H. (1994). System carrying capacity and flow estimation. *Developments in Aquaculture and Fisheries Science*, *27*, 9–60.

Lu, J., Zhang, Y., Wu, J., & Wang, J. (2020). Nitrogen removal in recirculating aquaculture water with high dissolved oxygen conditions using the simultaneous partial nitrification, anammox and denitrification system. *Bioresource Technology*, *305*, 123037.

Lu, Y., Slater, F., Bello-Mendoza, R., & Batstone, D. J. (2013). Shearing of biofilms enables selective layer based microbial sampling and analysis. *Biotechnology and Bioengineering*, *110*(10), 2600–2605.

Marais, J. P., Therion, J. J., Mackie, R. I., Kistner, A., & Dennison, C. (1988). Effect of nitrate and its reduction products on the growth and activity of the rumen microbial population. *British Journal of Nutrition*, *59*(2), 301–313.

Martins, C. I. M., Eding, E. H., Verdegem, M. C., Heinsbroek, L. T., Schneider, O., Blancheton, J.-P., d'Orbcastel, E. R., & Verreth, J. A. J. (2010). New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. *Aquacultural Engineering*, *43*(3), 83–93.

Masse, L., Massé, D. I., Kennedy, K. J., & Chou, S. P. (2002). Neutral fat hydrolysis and long-chain fatty acid oxidation during anaerobic digestion of slaughterhouse wastewater. *Biotechnology and Bioengineering*, *79*(1), 43–52.

Matěj\uu, V., Čižinská, S., Krejčí, J., & Janoch, T. (1992). Biological water denitrification—A review. *Enzyme and Microbial Technology*, *14*(3), 170–183.

Meriac, A., Eding, E. H., Schrama, J., Kamstra, A., & Verreth, J. A. (2014). Dietary carbohydrate composition can change waste production and biofilter load in recirculating aquaculture systems. *Aquaculture*, *420*, 254–261.

Metcalf, L., Eddy, H. P., & Tchobanoglous, G. (1991). *Wastewater engineering: Treatment, disposal, and reuse* (Vol. 4). McGraw-Hill New York.

Monsees, H., Klatt, L., Kloas, W., & Wuertz, S. (2017). Chronic exposure to nitrate significantly reduces growth and affects the health status of juvenile Nile tilapia (Oreochromis niloticus L.) in recirculating aquaculture systems. *Aquaculture Research*, *48*(7), 3482–3492.

Montanhini Neto, R., & Ostrensky, A. (2015). Nutrient load estimation in the waste of Nile tilapia Oreochromis niloticus (L.) reared in cages in tropical climate conditions. *Aquaculture Research*, *46*(6), 1309–1322.

Mook, W. T., Chakrabarti, M. H., Aroua, M. K., Khan, G. M. A., Ali, B. S., Islam, M. S., & Hassan, M. A. (2012). Removal of total ammonia nitrogen (TAN), nitrate and total organic carbon (TOC) from aquaculture wastewater using electrochemical technology: A review. *Desalination*, *285*, 1–13.

Moosbrugger, G. vR. (1993). Alkalinity measurement: Part 1-A 4 pH point titration method to determine the carbonate weak acid/base in an aqueous carbonate solution. *Water SA*, *19*(1), 11–22.

Morf, W. E. (1981). The Principles of Ion-selective Electrodes and of Membrane Transport, Akad. *Kiado, Budapest*, 430.

Morgenroth, E., Kommedal, R., & Harremoës, P. (2002). Processes and modeling of hydrolysis of particulate organic matter in aerobic wastewater treatment–a review. *Water Science and Technology*, *45*(6), 25–40.

Narihiro, T., Terada, T., Ohashi, A., Kamagata, Y., Nakamura, K., & Sekiguchi, Y. (2012). Quantitative detection of previously characterized syntrophic bacteria in anaerobic wastewater treatment systems by sequence-specific rRNA cleavage method. *Water Research*, *46*(7), 2167–2175.

Negral, L., Marañón, E., Castrillón, L., & Fernández-Nava, Y. (2015). Differences in soluble COD and ammonium when applying ultrasound to primary, secondary and mixed sludge. *Water Science and Technology*, *71*(9), 1398–1406.

Negri, E. D., Mata-Alvarez, J., Sans, C., & Cecchi, F. (1992). A mathematical model of VFA production in a plug-flow reactor treating the organic fraction of municipal solid waste. *Proc." IAWPRC Intern. Symp. on Anaerobic Digestion of Solid Waste", Bologna, Italy*.

Ng, L. Y., Ng, C. Y., Mahmoudi, E., Ong, C. B., & Mohammad, A. W. (2018). A review of the management of inflow water, wastewater and water reuse by membrane technology for a sustainable production in shrimp farming. *Journal of Water Process Engineering*, *23*, 27–44.

Orhon, D., Çokgör, E. U., & Sözen, S. (1999). Experimental basis for the hydrolysis of slowly biodegradable substrate in different wastewaters. *Water Science and Technology*, *39*(1), 87–95.

O'ROURKE, J. T. (1968). *Kinetics of anaerobic waste treatment at reduced temperatures*. Stanford University.

Palmowski, L. M., & Müller, J. A. (2003). Anaerobic degradation of organic materials-significance of the substrate surface area. *Water Science and Technology*, *47*(12), 231–238.

Pilli, S., Yan, S., Tyagi, R. D., & Surampalli, R. Y. (2015). Thermal pretreatment of sewage sludge to enhance anaerobic digestion: A review. *Critical Reviews in Environmental Science and Technology*, *45*(6), 669–702.

Police, A., Canziani, R., Bortone, G., & Andreottola, G. (1993). Nutrient removal at the wastewater treatment plant of Fusina (VE). *INGEGNERIA AMBIENTALE*, *22*, 462–462.

Poulsen, R., Cedergreen, N., Hayes, T., & Hansen, M. (2018). Nitrate: An environmental endocrine disruptor? A review of evidence and research needs. *Environmental Science & Technology*, *52*(7), 3869–3887.

Preena, P. G., Rejish Kumar, V. J., & Singh, I. S. B. (2021). Nitrification and denitrification in recirculating aquaculture systems: The processes and players. *Reviews in Aquaculture*, *13*(4), 2053–2075.

Pu, Y., Tang, J., Wang, X. C., Hu, Y., Huang, J., Pan, S., & Li, Y. (2019). Enhancing nitrogen removal from wastewater in sequencing batch reactors (SBRs) using additional carbon source produced from food waste acidogenic fermentation at different temperatures. *Environmental Science and Pollution Research*, *26*(33), 34645–34657.

Pungrasmi, W., Playchoom, C., & Powtongsook, S. (2013). Optimization and evaluation of a bottom substrate denitrification tank for nitrate removal from a recirculating aquaculture system. *Journal of Environmental Sciences*, *25*(8), 1557–1564.

Qi, W., Taherzadeh, M. J., Ruan, Y., Deng, Y., Chen, J.-S., Lu, H.-F., & Xu, X.-Y. (2020). Denitrification performance and microbial communities of solid-phase denitrifying reactors using poly (butylene succinate)/bamboo powder composite. *Bioresource Technology*, *305*, 123033.

Qi, W., Zhu, S., Shitu, A., Ye, Z., & Liu, D. (2020). Low concentration peroxymonosulfate and UVA-LED combination for E. coli inactivation and wastewater disinfection from recirculating aquaculture systems. *Journal of Water Process Engineering*, *36*, 101362.

Qin, Y., Cao, Y., Ren, J., Wang, T., & Han, B. (2017). Effect of glucose on nitrogen removal and microbial community in anammox-denitrification system. *Bioresource Technology*, *244*, 33–39.

Ravndal, K. T., & Kommedal, R. (2017). Starch degradation and intermediate dynamics in flocculated and dispersed microcosms. *Water Science and Technology*, *76*(11), 2928–2940.

Richardson, D. J., Van Spanning, R. J., & Ferguson, S. J. (2007). The prokaryotic nitrate reductases. *Biology of the Nitrogen Cycle*, 21–35.

Rinaldo, S., & Cutruzzolà, F. (2007). Nitrite reductases in denitrification. In *Biology of the nitrogen cycle* (pp. 37–55). Elsevier.

Ristow, N. E., Sötemann, S. W., Wentzel, M. C., Loewenthal, R. E., & Ekama, G. A. (2006). The effects of hydraulic retention time and feed COD concentration on the rate of hydrolysis of primary sewage sludge under methanogenic conditions. *Water Science and Technology*, *54*(5), 91–100.

Ruiz, P., Vidal, J. M., Sepúlveda, D., Torres, C., Villouta, G., Carrasco, C., Aguilera, F., Ruiz-Tagle, N., & Urrutia, H. (2020). Overview and future perspectives of nitrifying bacteria on biofilters for recirculating aquaculture systems. *Reviews in Aquaculture*, *12*(3), 1478–1494.

Sanders, W. T. M., Geerink, M., Zeeman, G., & Lettinga, G. (2000). Anaerobic hydrolysis kinetics of particulate substrates. *Water Science and Technology*, *41*(3), 17–24.

Sanders, W. T., Veeken, A. H. M., Zeeman, G., & Van Lier, J. B. (2003). Analysis and optimisation of the anaerobic digestion of the organic fraction of municipal solid waste. *Biomethanization of the Organic Fraction of Municipal Solid Waste. London: IWA Publishing*, 63–88.

Schomburg, D., & Stephan, D. (1997). Adenosine kinase. In *Enzyme Handbook 13* (pp. 663–667). Springer.

Shi, L., Du, R., & Peng, Y. (2019). Achieving partial denitrification using carbon sources in domestic wastewater with waste-activated sludge as inoculum. *Bioresource Technology*, *283*, 18–27.

Shi, L., Du, R., Peng, Y., & Li, Y. (2020a). Simultaneous carbon reutilization for primary sludge and stable nitrite production in a hydrolytic acidification coupled with partial denitrification system to treat nitrate contaminant. *Bioresource Technology*, *318*, 124062.

Shi, L., Du, R., Peng, Y., & Li, Y. (2020b). Successful establishment of partial denitrification by introducing hydrolytic acidification of slowly biodegradable organic matter. *Bioresource Technology*, *315*, 123887.

Shnel, N., Barak, Y., Ezer, T., Dafni, Z., & van Rijn, J. (2002). Design and performance of a zero-discharge tilapia recirculating system. *Aquacultural Engineering*, *26*(3), 191–203.

Soares, M. I., Brenner, A., Yevzori, A., Messalem, R., Leroux, Y., & Abeliovich, A. (2000). Denitrification of groundwater: Pilot-plant testing of cotton-packed bioreactor and post-microfiltration. *Water Science and Technology*, *42*(1–2), 353–359.

Sorokin, D. Y. (2005). Is there a limit for high-pH life? *International Journal of Systematic and Evolutionary Microbiology*, *55*(4), 1405–1406.

Stief, P. (2001). Influence of sediment and pore-water composition on nitrite accumulation in a nitrate-perfused freshwater sediment. *Water Research*, *35*(12), 2811–2818.

Stryer, L. (1988). *Biochemistry, 3'a ed.; W. H*. Freeman and Co.: New York.

Su, X.-L., Tian, Q., Zhang, J., Yuan, X.-Z., Shi, X.-S., Guo, R.-B., & Qiu, Y.-L. (2014). Acetobacteroides hydrogenigenes gen. Nov., sp. Nov., an anaerobic hydrogen-producing bacterium in the family Rikenellaceae isolated from a reed swamp. *International Journal of Systematic and Evolutionary Microbiology*, *64*(Pt_9), 2986–2991.

Suri, N., Zhang, Y., Gieg, L. M., & Ryan, M. C. (2021). Denitrification biokinetics: Towards optimization for industrial applications. *Frontiers in Microbiology*, *12*, 610389.

Turcios, A. E., & Papenbrock, J. (2014). Sustainable treatment of aquaculture effluents—What can we learn from the past for the future? *Sustainability*, *6*(2), 836–856.

Turk, O., & Mavinic, D. S. (1987). Benefits of using selective inhibition to remove nitrogen from highly nitrogenous wastes. *Environmental Technology*, *8*(1–12), 419–426.

Van Loosdrecht, M. C., & Henze, M. (1999). Maintenance, endogeneous respiration, lysis, decay and predation. *Water Science and Technology*, *39*(1), 107–117.

Van Rijn, J., Tal, Y., & Schreier, H. J. (2006). Denitrification in recirculating systems: Theory and applications. *Aquacultural Engineering*, *34*(3), 364–376.

van Spanning, R. J., Richardson, D. J., & Ferguson, S. J. (2007). Introduction to the biochemistry and molecular biology of denitrification. In *Biology of the nitrogen cycle* (pp. 3–20). Elsevier.

Vavilin, V. A., Fernandez, B., Palatsi, J., & Flotats, X. (2008). Hydrolysis kinetics in anaerobic degradation of particulate organic material: An overview. *Waste Management*, *28*(6), 939–951. https://doi.org/10.1016/j.wasman.2007.03.028

Vavilin, V. A., Rytov, S. V., & Lokshina, L. Y. (1996). A description of hydrolysis kinetics in anaerobic degradation of particulate organic matter. *Bioresource Technology*, *56*(2–3), 229–237.

Veeken, A., & Hamelers, B. (1999). Effect of temperature on hydrolysis rates of selected biowaste components. *Bioresource Technology*, *69*(3), 249–254.

Wang, J., & Chu, L. (2016). Biological nitrate removal from water and wastewater by solid-phase denitrification process. *Biotechnology Advances*, *34*(6), 1103–1112.

Wang, L., Li, G., & Li, Y. (2019). Enhanced treatment of composite industrial wastewater using anaerobic-anoxic-oxic membrane bioreactor: Performance, membrane fouling and microbial community. *Journal of Chemical Technology & Biotechnology*, *94*(7), 2292–2304.

Warneke, S., Schipper, L. A., Bruesewitz, D. A., McDonald, I., & Cameron, S. (2011). Rates, controls and potential adverse effects of nitrate removal in a denitrification bed. *Ecological Engineering*, *37*(3), 511–522.

Wen, Z., Liao, W., & Chen, S. (2004). Hydrolysis of animal manure lignocellulosics for reducing sugar production. *Bioresource Technology*, *91*(1), 31–39.

White, D., Drummond, J. T., & Fuqua, C. (1995). *The physiology and biochemistry of prokaryotes*.

Wilderer, P. A., Jones, W. L., & Dau, U. (1987). Competition in denitrification systems affecting reduction rate and accumulation of nitrite. *Water Research*, *21*(2), 239–245.

Xu, Z., Shao, L., Yin, H., Chu, H., & Yao, Y. (2009). Biological denitrification using corncobs as a carbon source and biofilm carrier. *Water Environment Research*, *81*(3), 242–247.

Yang, S., Wang, Z., Han, Z., & Pan, X. (2019). Performance modelling of seawater electrolysis in an undivided cell: Effects of current density and seawater salinity. *Chemical Engineering Research and Design*, *143*, 79–89.

Yogev, U., & Gross, A. (2019). Reducing environmental impact of recirculating aquaculture systems by introducing a novel microaerophilic assimilation reactor: Modeling and proof of concept. *Journal of Cleaner Production*, *226*, 1042–1050.

Yu, J., Wang, Y., Xiao, Y., Li, X., Zhou, L., Wang, Y., Du, T., Ma, X., & Li, J. (2021). Investigating the effect of nitrate on juvenile turbot (Scophthalmus maximus) growth performance, health status, and endocrine function in marine recirculation aquaculture systems. *Ecotoxicology and Environmental Safety*, *208*, 111617.

Zavarzin, G. A. (1986). Trophic relationships in methanogenic consortium. *Izvestia AN SSSR, Serie Biology*, *3*, 341–360.

Zhang, G., Yang, J., Liu, H., & Zhang, J. (2009). Sludge ozonation: Disintegration, supernatant changes and mechanisms. *Bioresource Technology*, *100*(3), 1505–1509.

Zhang, P., Zhang, G., & Wang, W. (2007). Ultrasonic treatment of biological sludge: Floc disintegration, cell lysis and inactivation. *Bioresource Technology*, *98*(1), 207–210.

Zhang, Y., Zhang, P., Zhang, G., Ma, W., Wu, H., & Ma, B. (2012). Sewage sludge disintegration by combined treatment of alkaline+ high pressure homogenization. *Bioresource Technology*, *123*, 514–519.

Zohar, Y., Tal, Y., Schreier, H. J., Steven, C. R., Stubblefield, J., & Place, A. R. (2005). Commercially feasible urban recirculating aquaculture: Addressing the marine sector. In *Urban aquaculture* (pp. 159–171). CABI Publishing Wallingford UK.