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Aerobic polishing of liquid digestate for the preparation of hydroponic fertiliser

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Master of Engineering in Chemical Engineering

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Abstract

Nitrogen and phosphorus form the backbone of most lifeforms on earth, however, improper management of these nutrients has devastating effects on the environment. These effects can be lessened by the use of the anaerobic digestion process, which provides methane-rich biogas and nitrogen-rich liquid biofertiliser. The use of liquid digestate (LD) in hydroponics can be problematic given that these systems are prone to microbial contamination. Thus, minimising the organic carbon present in the digestate before nitrification would make it ideal for hydroponic use.

By investigating the effect of pH on the use of a biological aerobic polishing unit for the removal of organic carbon with the retention of nitrogen and, to some extent phosphorus, the repeat-batch experiments displayed high levels of organic carbon removal at all investigated pH values (6, 7, and 8). Although ammonium losses were observed at pH 6 and 7, at pH 8, approximately 27% of the ammonium was recovered. Comparatively, the continuous process, wherein different three hydraulic retention times were investigated, performed better than the repeat-batch experiments as the TOC in the effluent was <100 mg L^{-1} which accounts for 90% of TOC removal from the LD. In this process, approximately 53% of the ammonium was recovered with the ammonium concentration in the polished product being >220 mg L^{-1} with the continuous addition of digestate. Excluding the nitrate, the nitrogen content in the polished product surpasses that of a standard Hoagland medium ($\approx$ 210 mg L^{-1}).

Findings from the metagenomic analysis of the biofilm indicate that a large fraction of the bacteria present in the biofilm are heterotrophic, hence the rapid decrease in TOC. The presence of other microorganisms responsible for the rapid uptake of phosphorus (*Gemmatimonas* spp.), nitrate assimilation (*Aquamicrobium* spp.) and nitrogen acquisition (*Luteimonas* spp.) are also documented in the findings. The combined effect of these microorganisms is the driving force behind the rapid carbon removal and nitrogen recovery seen in the findings.

This study provides a novel approach to the preparation of liquid digestate for hydroponic systems, which is not only more efficient but also minimises the risk of contamination in downstream processes, which will ultimately improve crop yield and quality. Although

phosphate losses were observed throughout all experiments, the phosphate levels can be supplemented prior to hydroponic use. The continuous process provides a greater deal of nutrient recovery, while simultaneously converting a large fraction of the organic carbon present in the liquid digestate and is the suitable method for the implementation of a circular economy.

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Publication of Work

The author has submitted the major findings of this study for publication in MDPI - Water. The first page of the publication is included in Appendix C.

Contents

1 Introduction

Nutrients form the basis for all life on earth and are integral to the continued growth and development of plants, animals, and humans. The nutrients in question – nitrogen, phosphorus and carbon exist in cycles which ensure that they are assimilated at the necessary points without causing damage to the environment. The carbon cycle is the exchange of carbon between different reservoirs on the earth's surface (Fung, 2003). The CO_2 in the atmosphere contributes to climate change by adjusting the concentration of water vapour in the atmosphere which causes either greenhouse warming or cooling. Burt (2013) states that second to the carbon cycle, the nitrogen cycle is the most important cycle to living organisms and is comprised of five main steps namely fixation, assimilation, ammonification, nitrification, and denitrification (Burt, 2013). Most of the steps in the cycle are carried out by microorganisms – e.g., plants absorb nitrogen from the soil and are then consumed by humans and animals; when the bodies decompose, the absorbed nitrogen is returned to the land and the water for the cycle to recommence (Prasad *et al*, 2017).

When these nutrients exist in quantities that surpass those that can be captured in their respective cycles, they cause damage to the environment. Bodies of water have been damaged by eutrophication which is the process wherein excess plant nutrients, primarily nitrogen and phosphorus lead to the occurrence of algal blooms (Istvánovics, 2009). Eutrophication also reduces the amount of dissolved oxygen in water and, reduces biodiversity and harms aquatic life. Runoff of these nutrients from agriculture produces leachate which pollutes groundwater sources and reduces the overall amount of potable water available to people. Increased amounts of CO_2 in the atmosphere also pose a danger to marine ecosystems due to ocean water acidification (Kroeker *et al*, 2013). This occurs via the chemical diffusion of carbon dioxide into the ocean waters which releases carbonic acid and disrupts the pH (Kroeker *et al*, 2013). The Haber-Bosch process, which is the primary method used to produce the ammonia used in fertilisers, is highly energy intensive and accounts for approximately 1.8% of global carbon emissions (Mohamed & Bicer, 2021). Therefore it is imperative to lessen, if not eradicate, the amount of additional synthetic fertiliser, specifically nitrogen, that is required in fertilisers and to avoid processes which have negative impacts on the environment.

These concerns can be alleviated by the use of anaerobic digestion, where organic waste is decomposed by microorganisms in the absence of oxygen (Achinas, Achinas & Euverink, 2020). The anaerobic digestion process is one of the most viable methods of sustainable biogas production from waste. Another by-product is solid digestate which is the nutrient-dense solid fraction of the digestion effluent which ideal for supplementing soil minerals

(Achinas *et al*, 2020). Liquid digestate, yet another by-product of the anaerobic digestion process, is a nutrient-rich liquid that has many applications as a sustainably produced biofertiliser (Akhiar, Battimelli, *et al*, 2017a; Akhiar, Guilayn, *et al*, 2021a). Although soil production is the prevalent method of crop production, an increase in population has also affected the availability of arable land. Soilless agriculture, specifically hydroponic systems alleviates this concern seeing as they are suspended growth systems that make use of nutrient-rich liquid mediums like the Hoagland's medium (Hoagland & Arnon, 1950).

Liquid digestate is rich in ammonium nitrogen and since plants prefer nitrate to ammonium (Rooyen & Nicol, 2021; Rooyen & Nicol, 2022) a nitrification step needs to be added to the process. Nitrification is the oxidation of ammonium to nitrate conducted by chemoautotrophic nitrifiers (Ergas & Aponte-Morales, 2014). The nitrifiers feed on inorganic sources of carbon such as CO_2 (Sage, 2008) while the heterotrophic bacteria present in digestate feed on organic matter (Theradimani & Ramamoorthy, 2022). Although hydroponic systems have many advantages these systems are also prone to microbial contamination (Saldinger *et al*, 2023). The organic matter present in the LD can feed heterotrophic organisms that adhere to plant roots and inhibit nutrient uptake (Patwardhan *et al*, 2023), or can also lead to root rot (Sutton *et al*, 2006). Therefore, it is imperative to employ a system aimed at removing organic carbon while retaining the nutrients required for plant growth.

Studies conducted on the use of liquid digestate in hydroponic systems indicate the feasibility of this process while providing competitive rates as compared to commercial fertilisers (Ronga *et al*, 2019; Bergstrand, Asp & Hultberg, 2020; Weimers *et al*, 2022). However, the pre-hydroponic steps conducted in these studies, i.e. the anaerobic digestion followed by nitrification can take more than 100 days (Lind *et al*, 2021; Ronga *et al*, 2019). This is done in order to minimise the organic carbon content prior to hydroponic use, however, this study investigates the optimisation of this process by making use of an aerobic polishing unit aimed at removing the organic carbon present in organic fertilisers while retaining nutrients, primarily nitrogen and phosphorus, in the medium. Polishing is used as a final treatment to anaerobically digested wastewater to improve the quality of the effluent (Arthur *et al*, 2022) however in this design the polishing treatment is modified to remove organic carbon whilst retaining some nitrogen and phosphorus. This study provides a novel approach to the use of aerobic polishing for nutrient recovery.

In this study, the results from the implementation of an aerobic polishing unit to oxidize the organic carbon present in liquid digestate, while the nutrients nitrogen and phosphorus are retained are reported. The effect of pH was investigated in an aerated, temperature and pH-controlled polishing unit modified from the design used by Swart,

Roux, *et al* (2020) and Swart, Brink & Nicol (2022). A biofilm was allowed to accumulate in the reactor throughout all the experiments, however, it remains nearly constant due to the removal of the shedded biomass with the effluent. The repeat-batch experiments and the results thereof were used to design the continuous implementation of this same system. It was observed that ammonium contributed the largest amount of nitrogen to the retained nitrogen and made up the rest. A comparison of repeat-batch and continuous operation showed that continuous operation of this process is ideal for the removal of significant levels of organic carbon while large amounts of nitrogen are retained which fulfils the overarching objective of the study. It will also be shown through discussion that the results from these experiments form the basis of a system that – with proper implementation – will alleviate the issues discussed previously.

2 Theory (Literature)

2.1 Importance of Nitrogen and Phosphorus

Nitrogen and phosphorus are essential nutrients for plant growth and formulate the basis of integral plant networks. Nitrogen is responsible for plant metabolism and resource allocation and is vital to plant growth and development (Yousaf *et al*, 2021). It is taken up in the form of nitrate (NO_3^-) or ammonium (NH_4^+), and is an essential constituent of amino acids used to build plant protein and chlorophyll which enables plants to photosynthesise (Leghari *et al*, 2016). This nutrient is immensely beneficial in agriculture as it contributes to enhanced crop yield and food quality (Leghari *et al*, 2016).

Phosphorus is commonly referred to as the limiting nutrient in most plant systems and performs specialised functions in the metabolism, structure and plant reproductive system that cannot be performed by another other nutrient (Day & Ludeke, 1993). The concentration thereof is usually lower than nitrogen, potassium and calcium and can give rise to unsatisfactory and stunted growth (Day & Ludeke, 1993). It is also a major component of energy-rich compounds such as adenosine triphosphate which is crucial to cellular energy transport and is also a constituent of DNA and RNA which are vital for reproduction and protein synthesis (Malhotra *et al*, 2018).

The importance of these nutrients cannot be understated, however, the production of these minerals required to meet the demand for crops has a devastating impact on the environment. Although nitrogen is easily available in the atmosphere (as inert N_2 gas), this form is unusable to plants incapable of fixating atmospheric nitrogen to a plant-ready form such as ammonium or nitrate (Leghari *et al*, 2016). This therefore constitutes the need for an external supply of nitrogen to the plants which in most instances needs to be synthetically produced in order to meet the demand of an ever-increasing population (Rouwenhorst *et al*, 2021). Nitrogen-based fertilisers largely use ammonium nitrogen which is synthetically produced using the Haber-Bosch process which is highly energy intensive and thus has drastic effects on the environment (Mohamed & Bicer, 2021).

Although the demand for phosphate is largely attributed to fertiliser production, there is a steady growth of other applications in the chemical industry, i.e. for the production of flame retardants, soft drinks and some pharmaceuticals (de Boer, Wolzak & Slootweg, 2019). Phosphate is mined from phosphate rock and treated with sulphuric acid to produce superphosphate which can be further treated to produce ammonium polyphosphate (Green, 2015). Although there are some studies that have reported the presence of radionuclides in phosphate rock, chemical analysis of these compounds has shown that they

are below tolerable limits (Fayiga & Nwoke, 2016).

However, along with the production, the spillage of these nutrients can also have detrimental effects on the environment. Accumulation of phosphate in soil can lead to soil erosion and high phosphate concentrations in runoff water (Bhattacharya, 2019). In addition to nitrogen, when introduced to bodies of water these nutrients can cause eutrophication which causes algal blooms and impacts the quality of the water (Istvánovics, 2009).

2.2 Organic Waste

Organic waste is the main contributor of these nutrients and without proper management, can have detrimental environmental implications. This form of waste mainly consists of food waste, agricultural waste, and human waste (Patel *et al*, 2021). It can also be defined as any form of waste that is capable of being decomposed either aerobically or anaerobically (Vis, 2017). Patel *et al* (2021) states that approximately a third of all food produced globally ends up as waste which amounts to 1.3 billion metric tons of waste and according to Pace *et al* (2018) over 46$\tilde{\%}$ of global solid waste is organic. It has also been predicted that the global rate of waste production will triple by 2100 (Mishra *et al*, 2023).

In addition to organic carbon, organic waste contains a great collection of nutrients such as nitrogen (N), potassium (K) and phosphorus (P) (Walling, Babin & Vaneeckhaute, 2019). Various methods exist for preserving these nutrients however, improper management of this organic waste also has detrimental effects on the environment. Landfills and incineration are existing methods of organic waste management which have been identified to be harmful to the environment.

The most common method of treating organic waste is composting which is the process wherein these solids are biologically degraded under controlled conditions (Dentel & Qi, 2014). However, this process is not without its drawbacks as it increases CO_2, CH_4, N_2O and NH_3 emissions (Oliveira *et al*, 2017). Composting can also be extended to vermicomposting which is the degradation of organic matter by earthworms and their respective gut-borne microorganisms (Soobhany, 2019).

A benefit of these streams is that they have the potential to be valorised into sustainable bio-materials. The composting and vermicomposting processes produce biofertilisers that can be used for soil remediation and minimise the use of external sources of nutrients thereby promoting nutrient recycling (Hussain, Paulraj & Nuzhat, 2022). The waste can

also be incinerated to produce some biogases, which unfortunately has an unfavourable impact on the environment due to the contribution of this process to the accumulation of greenhouse gases (Soobhany, 2019). Another valorisation method is anaerobic digestion which produces both methanated biogas and nutrient-rich digestate which has vast uses in agriculture, from soil-based to soil-less horticulture (Y Hu, Bassi & Xu, 2020). This method offers a viable solution to the issues at hand while contributing to a circular economy by promoting nutrient recycling.

2.3 Anaerobic Digestion

Anaerobic digestion, is the process wherein organic matter is broken down by microorganisms in the absence of oxygen (Achinas *et al*, 2020). Waste materials are used for this process which has been used for the past 2000 years (Negi *et al*, 2019) primarily produces biogas containing carbon dioxide and methane in a 60/40 ratio (Y Hu *et al*, 2020; Gonde *et al*, 2023). The methane present in this renewably produced biogas is mostly used for the generation of heat and energy and offers an electrical efficiency of 33% and a thermal efficiency of 45% (Appels *et al*, 2011). Renewable energy and fuel generation from biogas has since had a large increase in countries north of the equator namely, China, Germany and Sweden which are members of the IEA Bioenergy Task 37. Findings from the 2022 report show that China and Germany have the highest number of biogas plants among the IEA Bioenergy Task 37 members with 100,000 and 10,000 plants respectively (Bioenergy, 2022).

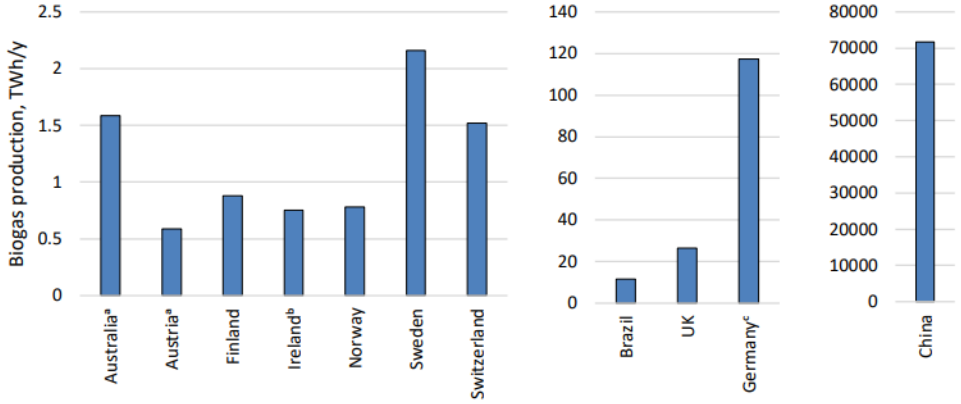

Figure 2.2. Annual biogas production in selected IEA Bioenergy Task 37-member countries

[a] Calculated from installed capacity
[b] Calculated from 80% of installed capacity for electricity production, assuming 35% efficiency
[c] Calculated assuming 35% efficiency in electricity production

Figure 1: IEA Bioenergy Task 37 biogas production by member countries (Bioenergy, 2022)

In recent years Germany and Sweden have had the largest market for biomethane and

in Sweden, more than half of the biogas is used for vehicle fuel (Bioenergy, 2022). The inclusion of financial support systems by means of feed-in tariffs, investment grants, and tax exemptions in these countries has increased the amount of biogas utilised (Bioenergy, 2022).

Another valuable by-product of the anaerobic digestion process is the nutrient-rich digestate which can be found as a slurry, solid or liquid. Rich in ammonium nitrogen as well as other plant-ready essential nutrients, the digestate is generally used in agriculture as a biofertilizer. Although China is a larger biogas producer than Germany, for the size of the country it has quite a substantial production capacity. It therefore follows that Germany is the largest biogas producer in Europe and has been used as a model for further implementation of biogas production. The digestate produced from biogas production in European countries approximated at 56 million tonnes per year (Stoyanov, 2016), has primarily been used as a biofertilizer, which has not only allowed the agricultural industry to behave more sustainably, but it has also contributed to the EU's commitment to reduce greenhouse gas emissions by 55% by 2030 (European Biogas Association, 2019). Less than 3% of digestate has undergone further processing to produce specialised products such as pellets or growth media constituents and Stoyanov (2016) illustrated that the digestate is treated differently across the EU where in central Europe, it is separated into its solid and liquid fraction and the solid fraction is used in agricultural land, whereas in Scandinavia, the digestate slurry is directly used in agriculture. Anaerobic digestion is also favoured for the mineralisation of nutrients that are vital to plant growth and a study by Reuland *et al* (2022) shows that the mineralisation of nitrogen in digestate ranges from 21% to 39% (European Biogas Association, 2019).

The solid fraction of the digestate consists of all the undigested feedstock and is still a nutrient-rich substrate. The solids are generally high in fibre the undigested material consists of lignin, cellulose and microbial biomass (Farm Energy, 2019). Farm Energy (2019) also states some of the various applications of solid digestate include but are not limited to, organic fertilisers, livestock bedding and compost among other valuable by-products. The high carbon content of the solid digestate makes it viable for soil augmentation since it adds to the organic matter present in the soil and improves its water-carrying capacity. Recent research indicates that solid digestate can also be used as a renewable construction material, especially in the production of medium-density fibreboard and wood/plastic composites (Farm Energy, 2019). The solid fraction of digestate has also been successfully used for the production of lignocellulosic enzymes and is shown in the work by Musatti *et al* (2017). Seeing as solid digestate is such an inexpensive substrate, the research into these enzymes can also be used to produce valuable by-products from the digestate and increase the biodegradation of lignocellulosic materials. One of the other valuable products from the anaerobic digestion process is the

liquid digestate which contains all the nutrients extracted from the feedstock and is more nutrient-dense than its solid counterpart.

2.4 Liquid Digestate

The liquid fraction of digestate also known as liquid digestate (LD) is commonly used as an organic fertiliser or fertiliser supplement due to its high nitrogen and phosphorus content which are vital for plant growth. Systemic (2017) gives various examples of experimental exploration where LD has been successfully used for plant growth and which shows promise in the field of sustainable agriculture. It also accounts for 94% of the digestate produced in Germany is used as a biofertilizer (Stoyanov, 2016) and is preferred by farmers due to its ease of handling and potential to mix with herbicides.

2.4.1 Composition of liquid digestate

Due to its applications in the agricultural sector, it is important to know the composition of the LD to be able to predict things like crop yield and nutrient content as well as ascertain the environmental and health impacts of the use of digestate as a fertiliser.

The composition of LD is not uniform and has a high variation in chemical, physical and biological characteristics which mainly depend on the feedstock, length of digestion and the methods used to separate the solid digestate from the liquid digestate. It is usually measured in terms of total organic carbon (TOC), inorganic carbon (IC), biological oxygen demand (BOD), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), ammonium (NH_4^+) and the carbon to nitrogen ratio (C/N). However, even though BOD is a common measurement in the wastewater treatment sector, it is not commonly measured in liquid digestate analysis partly due to the time it takes to complete the measurement and the fact that it is a fraction of the TOC and COD. Akhiar, Guilayn, *et al* (2021b) conducted a more in-depth analysis of the LD where the micronutrients present in the digestate namely sodium (Na^+), potassium (K^+), chlorine (Cl^-), phosphate (PO_4^{3-}) and sulphate (SO_4^{2-}) were measured.

Table 1 and Table 2 show a summary of the various feed stocks and their associated nutritional compositions. The method of separation from the solid digestate, if applicable, is also shown in Table 1 and Table 2 to illustrate the impact of separation on the composition of the final product.

Feedstock	TOC (g L^{-1})	IC (g L^{-1})	COD (g L^{-1})	TKN (g L^{-1})	NH_4^+ (g L^{-1})	C/N	Reference
Sludge (36%), manure (16%), crop residues (7%), fats (22%), blood (7%), pet food (5%), fruits and vegetables (7%)	0.47	2.32	1.50	-	-	0.2	(Akhiar, Battimelli, *et al*, 2017b)
Poultry manure	2.74	-	-	-	4.71	4.09	(Häfner, Hartung & Möller, 2022)
40% slaughterhouse waste, 45% waste from food processing industries, 15% organic waste	0.5	2.3	47.3	6.5	2.6	1.1	(Akhiar, Guilayn, *et al*, 2021b)
Bio-waste (100%)	1.45	2.25	4.36	-	-	0.7	(Akhiar, Battimelli, *et al*, 2017b)
75% manure (cow and pig), 25% industrial waste	1.5	2.7	9.2	4.7	1.3	2.5	(Akhiar, Guilayn, *et al*, 2021b)

Table 1: Summary of the compositions of liquid digestate found in literature

Feedstock	TOC (g L^{-1})	IC (g L^{-1})	COD (g L^{-1})	TKN (g L^{-1})	NH_4^+ (g L^{-1})	C/N	Reference
Liquid cow manure (50%), cow manure (2%), poultry manure (8%), crop residues (18%), cereal residues (11%), fruits and vegetables (11%)	3.16	3.16	2.21	-	-	0.9	(Akhiar, Battimelli, *et al*, 2017b)
Pig manure (64%), cereal residues (9%), crop residues (5%), fruits and vegetables (16%) and sludge (5%)	1.10	2.94	3.20	-	-	0.6	(Akhiar, Battimelli, *et al*, 2017b)
Undigested cattle slurry	2.50	-	-	-	1.92	11.5	(Häfner, Hartung & Möller, 2022)
Crop residues (20%), fats (20–30%), grain wastes (10–15%), glucose (5–10%), sludge (10–12%), bio-waste and slaughterhouse waste (3–4%)	0.452	1.6	0.99	-	-	0.3	(Akhiar, Battimelli, *et al*, 2017b)

Table 2: Summary of the compositions of liquid digestate found in literature concluded

Table 1 and Table 2 show the effect of substrate on the nutrient content of the digestate. This also implies that specific nutrient requirements for certain plants can be partially fulfilled given the correct choice of substrate.

2.5 Hydroponics

Hydroponics is a method of growing plants which involves suspending the roots in nutrient-rich growth mediums such as the Hoagland solution (Hoagland & Arnon, 1950). These systems have recently gained a lot of traction due to their environmentally friendly benefits and reliability in terms of crop production (Sharma *et al*, 2018). With the rapid increase in global population, soilless agriculture will soon become a norm since in most installations it does not take up a lot of space and is easily modifiable. Seeing as the hydroponic system makes use of liquids, it is also possible to use liquid digestate in these systems to further promote nutrient recovery (Weimers *et al*, 2022; Bergstrand *et al*, 2020; Ronga *et al*, 2019). The previous section has shown that depending on the feedstock used, the LD can possess all the necessary nutrients for plant growth, however, in some instances, it may need to be supplemented given that the Hoagland medium is the standard for hydroponic growth mediums and fulfils the nutrient requirements of the plants (Hoagland & Arnon, 1950).

2.5.1 Microbial contamination in hydroponic systems

Owing to the fact that hydroponic systems are pivotal to the production of consumable crops, it is essential to understand the ways in which microbial contamination can occur in these systems. Microbial contamination in a water-borne system also accounts for the rapid spread of the pathogens, which in the case of a hydroponic system would infiltrate plant cells then be used for human consumption (Saldinger *et al*, 2023). The presence of bacteria in plant systems has the potential to form a biofilm that adheres to plant roots (Patwardhan *et al*, 2023). These biofilms can be beneficial to plant systems or inhibit nutrient uptake while increasing the risk of pathogen spread to humans (Patwardhan *et al*, 2023).

Pythium root rot is a phenomenon observed in hydroponic systems, which threatens the productivity of the plants, and is often linked to environmental stress factors (Sutton *et al*, 2006). Although there may be various strains of this disease, they have the same effect which ultimately inhibits nutrient uptake to the plants (Sutton *et al*, 2006).

2.5.2 Use of LD in hydroponic systems

It is imperative to evaluate previous studies which utilised LD as a hydroponic growth medium in order to determine the feasibility of the approach and provide insight into areas that can be improved upon. In this section a brief summary of the relevant studies, showcasing the different types of produce that can be cultivated using this method, are discussed along with the outcomes and main findings of the relevant studies.

Ronga *et al* (2019) conducted a study into the use of liquid digestate for the cultivation of baby leaf lettuce (*latuca sativa L.*) by using nine different combinations of substrate and fertiliser for the hydroponic mediums. The results from the study show that digestate can be used for the growth of baby lettuce leaves in hydroponic systems. This study showed the possibility of using liquid digestate to produce high-yielding crops with low microbiological contaminants (Ronga *et al*, 2019).

Another study was conducted by Bergstrand *et al* (2020) where Pak Choi was cultivated in a hydroponically controlled climate. In this study, the organic mediums, consisting of varying concentrations of liquid digestate, were compared to commercial mineral fertilisers. Although the mineral fertilisers generally outperformed the organic fertilisers, Bergstrand *et al* (2020) found that the quality of the produce, with respect to vitamins, was improved in the crops that were grown using the organic solutions. Bergstrand *et al* (2020) also concluded that organic solutions containing liquid digestate are valuable for plant growth.

Pak Choi was also used by Weimers *et al* (2022) to investigate the feasibility of digestate as a sole nutrient source in hydroponic systems. A peat-based growing medium was used to assess the nutrient availability of the digestates at a macro and micro-nutrient level with a specific focus on phosphorus (P) and sulphur (S). The effect of amending the digestate by adding the lacking nutrients was also investigated in this study. Similar yields to commercial mineral fertiliser were obtained from the digestates that were supplemented with additional nutrients (Weimers *et al*, 2022). Weimers *et al* (2022) concluded that the use of nitrified digestate in hydroponic systems is a promising way to recycle organic nutrients from waste streams in systems that do not have to adhere to strict organic protocols.

However, a study conducted by Mupambwa *et al* (2019) showed that biogas digestate is not suitable for the hydroponic growth of tomatoes. The effects of the biogas digestates on crop phytotoxicity, as well as their fertiliser potential, were investigated in this study and it was found that the produce grown with biogas digestates supplemented with mineral fertilisers resulted in a 275 % lower yield as compared to the control treatment

(Mupambwa *et al*, 2019). Although the results of this study suggest that cow-based digestate may not be suitable for hydroponic tomato growth, there are still various plants that can be used for more studies for this application.

Similarly, in the study conducted by Kechasov *et al* (2021) on the hydroponic cultivation of tomatoes using nitrified pig manure based liquid digestate, it was found that although the digestate-grown crops had lower growth rates, they had a higher average fruit size which resulted in a comparable yield to commercial fertilisers. Kechasov *et al* (2021) ultimately concluded that the use of LD as a hydroponic fertiliser is possible with an additional nutrient supply.

The studies discussed above highlight the applications of LD in agriculture and furthermore illustrate its feasibility as a sustainably produced organic fertiliser. All of the LD that was supplied to the hydroponic units was first nitrified seeing as nitrate is the preferred form of nitrogen for these crops and to avoid the possibility of ammonium toxicity (Rooyen & Nicol, 2021; Rooyen & Nicol, 2022). The findings herein also indicate that treated LD is a suitable fertiliser for leafy greens, and with some additional nutrients can also be used for the growth of tomatoes. It is also important to note that these findings are applicable to the use of liquid digestate hydroponic systems and cannot be extended to soil applications which have different outcomes.

2.5.3 Preparation of LD for hydroponic use

Although various studies have been conducted on the use of liquid digestate for hydroponic use, not many studies detail the preparation of the liquid digestate as many studies made use of externally supplied digestate which was then characterised prior to use in the hydroponic systems. These processes are viable for standalone systems which utilise organic fertilisers in hydroponic systems, however, for a fully integrated system, it is ideal to have an indication of the duration of each step in the process to ascertain the effect on downstream processes.

In the study conducted by Weimers *et al* (2022), the digestate used in the experiments was sourced from a municipal biogas plant (Karpalund Biogas Plant, Kristianstad, Sweden) and it was also documented that the anaerobic digestion ran for 40 days at 55 °C. Following the digestion, the liquid fraction of the digestate was separated and placed into a nitrification unit for 51 days (Weimers *et al*, 2022). The digestate used by Bergstrand *et al* (2020) was sourced from the same biogas plant as Weimers *et al* (2022), only in this instance, after separation the digestate was only nitrified for two weeks.

2.6 Aerobic Digestion

Aerobic digestion is the degradation of organic matter in the presence of oxygen which is usually carried out by microbial populations and nitrate, phosphate and carbon dioxide are usually produced as a result of this process (Shabbir *et al*, 2021a). This process, which at times can also refer to composting, is mostly utilised for the treatment of biological waste (Albihn, 2009) and is also used for biological wastewater treatment more so in activated sludge systems and attached growth systems. Microbial growth in aerobic digesters is typically high and therefore has shorter retention times (Shabbir *et al*, 2021a). Aerobic digestion has also been used as a pretreatment to reduce the organic content of effluent and this mostly occurs in the mesophilic temperature range (25 °C–35 °C) which is optimal for the aerobic microbes (Shabbir *et al*, 2021a). The aerobic microbes thrive in pH values ranging between 6 and 8, therefore making pH control important in order to optimise the reduction of organic matter.

2.6.1 Wastewater polishing

Aerobic digestion is commonly used in wastewater treatment to minimise the presence of organic solids in the wastewater treatment process. This is also done by the use of wastewater polishing ponds which are mostly used to improve the effluents from anaerobic digestion sewerage treatment plants (Arthur *et al*, 2022). Wastewater polishing intends to reduce the amount of remaining suspended solids while improving the hygienic quality via the removal of faecal coliforms in the wastewater (Arthur *et al*, 2022). This method has also successfully been used for the revitalisation of oil-laden wastewater (Shabbir *et al*, 2021b; Liew *et al*, 2015).

2.6.2 Carbon removal in aerobic digestion

Conventional wastewater treatment and/or polishing decomposes all present nutrients to their simplest forms, CO_2 and N_2, by employing processes of decomposition and combinations of nitrification and denitrification (Shabbir *et al*, 2021a). However, the specific application of wastewater polishing for the specialised treatment of liquid digestate is yet to be documented thereby cementing this study as the first of its kind. The study aims to polish the LD by using aerobic digestion to eradicate the organic carbon while retaining nitrogen in the medium. Therefore understanding the ways in which aerobic digestion can be utilised for this purpose is crucial to the success of the study.

S Lee & J Lee (2015) states that one of the limitations of hydroponic systems is the rapid spread of pathogens and microbial growth which forms biofilm on plant roots subsequently preventing nutrient uptake. This can be alleviated by an aerobic digestion pretreatment step to reduce the organic content of the substrate if any which in the long run will promote healthy crop growth. Various methods can be used for this purpose which will be discussed below.

LQ Zhang *et al* (2022) made use of a membrane aerated bioreactor (MABR) to simultaneously remove nitrogen and carbon for low-strength municipal wastewater treatment. The MABR is believed to be capable of reducing COD, ammonia and inorganic nitrogen (TIN) to less than 50 mg L^{-1}, 5 mg L^{-1} and 15 mg L^{-1} respectively. Although the performance of the MABR was tested using synthetic municipal wastewater, the results obtained from the experiments showed great promise for the use of the MABR for simultaneous carbon and nitrogen removal (LQ Zhang *et al*, 2022).

A study was conducted by Pedros & Dobie (2006) where a submerged attached growth bioreactor was used for decentralised wastewater treatment in Massachusetts, Connecticut, Rhode Island and New Jersey. Pedros & Dobie (2006) states that the advantages of the SAGB include but are not limited to, a small volume requirement as well as the bypass of a downstream clarification unit in wastewater treatment. The data obtained from the five-year-long study showed that the SAGB is an effective process for the removal of biological nitrogen and all the effluent requirements were met for this system. However, no mention is made of the carbon removal efficiency of this system which should also be explored.

Magdum & V. (2019) made use of a high-rate moving bed biofilm reactor (MBBR) to optimise the carbon and nitrogen removal efficiency in wastewater treatment. The aim of the study was to evaluate the effect of organic loading on the carbon and nitrogen of the system. With a higher organic loading, the carbon and nitrogen reduction was improved to 87.8 % and 47.2 % respectively, which shows that an increase in the organic loading improves the removal efficiency (Magdum & V., 2019).

Table 3 below summarises a literature survey of the existing studies that target rapid organic carbon removal. A caveat of these systems, in line with our intended application is the simultaneous removal of nitrogen along with the carbon and therefore studies wherein the evaluated conditions promoted nitrification were excluded in the summary.

Reactor type	Surface loading rate (g COD m^{-2} day^{-1})	Organic loading rate (kg COD m^{-3} day^{-1})	HRT	Reference
Membrane Aerated Bioreactor MABR	19.4–25.9	-	3–4 h	(LQ Zhang *et al*, 2022)
Submerged Attached Growth Reactor SAGB	-	2.5 kg BOD_5 m^{-3} day^{-1}	3–5 h	(Pedros & Dobie, 2006)
Moving Bed Biofilm Reactor MBBR	4.9–24	1.21–4.59	2–3 h	(Magdum & V., 2019)
Aerobic Packed Bed Biofilm Reactor APBR	-	0.72–4.32	4–6.4 m^3 day^{-1} m^{-2}	(Dey & Mukherjee, 2010)
Moving Bed Biofilm Reactor MBBR	-	0.4–1.4	2.16–3.05 h	(Tomaszek & Grabas, 2007)

Table 3: Summary of the literature survey conducted on the existing processes for carbon removal using aerobic digestion where HRT refers to the hydraulic retention time

2.6.3 Nitrification

According to Ergas & Aponte-Morales (2014), nitrification can be described as the oxidation of ammonium to nitrite, then subsequently nitrate as is shown in Equation 1 below

$$NH_4{}^+ \longrightarrow NO_2{}^- \longrightarrow NO_3{}^- \tag{1}$$

The reactions are carried out in aerobic conditions by microbial organisms that are capable of oxidising ammonium and nitrite such as ammonia-oxidizing bacteria, ammonia-oxidizing archaea, nitrite-oxidizing bacteria (Ward, 2008). These organisms are also referred to as nitrifiers and are predominantly autotrophic meaning they are able to produce biomass from inorganic sources such as carbon dioxide and minerals (Sage, 2008). Dodds *et al* (2017a) states that nitrification is the only natural pathway that produces nitrogen within a system and nitrifiers are inherently chemoautotrophic and have lower rates than other nitrogen cycle processes. Their chemoautotrophic nature implies that they harvest energy from reduced molecules in the environment to produce biomass (Sage, 2008). There is, however, another group of microbes - the anammox organisms that are capable of directly oxidising ammonium to nitrogen gas (Ward, 2008).

Nitrification is an important process in agriculture which dominates the pathway of nitrogen flow and Subba Rao *et al* (2017) discovered that NO_3^- accounts for 95 % of nitrogen uptake. The process is very sensitive to changes in environmental conditions and in soil specifically, it can be affected by soil moisture, temperature, pH and rainfall among other factors. Subba Rao *et al* (2017) also states that the rate of nitrification in the soil is greatly accelerated by an increased pH and was found to reach its maximum at pH 8–9. Dodds *et al* (2017b) found that nitrification is also affected by the availability of ammonium, O_2 and organic carbon as well as light. According to Taslimi (2020) the optimum conditions for nitrification ential a pH of 7–8, protection from UV light *i.e* sunlight, temperature between 15–30 °C and high levels of O_2. These conditions apply to the growth of an aquatic biofilter that can be implemented in an aquaponic system to regulate nitrate levels in the system.

As stated, nitrification occurs under oxic conditions, however, in conditions of low oxygen it is coupled to denitrification which is the microbial process of converting nitrate and nitrite to nitrogen (N_2) and nitrous oxide (N_2O) in their gaseous forms (Skiba, 2008). The O_2 limitation causes the nitrifier to switch from aerobic respiration which produces nitrate, to anaerobic respiration where N_2O is used as an electron acceptor (Skiba, 2008).

In wastewater treatment, this is a valuable process due to the complete removal of nitrogen from the wastewater. The ammonium-rich wastewater is nitrified thus producing nitrite/nitrate which is subsequently denitrified in anoxic zones present in the system. Roy *et al* (2022) describe the denitrification reaction as shown in Equation 2

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2 \tag{2}$$

Although the is beneficial in wastewater treatment and the management of aquatic systems, it removes valuable nitrogen from soil-based systems and releases the greenhouse gasses N_2O and NO into the environment (Skiba, 2008).

3 Experimental

3.1 Medium and Medium Preparation

The liquid digestate used in the study was prepared according to the method described by Gonde *et al* (2023) such that the total solids in the medium are 5 % of the total mass. On the day of preparation, fresh cow manure was collected from the experimental farm on the Hillcrest Campus of the University of Pretoria, South Africa. The cow manure, used as the inoculum, along with IMBO®red spilt lentils and distilled water sourced from the University of Pretoria labs were the materials used in the digestate. The red lentils were chosen for their protein content, which during the digestion process would be broken down to ammonium which is the ideal since the objective of the study is to prepare a nitrogen-rich fertiliser (Gonde *et al*, 2023). Eight 1 L Schott bottles were prepared with each flask containing 600 mL of the digestion slurry containing 35.69 g of cooked red lentils, 100 g of fresh cow manure and 433.41 mL of distilled water - the slurry was blended to homogenise the mixture prior to digestion.

The specific quantities were added to ensure a 1:1 ratio of lentils to cow manure on a dry basis. The mixture was digested anaerobically in an incubator for 21 days at 35 °C and 150 rpm and the pH of the mixture was monitored daily and adjusted to a value of 7 using a 5M solution of NaOH and a 3M solution of HCl. The digestate was filtered using a fine mesh coffee filter to separate the solid undigested biomass from the liquid. The filtered liquid from all the bottles was homogenised and then refrigerated at 4 °C for further use. Analysis of the digestate indicated an average COD value of 12000 mg L^{-1} and an average ammonium concentration of 845 mg L^{-1}. 1.2 L of the process medium was prepared by diluting the liquid digestate by a factor of 4. The digestate was centrifuged for 10 minutes at 9000 rpm and 300 mL of the supernatant was added to 900 mL of distilled water to prepare the process medium. The centrifugation and dilution of the digestate were to minimize any suspended solids that would cause blockages in the piping of the reactor. The concentrations of the diluted digestate are shown in Table 4 along with the concentrations of the nutrients added which were calculated by multiplying each HRT with the relevant concentration.

		Concentration (mg day^{-1})		
Component	Concentration (mg L^{-1})	$\tau = 7.38$	$\tau = 3.18$	$\tau = 1.63$
COD	3500	511.88	1187.59	2319.42
TOC	600	87.75	203.59	397.61
NH_4^+	220	32.18	74.65	145.79
NO_3^-	24	3.51	8.14	15.9
PO_4^{3-}	23	3.36	7.8	15.24

Table 4: Concentrations of the diluted digestate and added nutrients at the different hydraulic retention times (τ = HRT in days) explored in the continuous experiments

3.2 Polishing Unit Design and Operation

The polishing unit was modified from the fermenter design used for the production of fumaric acid from *Rhizopus oryzae* by Swart, Roux, *et al* (2020) and Swart, Brink, *et al* (2022) which consists of a glass tube within a stainless-steel housing unit and has a liquid volume of 1.08 L and a gas volume of 0.380 mL. A polypropylene tube where the biofilm would grow, with a length of 386.5 mm and inner and outer diameters of 32 mm and 40 mm respectively, was located in the middle of the reactor and was notched using a scalpel to ensure adherence of the biofilm to the surface of the tube. Lab grade air containing 20% oxygen and 80% nitrogen was continuously sparged at a rate of 200 mL min^{-1} for all runs. The flow rate of the feed gas was controlled by an SLA5850 Brooks Mass Flow Controller (Hatfield, PA, USA). Temperature and pH were measured online using the Endress + Hauser CPS171 pH-probe (Gerlingen, Germany) and the gas and liquid phases present in the reactor were recycled to avoid concentration gradients (Swart, Brink, *et al*, 2022).

The pH was maintained at pH 6, 7 and 8 by using 0.5 mm marprene tubing with two 120U Watson-Marlow (Johannesburg, South Africa) pumps for the continuous addition of 1M NaOH and 1M HCl solutions. The temperature was maintained at 35 °C. The reactor was sampled daily in the same time interval and the samples were immediately stored at -40 °C to prevent any further digestion prior to analysis. A schematic representation of the reactor, modified from the configuration used by Swart, Roux, *et al* (2020) and Swart, Brink, *et al* (2022) is shown in Figure 2 below.

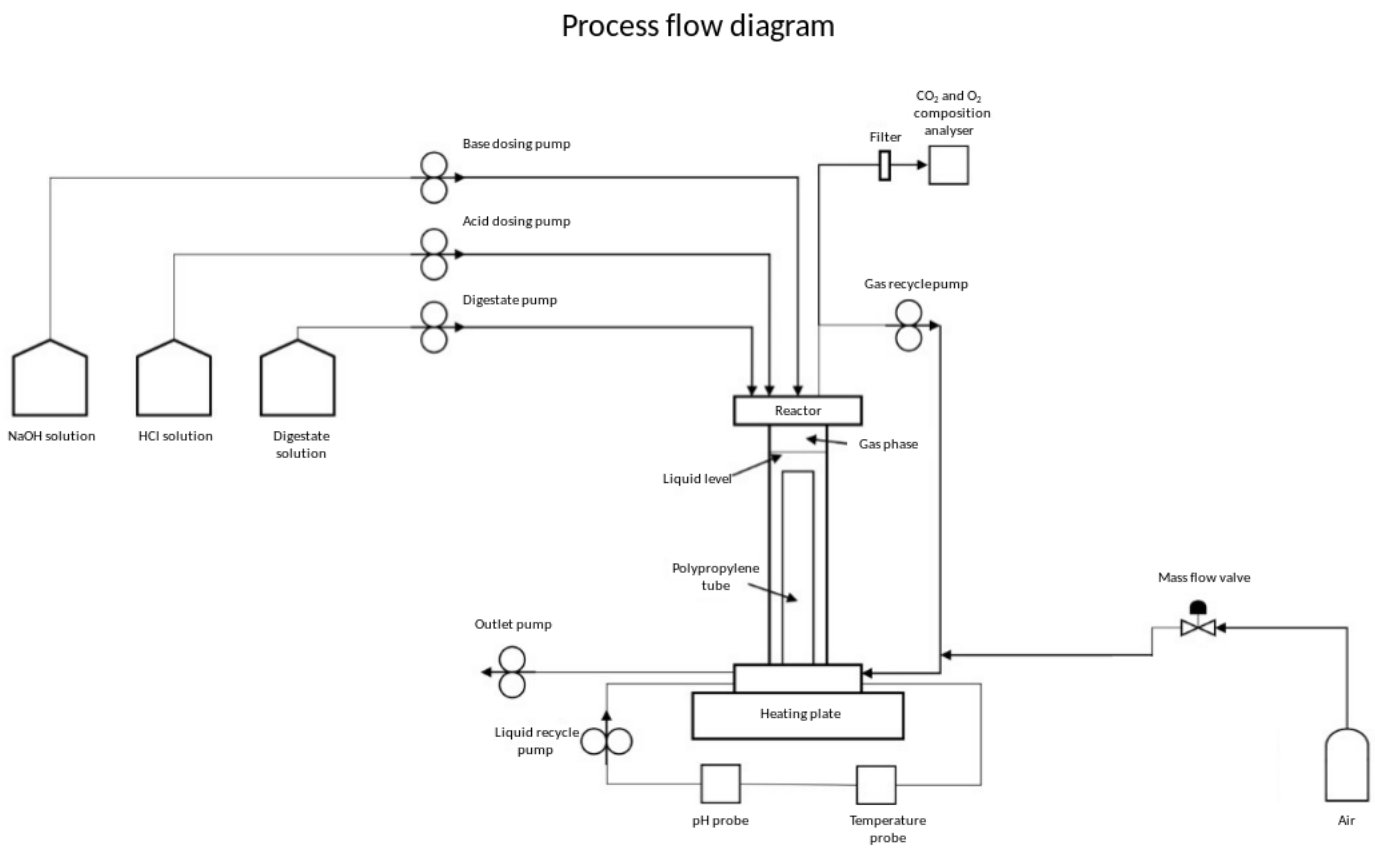

Figure 2: Schematic representation of the aerobic polishing unit showing all equipment and their relevant control mechanisms

For continuous operation an additional 120U Watson pump was added to the setup to allow for the continuous addition of the process medium, i.e the digestate depicted in Figure 2 at a flow rate which was incrementally increased and set to the outlet flow rate in order to maintain a constant volume. The process was initially implemented in batch mode at pH 6 and was increased to 7 after four days at which point the digestate was continuously added. The rate of the continuous addition of substrate was determined from the results of the repeat batch process. The unit in operation as shown in Figure 3, shows the reactor filled with the diluted liquid digestate during the progression of the run.

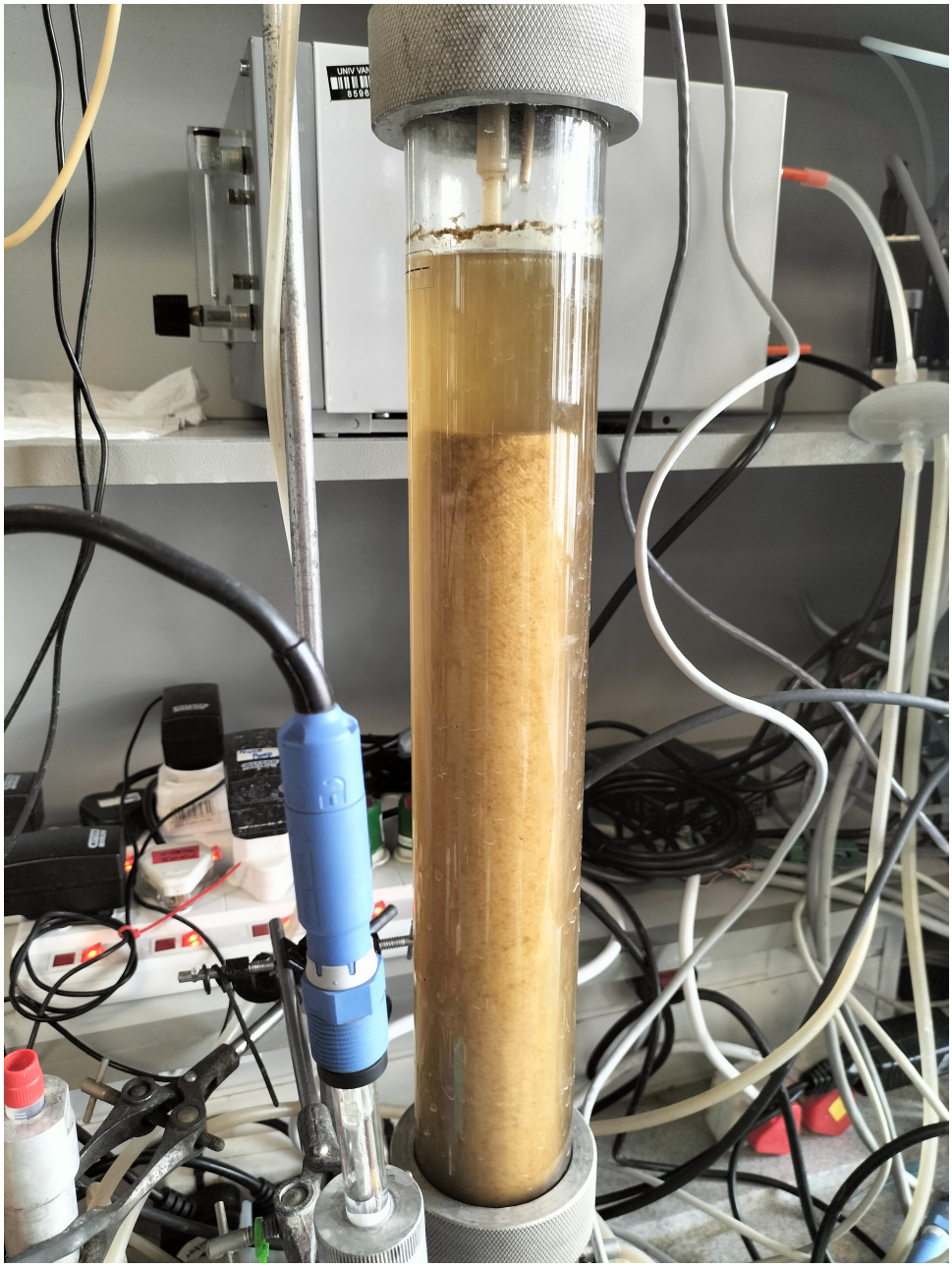

Figure 3: Polishing unit in operation filled with liquid digestate

3.3 Analytical Methods

Analysis of the samples taken from the reactor was performed using the Nitrate Test Kit, Phosphate Test Kit, Ammonium Test Kit, COD Cell Test Kit, and the Total Nitrogen Cell Test Kit which were all procured from MERCK®. Ammonium, nitrate and phosphate concentrations were determined using photometric test kits and were prepared according to the package inserts and subsequently analysed using an Agilent Technologies Cary 60 UV-Vis spectrophotometer. The ammonium and phosphate solutions were placed in 4 mL microcuvette and were analysed at a wavelength of 690 nm. For the nitrate tests the wavelength was set to 340 nm. The cell test kits for the COD, TOC and total nitrogen were analysed using the Spectrosquant CombiCheck 700. TOC analysis was conducted using a Shimadzu TOC-V analyser (Kyoto, Japan).

3.3.1 DNA Sequencing

16s rRNA characterisation was conducted on a sample from the biofilm to determine the metagenomics of the microbial colony in the reactor. The metagenomic sequencing is initiated by the PCR amplification of the bacterial genes across the V1-V9 regions, which utilises barcoded 16s forward and reverse primers (PacBio, 2022). After amplification, the samples are transferred to SMRT ®bell Templates prior to purification PacBio (2018) and are subsequently sequenced using the Circular Consensus Sequencing (CCS) algorithm which can be accessed through the latest SMRT ®Link software wherein the data can also be analysed (de Zoete *et al*, 2021).

4 Results and Discussion

In order to assess the feasibility of this process in hydroponic systems, it is important to investigate the nutrients in question, nitrogen and phosphorus, in the forms they would most likely be found in agricultural settings. Nitrogen is commonly introduced in the form of ammonium which is transformed to nitrate by the nitrifiers present in soil-based systems (Dodds *et al*, 2017b). Although either form of nitrogen (ammonium or nitrate) can be taken up by plants, studies conducted on the use of liquid digestate in hydroponic systems have included a nitrification step (Lind *et al*, 2021; Bergstrand *et al*, 2020; Weimers *et al*, 2022), due to the fact that at high concentrations ammonium (NH_4^+) can be toxic to plants (Tabatabaei, Fatemi & Fallahi, 2006). Both forms of nitrogen are measured in this study, to determine the occurrence of nitrification and phosphate is measured since it is the form of phosphorus that is readily taken up by organisms and plants. Additionally, COD which is a measure of the chemical oxygen demand of a given sample and TOC, a measure of the total organic carbon were also measured to quantify the removal of organic matter as they are prevalent measurements in wastewater treatment. The objective of the process is to assess the capability of an aerobic polishing unit to remove the organic carbon from liquid digestate to minimize any microbial growth with hydroponic systems and improve the efficacy of the nitrification process. This process is pivotal to the implementation of a circular economy and the maximisation of nutrient recovery.

4.1 Repeat-batch Experiments

Figure 4 illustrates the results of the analysis of the TOC, ammonium, nitrate, and phosphate profiles from the investigation of the effect of pH on the aerobic polishing of liquid digestate in a repeat batch process. The process conditions – temperature, air flow rate, were kept constant throughout the experiments at 35 °C and 200 mL min^{-1} respectively.

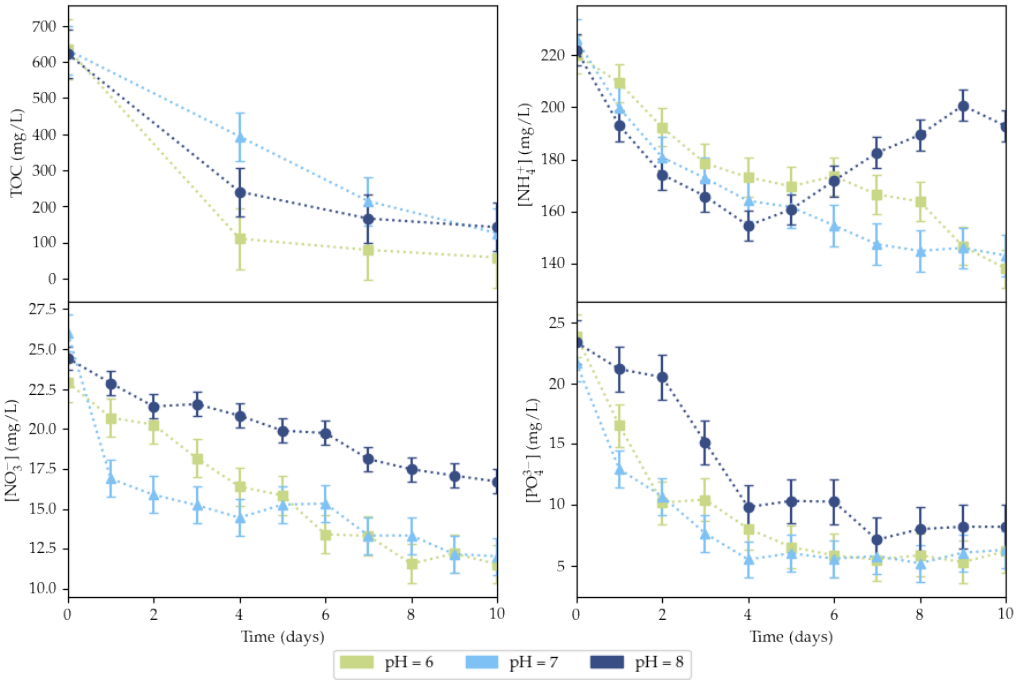

Figure 4: Changes in the TOC, ammonium, nitrate, and phosphate concentration as a function of time due to a change in pH

The majority of the bacteria present in the LD (liquid digestate) is heterotrophic as is shown by the 16S metagenomic sequencing (discussed further in Appendix D) since they use organic and inorganic compounds for growth (Theradimani & Ramamoorthy, 2022), which is discussed further in Subsection 4.2.1.

The profiles shown in Figure 4 indicate the consumption of all nutrients that were accounted for, and it would follow that microbial growth was established due to the utilisation of organic and nitrogenous compounds as well as phosphate. The COD measured in any given sample the amount of oxygen needed to oxidise the organic carbon to CO_2 as well as other reduceable material present in the LD. The anaerobic digestion process mineralizes nitrogen to ammonium (Bergstrand *et al*, 2020), which apart from the organic carbon, is the only other mineral (of notable concentration) that can be further oxidized (to NO_3^-) (Ergas & Aponte-Morales, 2014). However, the COD tests make use of potassium dichromate which is a very strong oxidising agent that does not account for the oxygen demand due to nitrification (Wilhelm, 2009). Therefore, the COD profiles depict the depletion of all oxidisable materials in the medium over time and allude to the continuous consumption of organic matter for microbial growth. The trends observed in the profiles for all the pH values indicate a consumption of nutrients which is attributed to bacterial growth. Similar to an attached growth system used in wastewater treatment, the bacteria consume the nutrients in the presence of oxygen to initiate the metabolic growth process, however, this system remains continuously aerated and mixed to avoid

anoxic zones which would promote denitrification (Skiba, 2008). The depletion of the nitrate concentration throughout all the pH values is evidence of the absence of nitrification taking place in the reactor. Nitrification would increase the concentration of nitrate due to the conversion of ammonium to nitrate (Ergas & Aponte-Morales, 2014). The decrease in concentration over time is attributed to the assimilation of nitrate by NAB (nitrate assimilating bacteria) (Jiang & Jiao, 2016) which can then be used for growth. *Aquamicrobium*, which has been identified in the biofilm, is capable of assimilating nitrate.

In terms of quantity, apart from the organic carbon, the ammonium is the largest contributor of nutrients to the system where the concentration is approximately ten times that of the nitrate and the phosphate. Kirchman & Wheeler (1998) states that heterotrophic bacteria frequently account for a large fraction of ammonium uptake in aquatic systems, and this is also observed in these experiments where, at pH 6 and pH 7, 36% of ammonium is taken up which is similar to the figure reported in the study. Ammonium is the preferred form of nitrogen for the bacteria present in the reactor and like the phosphate, less is needed for cell maintenance than for growth. However, this is not the case for pH 8 and this anomaly can be attributed to microbial stress induced by the high pH. Decreasing trends are observed in all experiments due to nutrient uptake for microbial growth. pH 6 and pH 7 follow similar trends however, halfway through the established runtime, pH 8 experiences an increase in ammonium concentration while all other nutrients are still decreasing. This behaviour could be attributed to endogenous respiration, where active cells utilise cell material (L Li, Song & Visvanathan, 2019) however, this only occurs in the complete absence of substrate. Figure 4 indicates that on Day 4, there are still sufficient amounts of all nutrients available to allow for continued microbial growth. Ammonification is a step in the nitrogen cycle, where organic nitrogen is converted to ammonia (ammonium) (Stefanakis, Akratos & Tsihrintzis, 2014). The data suggests that this is what is occurring at pH 8 which is induced by the alkali conditions and is within the optimum pH range for ammonification (Stefanakis *et al*, 2014). However, the organic nitrogen being converted to ammonium is sourced from decaying microorganisms whose survival rates are impacted by ammonium toxicity at the high pH. Wurts (2003) states that the concentration of the more toxic form of ammonia-nitrogen, unionized ammonia, which would be harmful to the microbes, increases with increasing pH and therefore clarifies the cause of the microbial decay at pH 8.

Kirchman (1994) also states that heterotrophic bacteria account for a large portion of total phosphate and ammonium uptake which was also observed throughout the experiments. Figure 4 shows a 73% decrease in phosphate at pH 6 and pH 7 and a 64% decrease at pH 8. A study by Sidat, Bux & Kasan (1999) shows that heterotrophic organisms store phosphate intracellularly for periods of phosphate starvation which explains why pH 6

and 7 have a higher phosphate consumption rate. The faster microbial growth rates at these conditions would cause the organisms to store more phosphate because it would be depleted sooner. For this same reason, there is no notable increase in phosphate concentration at pH 8 even with the detected ammonium release because it is believed that the remaining microbes aggressively take up whatever phosphate is released due to decay so that they may not encounter phosphate starvation.

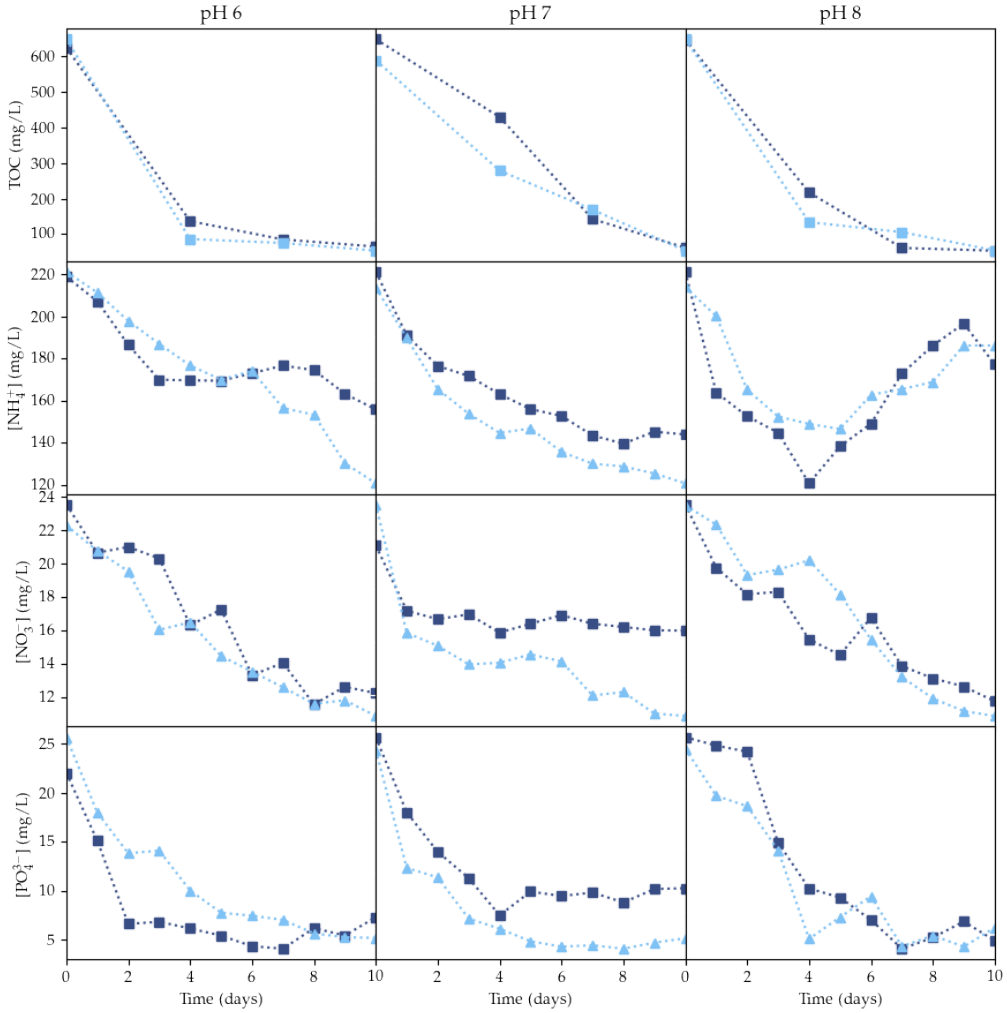

Figure 5: Indication of the repeatability of the trends observed in Figure 4. Run 1 is shown by the dark blue line and Run 2 by the light blue line. The vertical represents the different measurements, and the horizontal axis represents the different conditions

The trends shown in Figure 4 are better shown in Figure 5. Throughout all the experiments it is evident that at pH 6, the highest amount of nutrient loss is observed whereas, conversely, at pH 8 nutrient recovery is observed due to the ammonification that takes place. The phosphate, nitrate and TOC measurements indicate that this behaviour is not attributed to the absence of these nutrients. The slight upward trends observed in the phosphate profile at pH 8 also indicate the release of nutrients into the system, how-

ever, not to the same extent as observed with the ammonium. A total of 6 runs were conducted in the repeat-batch reactor where the biofilm was unchanged at the end of each run. Therefore, it follows that the microbial population would also increase after each run and would therefore alter the rates of consumption of the nutrients within the system. The order of the experiments is shown in Table 5.

pH	Experiment number	Runtime (days)	Temperature (°C)	Average TOC consumption (%)
6	2, 5	10	35 °C	≈ 90%
7	1, 4	10	35 °C	≈ 79%
8	3, 6	10	35 °C	≈ 76%

Table 5: Summary of the order of the experiments and their associated conditions

The profiles shown in Figure 5 support this concept as it can be seen that majority of the repeat batch runs (depicted in light blue by triangular markers) have a higher consumption of nutrients. Figure 5 shows the repeatability of the trends observed at the various conditions. Between each run, there was a pH adjustment period of 2–3 days to allow the bacteria to acclimatise to the new pH in the presence of some nutrients prior to the experiment being conducted. This figure also illustrates the effect of pH on the nutrient removal capabilities of the heterotrophs present in the reactor.

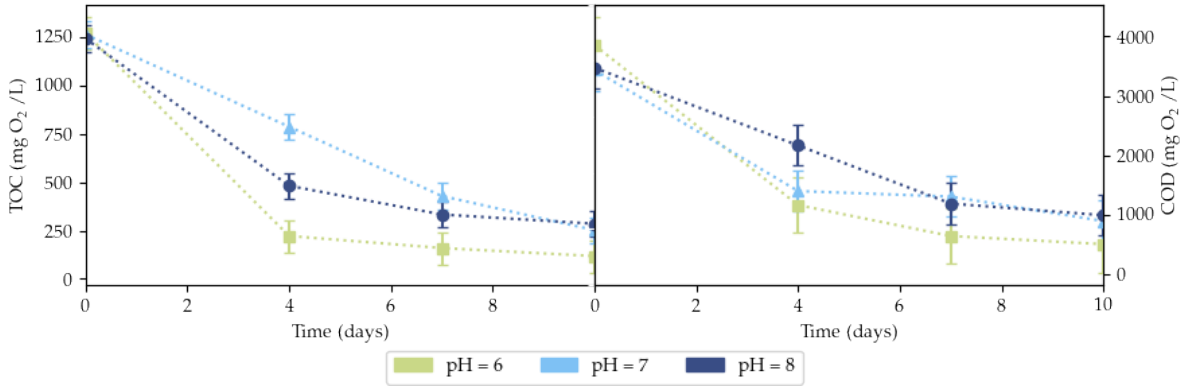

Figure 6: Comparison of the TOC (left) and the COD (right) on Day 0, 4, 7 and 10. The values shown are the averaged values of the repeats and the error bars depict the standard error

Figure 6 shows a comparison of the measured TOC and the measured COD. COD and TOC (Total Organic Carbon) are measurements that are commonly used in wastewater treatment for the characterization of NOM (Natural Organic Matter) (Park *et al*, 2023).

TOC is generally favoured due to the hazardous materials produced from COD analysis. COD measures the amount of carbon that can be oxidised to CO_2 whereas TOC measures the amount of carbon which is bound to the organic compounds (Orellana *et al*, 2011; Z Hu & Grasso, 2005). COD is said to be one of the most accurate quantifications of chemically oxidisable material while the TOC is a measure of all the organic carbon present in the dissolved sample (Aguilar-Torrejón *et al*, 2023). It therefore follows that the TOC and COD would have a proportional relationship as described by Dubber & Gray (2010) and is also shown in Appendix A. Aguilar-Torrejón *et al* (2023) also states that COD is greater than COD which has also been observed in Figure 6 where the trends observed for the TOC and COD paint a clearer picture of the nutrient uptake taking place in the reactor. In tandem, these measurements allow for the holistic characterization of the nutrient distribution in each sample. D Li & Liu (2019) states that COD is a measure of the reducing substances present in a sample which are organic, nitrite, sulphide, and ferrous salts; the bulk of which is organic matter.

Minimizing the organic carbon is essential to optimise the nitrification process and limit the possibility of bacterial growth, especially in closed hydroponic systems (S Lee & J Lee, 2015). In addition to the nutrients quantified in Figure 4, the organic matter represented by the COD was also consumed to different extents throughout all the experiments. The COD profile in Figure 6 shows a substantial decrease in concentration over the first four days and thereafter decreases at a slower pace over the rest of the runtime. The largest decrease is observed at pH 6 which shows near complete consumption of the organic carbon. Similar trends are observed for pH 7 and pH 8 however the removal of organic carbon is not to the same extent as pH 6. This is analogous with the findings from Figure 4 where pH 6 showed the largest fraction of nutrient loss.

4.2 Continuous Experiment

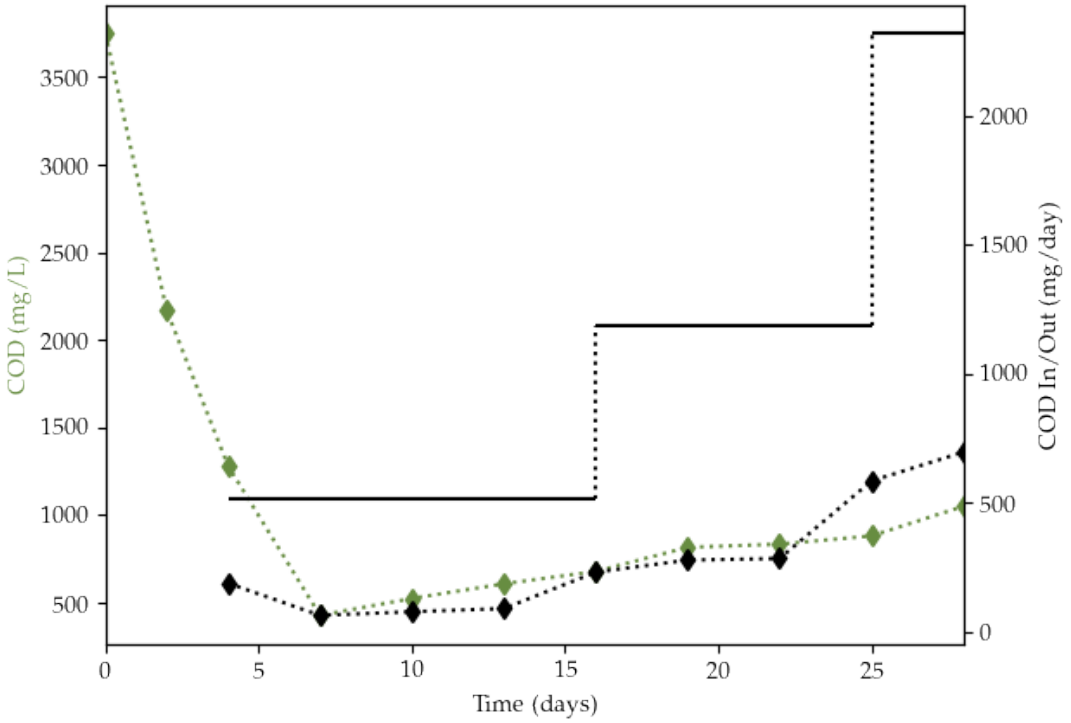

Figure 7: COD data obtained from the continuous run with measured concentration shown on the left axis and nutrient amounts added and withdrawn shown on the right axes

The COD added shown in Figure 7 added was derived from the concentrations shown in Table 4. The continuous run was first conducted in batch at pH 6 to minimise the COD to approximately 1000 mg L^{-1} and thereafter the pH was changed to 7. The flow rate of digestate added was doubled on two instances to determine the effect of additional nutrients on the microbial uptake of nutrients. This was largely done to observe the consumption of the COD and how much can still be added to the reactor before large accumulations occur. This was largely done to observe the consumption of the organic carbon and how much can be added to the reactor before large accumulations occur. From batch operation, the hydraulic retention time (τ) was changed from 7.38 days to 3.18 days and finally 1.63 days. COD breakthrough is observed from Day 13 onwards where the concentration begins to increase over time. Figure 7 clearly illustrates the COD removal capabilities of the microbial population present in the reactor as, by day 25, more than 70% of the COD added is removed within 4 days. In comparison, in the continuous run COD was removed to a much larger extent than the batch runs which indicates the suitability of this method for the minimisation of organic carbon. The results indicate consumption of organic carbon which is one of the main purposes of this study. Even with the continuous addition of substrate, organic matter readings remain

low which leaves little feed for heterotrophic bacteria in further steps (Theradimani & Ramamoorthy, 2022).

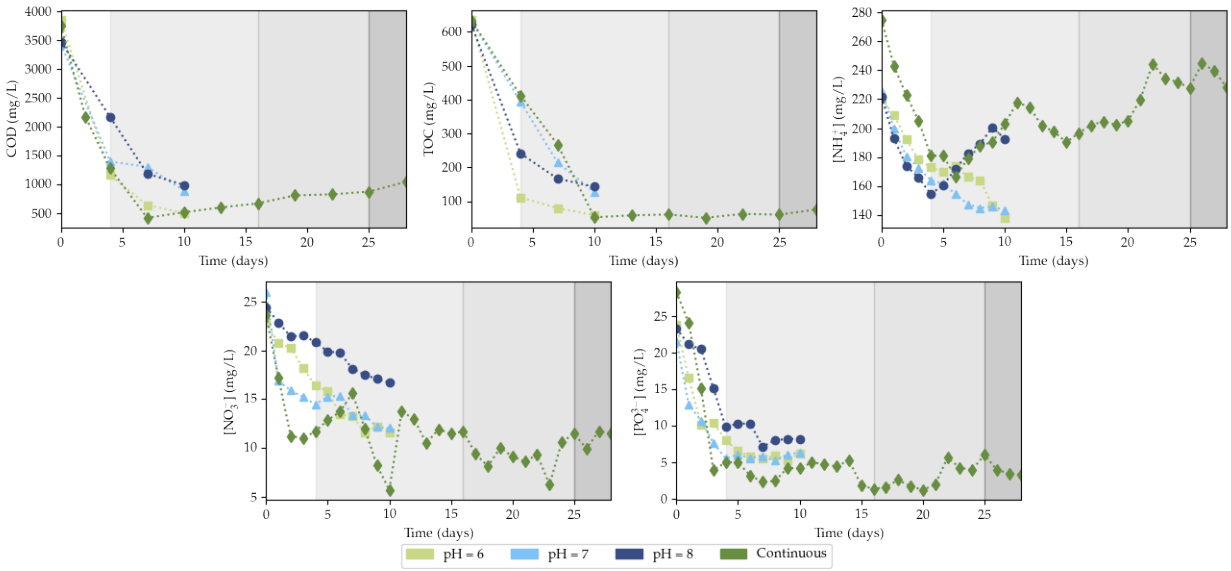

Figure 8: Comparison of the batch and continuous profiles. The shaded regions depict the various hydraulic retention times ($\tau = 7.38$ days, $\tau = 3.18$ days and $\tau = 1.63$ days) from light to dark respectively.

As shown in Figure 8, the TOC data for all the runs decreases which further proves the hypothesis of the consumption of depletion of organic matter in the system, specifically organic carbon. However, compared to the COD, especially for the continuous run, an increase is observed which is not present in TOC data. The nutrient content of LD varies depending on feed, manure, and retention time, however for reference, as discussed by Weimers *et al* (2022) it contains (per kilogram fresh weight of digestate) 0.28 g/kg sulphur (S) and 325 mg/kg iron (Fe). Seeing as these are the components expected to be present in COD analysis, these figures give an approximation of the remaining fraction which is measured in COD. The COD and TOC profiles shown in Figure 8 emphasise the presence of non-organic minerals that are chemically oxidisable as proposed by L Li *et al* (2019) - the increase in COD while TOC remains low would stem from the accumulation of said nutrients in the medium. The findings also suggest that these minerals are not as easily taken up by the microbial colony even though they may be present in smaller quantities. It would be ideal to fully characterise the minerals present in the medium.

The ammonium profile shows clear evidence of accumulation in the system whilst low concentrations of TOC and COD are maintained. However, in this instance, the increase in ammonium concentration is not attributed to ammonification as the repeat batch experiments have shown that pH 7 is optimal for growth. In terms of production of a hydroponic fertiliser, this would be ideal since the organic carbon is minimised while the

N, which is a major component of fertiliser, is maintained which fulfils the objective of the study. Only a 15% loss in nitrogen concentration is observed for the continuous run, while the organic carbon is sufficiently removed even with the increases in HRT. The N content in the polished product is > 220 mg L^{-1} NH_4^+ which is higher than a standard Hoagland medium which contains roughly 210 mg L^{-1} total N (Hoagland & Arnon, 1950). In addition, with the remaining NO_3^-, the total N of the polished product is increased which would make it suitable for systems which make use of a full-strength Hoagland medium, which findings show is most hydroponic systems use (H Li & Cheng, 2015).

However, phosphate losses are significant, with a noted 88% decrease for the continuous and a maximum of 75% for the batch runs. Yao *et al* (2016) states that phosphorus is vital for biological information storage and energy transfer among other crucial systems. Therefore, it is imperative to maintain phosphorus levels in the polished product, as phosphate starvation in plants leads to severe damage to roots and plant immunity is also repressed (Finkel *et al*, 2019). A standard Hoagland solution contains 31 mg L^{-1} P (Hoagland & Arnon, 1950) which is far greater than the P present in the polished product. Therefore, before the polished product is used in a hydroponic system, the phosphate levels would need to be replenished.

The data for the continuous run shows that whatever organic carbon is added, is rapidly utilised while the N content in the polished product remains high, and on par with a Hoagland medium (Hoagland & Arnon, 1950). The analysis shows that even with the continued addition of LD, the organic carbon is rapidly taken up as TOC values remain below 100 mg L^{-1} from day 10 onwards. COD accumulates as a result of the presence of other oxidisable materials in the LD and a fraction of the nitrate is also recovered, however, the phosphate is rapidly taken up. Findings from this run indicate that the continuous run is the superlative method for using the aerobic polishing process to prepare a hydroponic medium.

4.2.1 16s rRNA Sequencing

Bacterial identification was outsourced to Inqbaba Biotec (Pretoria, South Africa) where a 16S metagenomic analysis was conducted on a sample from the biofilm, identifying the following prevalent species summarised in Table 6. Most of the species identified in the analysis were found to be aerobic, with some exceptions for facultative anaerobes and the prevalent species exist in soil. The analysis identified 83 different bacterial species which are documented in detail in Appendix D.

The findings summarised in Table 6 indicated that most of the bacteria in the biofilm are

Genus	Heteroptrophic	Optimum pH	Applications	Reference
Proteiniphilum	Yes	7.1–7.8	Facultative anaerobe found in anaerobic digesters	(Yi *et al*, 2014; Hahnke *et al*, 2016)
Gemmatimonas	Yes	7.0	Aerobic bacteria linked to rapid phosphorus removal in wastewater treatment	(H Zhang, 2003)
Planctomicrobium	Yes	6.0–6.5	Aquatic aerobe found in wetlands with a preference for organic sugars	(Dedysh, Ivanova & Kulichevskaya, 2022; Kulichevskaya *et al*, 2015)
Aquamicrobium	Yes	6.5–7.5	Strictly aerobic marine bacteria capable of reducing nitrate	(Jin, Kim & Jeon, 2013)
Luteimonas	Yes	7.0	Aerobic bacteria found in wastewater treatment plants capable of increasing nitrogen acquisition	(Ulrich *et al*, 2022; Siddiqi *et al*, 2020)

Table 6: Summary of the prevalent species detected in the 16S metagenomic analysis of the biofilm

heterotrophic and thus account for the rapid decrease in TOC at all observed pH values. This conclusion is valid seeing as heterotrophic organisms consume organic matter for growth (Theradimani & Ramamoorthy, 2022). The rapid removal of the TOC is onset by the combined effect of all the heterotrophs in the biofilm.

Along with *Gemmatimonas* spp., the rapid phosphate removal observed in Figure 8, is also attributed to *Terrimonas* spp. which has been identified as a phosphorus mobilizing bacteria (Nassal *et al*, 2018). The optimal pH ranges of the bacteria listed in Table 6 illustrate that most of the microbes thrive at near neutral pH values (H Zhang, 2003; Dedysh, Ivanova & Kulichevskaya, 2022; Jin, Kim & Jeon, 2013; Siddiqi *et al*, 2020). It follows that the survival of these microbes would be affected by the alkali conditions at pH 8. The nitrogen acquisition effect of the *Lutemonias* spp. genus is observed in the continuous run with the increases in nitrogen concentration at pH, which adheres to the optimal pH for this genus (Siddiqi *et al*, 2020).

The bacterial characterisation shows that all the microbes present in the biofilm play a part in achieving the levels of nutrient removal observed in the study. The rapid removal of TOC, while nitrogen is retained follows as a result of all the heterotrophic bacteria and all species of the *Luteimonas* genus present in the biofilm (Ulrich *et al*, 2022).

5 Conclusions and Recommendations

This study has successfully implemented an aerobic polishing unit for the removal/minimization of organic carbon while high levels of nitrogen were retained in the medium for the preparation of a hydroponic fertiliser. The use of such a system, for this purpose, is the first of its kind as previous studies, used longer anaerobic digestion periods, various separation techniques on untreated liquid digestate, and lengthy nitrification periods. This study has also shown that the time taken for the entire process - from anaerobic digestion to nitrification, can be minimised using an aerobic polishing unit which swiftly removes organic carbon that can cause problems in downstream processes. The investigation of the effect of pH has shown that, for the microbial population in the system, pH 6 was optimal for rapid growth followed by pH 7 while pH 8 was ideal for nutrient recovery, as ammonium was recovered, however, this was at the expense of a fraction of the microbial colony.

It is important to minimise the organic carbon to limit the substrate available to pathogens that cause root rot in hydroponic systems. Repeat-batch experiments provide a quick and easy method of removing organic carbon while retaining nitrogen, and to some extent phosphorus, however, the findings have definitively shown that continuous polishing is the best option for the production of a carbon-scarce and nitrogen-rich effluent In the continuous system the organic carbon remains below 100 mg L^{-1} while the final N concentration is above 220 mg L^{-1} These nitrogen concentrations are analogous to a Hoagland solution which is the primary growth medium in hydroponic systems, therefore it can also be stated that the objective of the study has been achieved.

The only caveat of the system is that the phosphate concentration in the effluent was low throughout all the experiments. This would necessitate the addition of phosphate to the medium prior to hydroponic use to ensure all nutrient requirements are fulfilled. It would also be ideal to fully characterise the minerals present in the effluent to determine if any other micronutrients need to be replenished. Although nitrification was not achieved within the reactor, high levels of ammonium were recovered which can easily be nitrified in a nitrification unit or a hydroponic system inoculated with nitrifying bacteria can be used for this same purpose to minimise the time-span of the entire process.

The findings from this study can be improved by further investigation into the continuous process to maximise the removal of organic carbon and recovery of nitrogen. The biofilm used in this study should also be preserved for further studies to ensure the extent of nutrient removal and recovery observed in these studies. In this regard, it would be ideal to identify the hydraulic retention times that drive the microbial pollution into

cell maintenance as this would ensure that organic carbon is consistently consumed with limited consumption of nitrogen, which is not heavily relied upon for cell maintenance.

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A Appendix A: TOC and COD proportionality

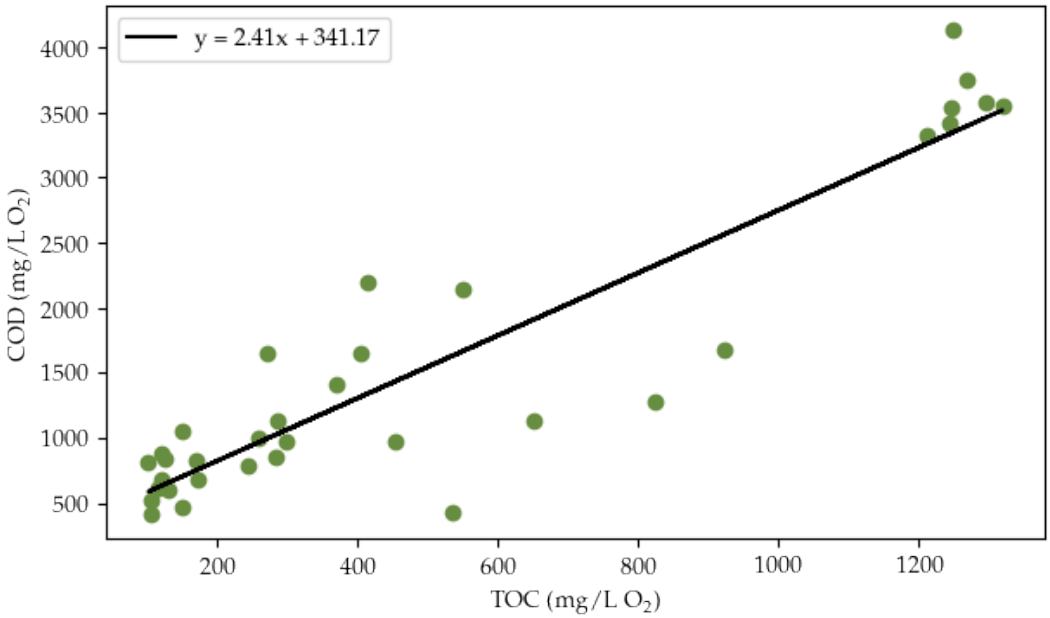

Figure A.1: Correlation between TOC and COD for all experimental measurements with line of best fit indicated in black

The COD measured in any given sample the amount of oxygen needed to oxidise the organic carbon to CO_2 as well as other reduceable material present in the LD. The anaerobic digestion process mineralizes N to ammonium (Bergstrand *et al*, 2020), which apart from the organic carbon, is the only other component (of notable concentration) that can be further oxidized (to NO_3^-) (Ergas & Aponte-Morales, 2014). However, the COD tests make use of potassium dichromate which is a very strong oxidising agent that does not account for the oxygen demand due to nitrification (Wilhelm, 2009). Therefore, the COD profiles depict the depletion of all oxidisable materials in the medium over time and allude to the continuous consumption of organic matter for microbial growth. A statistical correlation between TOC and COD was conducted on n sample points and along with the equation for the linear regression shown in Figure A1, it was found that $R^2 = 0.77$ which proves a statistically significant relationship between the TOC and COD measurements (Chicco, Warrens & Jurman, 2021). It should also be noted that the measurements for all the conditions were used to determine this correlation which also shows that this finding is relevant for all the tested conditions. This data is consistent with the findings from Dubber & Gray (2010) where the proportionality between TOC and COD is reported.

B Appendix B: Synthetic digestate

In order to model the carbon uptake and possible nutrient retention of a microbial aerobic system, a lab-scale 1 L aerated vessel with pH and temperature was constructed. The aerobic digestion step of the process is aimed at removing the organic carbon present in liquid digestate in order to prepare the medium for use in a hydroponic system. If not removed, the carbon present in the digestate along with the naturally occurring microbes will cause a biofilm to establish on the roots of the plants, therefore, hindering nutrient uptake and subsequently killing the plants.

Table B.1: Stock solution concentrations (SS conc.) and amounts to prepare a modified Hoagland's solution

Component	SS conc. (g L^{-1})	mL SS L^{-1} Hoagland's	mg L^{-1} Hoagland's
2M $(NH_4)_2SO_4$	264	3.75	990
2M $Ca(NO_3)_2 \cdot 4H_2O$	472	2.5	1180
2M $MgSO_4 \cdot 7H_2O$	493	1	493
1M KH_2PO_4	136	1	136
H_3BO_3	2.86	1	2.86
$MnCl_2 \cdot 4H_2O$	1.81	1	1.81
$ZnSO_4 \cdot 7H_2O$	0.22	1	0.22
$CuSO_4 \cdot 5H_2O$	0.08	1	0.08
$Na_2MoO_4 \cdot 2H_2O$	0.12	1	0.12
Iron	15	1	15

The liquid digestate was modelled using a modified Hoagland's as shown in Table B.1 in order to account for all the macro and micronutrients that could be present in the digestate. Due to the absence of carbon in the medium 5 mL of a 2M sodium acetate stock solution was added to the synthetic digestate (SD). Acetic acid was chosen since liquid digestate contains a large fraction of it and since it is a simple hydrocarbon it will be easily taken up by the microbes present in the system. The nitrogen content of the SD was increased to 10 mM in order to up the C/N ratio of the SD to 5 to observe a larger response in the carbon uptake profiles.

The experiments were conducted at pH 6, 7 and 8 in line with the optimum range suggested in literature with an additional experimental uncontrolled run as the control. The temperature of the system was maintained at 35 °C with a water bath in order to operate the reactor in the mesophilic temperature range which, according to literature, is optimum for aerobic digestion. The results which are shown in Figure B.1 and Figure B.2 are the averages of three repeat experiments.

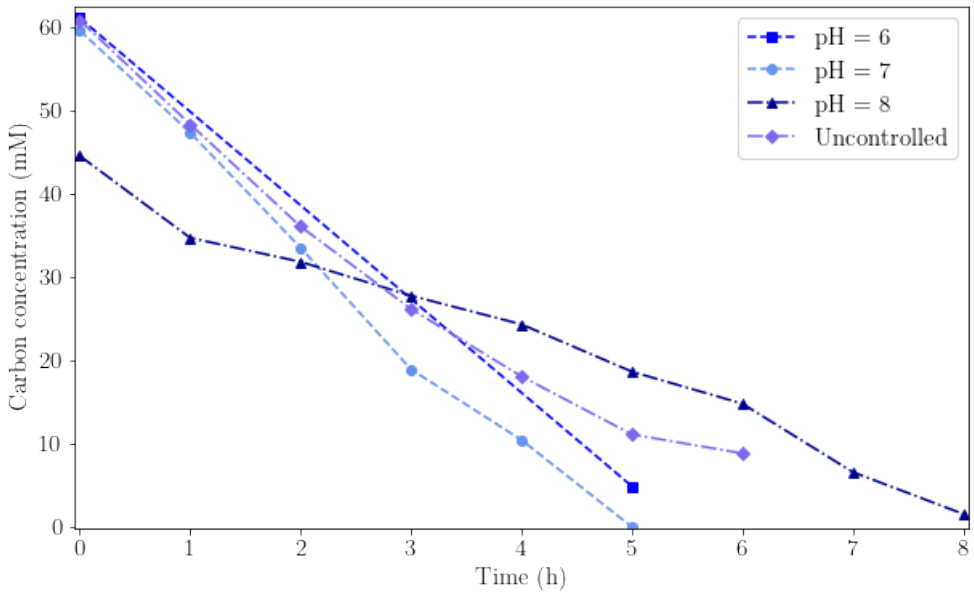

Figure B.1: Summary of the carbon profiles obtained from the aerobic digestion of liquid digestate

Figure B.1 shows the carbon uptake profiles obtained throughout the experiments. The primary goal of these experiments was to remove all the carbon that was present in form of sodium acetate and it is evident from Figure B.1 that pH = 7 was the optimum pH for carbon removal. Even though pH 6 also took 5 hours, not all the carbon was removed in that time and although the pH 8 run removed most of the carbon it took 8 hours to remove most of the carbon.

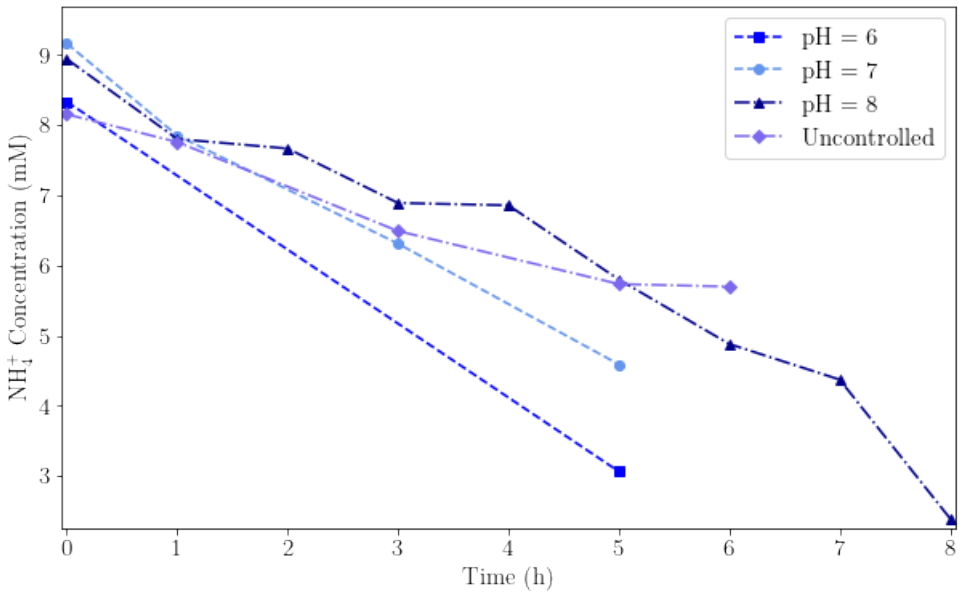

Figure B.2: Summary of the ammonium profiles obtained from the aerobic digestion of liquid digestate

The ammonium profiles shown in Figure B.2 show that in most instances nutrient retention did occur except for the pH 8 run which consumed all the nitrogen present. Although the uncontrolled run retained the most nitrogen, it took longer than the pH 7 run and Figure B.1 shows that not all the carbon was consumed in that run.

The results from these experiments theoretically indicate that the removal of organic carbon *via* aerobic digestion with nutrient retention in the form of nitrogen is possible and this was used as a basis for further experimental exploration.

C Appendix C: Publication

The first page of the publication submitted to MPDI-Water is shown below. At this time, the manuscript is under review.

water

MDPI

Article

Aerobic polishing of liquid digestate for the preparation of hydroponic fertilizer

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Abstract: Nutrient pollution – mainly nitrogen and phosphorus – caused by organic waste continues to impact the environment. The implementation of a circular economy is integral to alleviating these effects. Liquid digestate, which is a byproduct of anaerobic digestion (a waste valorising process), is a nutrient dense organic fertiliser with vast applications in agriculture. Using an aerobic polishing unit this study developed a viable method for the preparation of a hydroponic fertiliser by investigating the effect of pH on the nutrient recycling capabilities of said system. The heterotrophic bacteria present in the biofilm, identified by 16S gene sequencing are responsible for 90% of organic carbon (as TOC) removal with minimal ammonium loss. This is ideal to promote optimal nitrification in hydroponic systems in the absence of organic carbon to ensure plant growth is not affected. Although pH 8 was found to be ideal for batch operation, continuous operation at pH 7 proved to be a better option owing to the ammonium rich effluent (> 220 mg/L) which was produced which is on par with the nitrogen concentration of Hoagland solution. Continuous carbon polishing of liquid digestate provides an efficient way of utilising organic fertilisers in hydroponic systems.

Keywords: nutrient recovery; carbon polishing; nitrogen retention; heterotrophs

Citation: To be added by editorial staff during production.

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Accepted: date
Published: date

1. Introduction

The circular economy is integral to the future of sustainable practices, especially in the agricultural sector where the recycle of essential nutrients and minimization of nutrient spillage are the main concern. Nitrogen (N) and phosphorus (P) are essential macronutrients in agriculture and along with other element s contribute to the growth of healthy plants. N is one of the essential compounds in all plant proteins while P is crucial to biological information and energy storage [1]. Although numerous other elements are required for plant growth, N and P spillage are typically considered as the main contributors to nutrient pollution in groundwater and downstream aquatic ecosystems [2].

As vital as N and P are, when released into the environment they have the potential to severely disturb ecosystems. Bodies of water have been damaged by eutrophication which is the process wherein excess plant nutrients, primarily N and P lead, to the occurrence of algal blooms [3] which reduce the amount of dissolved oxygen in water and ultimately reduce biodiversity and harm aquatic life. Agricultural runoff produces leachate rich in N and P, which pollutes groundwater sources. The worldwide trend is to reduce the spillage of N and P by decreasing the synthetic production and unsustainable usage while simultaneously enhancing the recycle of these compounds. The treatment of organic waste plays an imperative role in facilitating nutrient recycle.

Organic waste, commonly referred to as biodegradable waste, is mainly produced from living organisms and consists of food waste, agricultural waste, livestock waste, and human waste, among others [4]. A benefit of organic waste is that it is nutrient rich and

D Appendix D: ngDNA Sequencing

The complete detailed report of the 16S RNA characterisation carried out by Inqbaba Biotec (Pretoria, South Africa) is documented in the report below.

inqaba biotec metagenomic report

Sample Information

Index:	M13_bc1010_F--M13_bc1066_R
Sample Name:	JM-Biofilm-16S
Run Name:	231127_Cell1
Report Date:	Tue Nov 28 11:22:22 2023

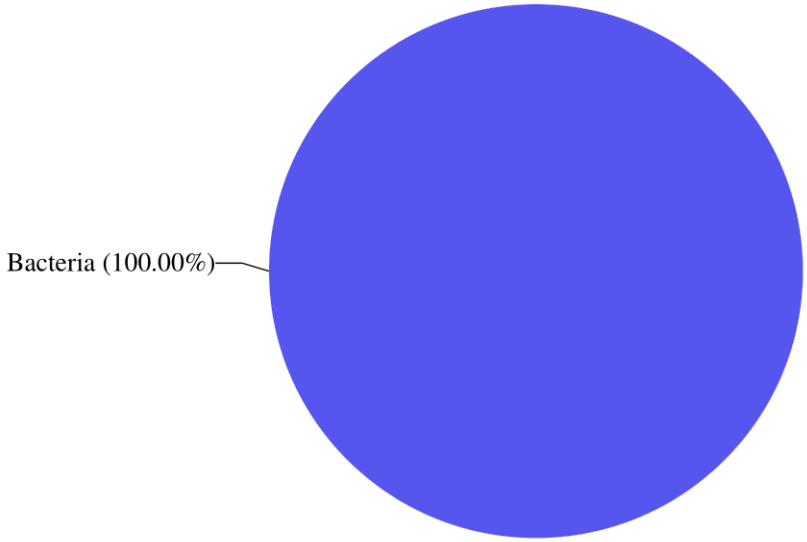
This report contains the summarized metagenomic analysis of full length 16s gene amplicons. Samples were sequenced on the Sequel IIe system by PacBio (www.pacb.com). Raw sub-reads were processed through the SMRTlink (v11.0) Circular Consensus Sequences (CCS) algorithm to produce highly accurate reads (>QV40). These highly accurate reads were then processed through vsearch (https://github.com/torognes/vsearch) and taxonomic information was determined based on QIMME2. Report generation command used :$create_vsearch_single_sample_pdf_report_pacbio.py create_vsearch_single_sample_pdf_report_pacbio.py JM-Biofilm-16S_M13_bc1010_F--M13_bc1066_R.hifi_reads-filtered-feature-table-asv.tsv M13_bc1010_F--M13_bc1066_R JM-Biofilm-16S 231127_Cell1 16S

Taxanomical Classification

Kingdom Classification

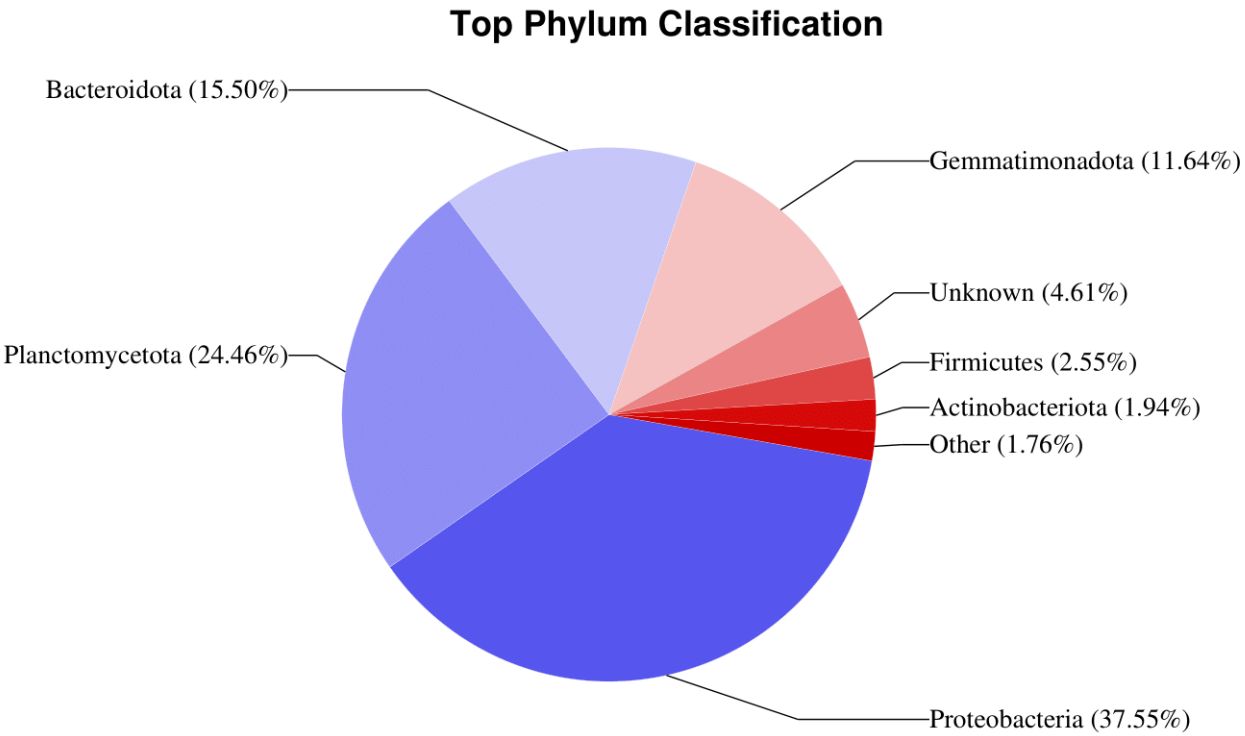
Kingdom	Read Count	%
Bacteria	44840.0	100.00

Top Kingdom Classification



Phylum Classification

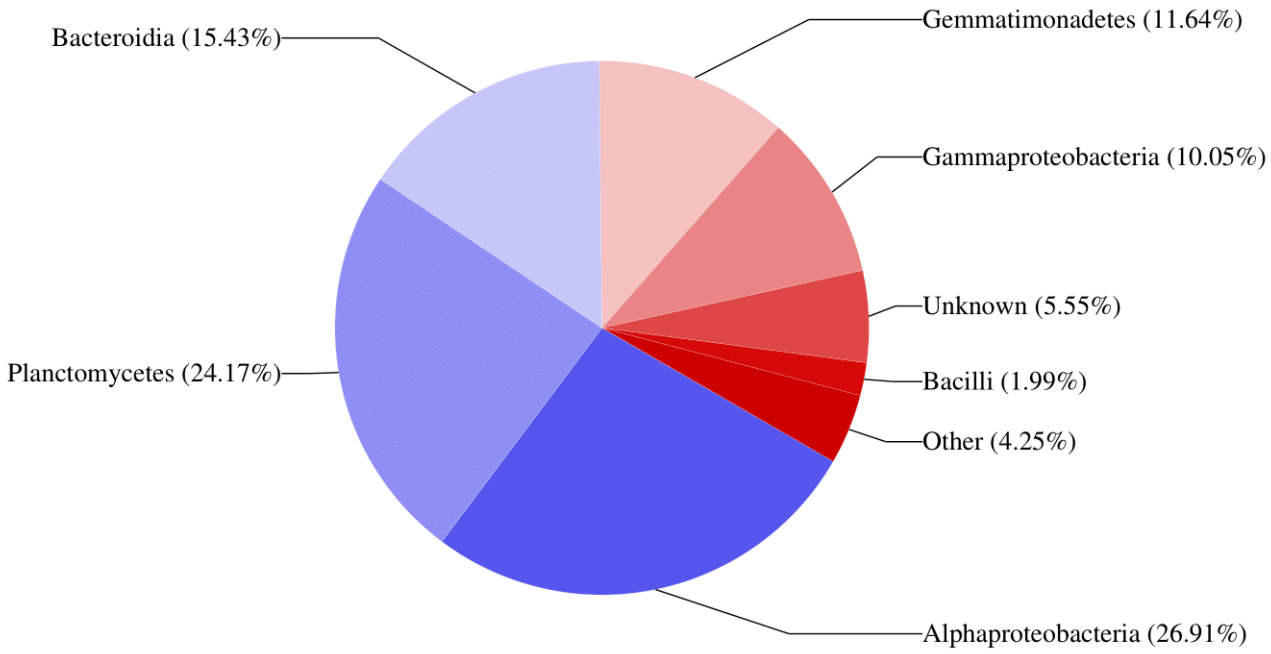
Phyla Classification	Read Count	%
Proteobacteria	16835.0	37.55
Planctomycetota	10969.0	24.46
Bacteroidota	6949.0	15.50
Gemmatimonadota	5220.0	11.64
Unknown	2066.0	4.61
Firmicutes	1143.0	2.55
Actinobacteriota	868.0	1.94
Chloroflexi	606.0	1.35
Myxococcota	95.0	0.21
Patescibacteria	54.0	0.12
SAR324_clade	24.0	0.05
Desulfobacterota	10.0	0.02



Class Classification

Class	Read Count	%
Alphaproteobacteria	12066.0	26.91
Planctomycetes	10835.0	24.17
Bacteroidia	6918.0	15.43
Gemmatimonadetes	5220.0	11.64
Gammaproteobacteria	4507.0	10.05
Unknown	2489.0	5.55
Bacilli	894.0	1.99
Anaerolineae	413.0	0.92
Acidimicrobiia	397.0	0.89
Actinobacteria	379.0	0.85
Clostridia	194.0	0.43
Chloroflexia	184.0	0.41
Polyangia	90.0	0.20
Thermoleophilia	86.0	0.19
Saccharimonadia	54.0	0.12
Limnochordia	45.0	0.10
SJA	31.0	0.07
SAR324_clade	24.0	0.05
Desulfovibrionia	10.0	0.02

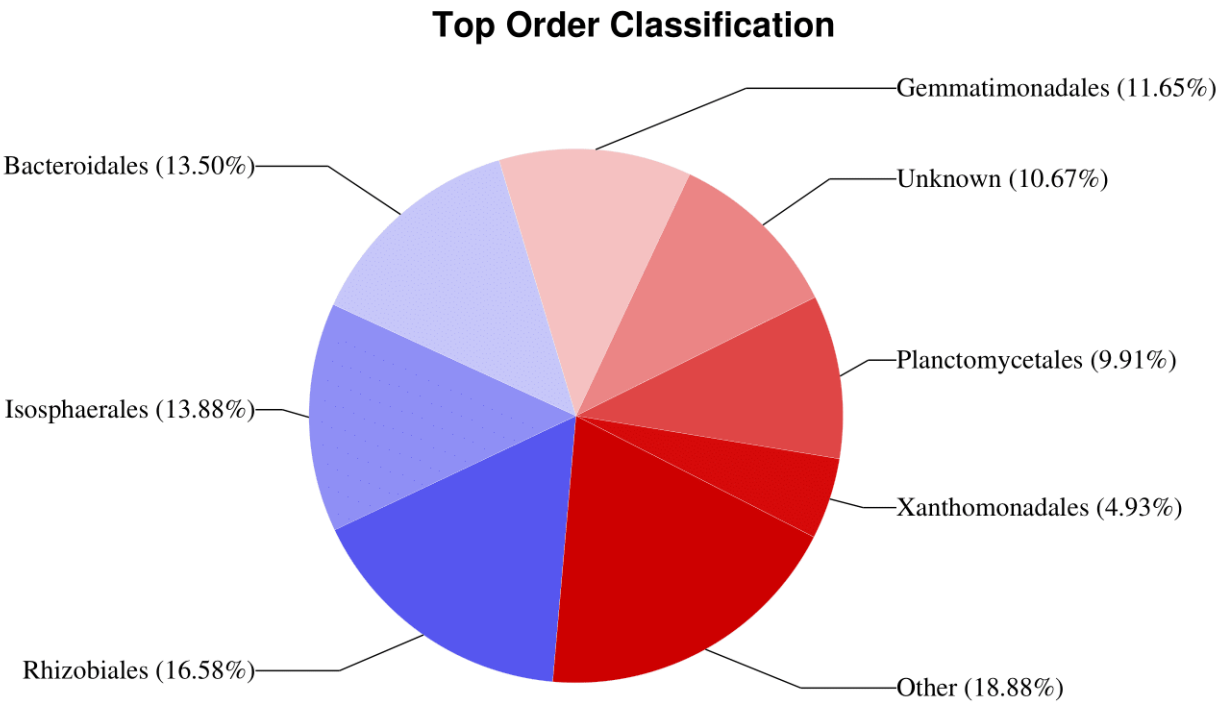
Top Class Classification



Order Classification

Order	Read Count	%
Rhizobiales	7427.0	16.58
Isosphaerales	6218.0	13.88
Bacteroidales	6048.0	13.50
Gemmatimonadales	5220.0	11.65
Unknown	4781.0	10.67
Planctomycetales	4438.0	9.91
Xanthomonadales	2208.0	4.93
Burkholderiales	1902.0	4.25
Reyranellales	819.0	1.83
Chitinophagales	740.0	1.65
Sphingomonadales	698.0	1.56
Paenibacillales	693.0	1.55
Caulobacterales	442.0	0.99
SBR1031	388.0	0.87
Microtrichales	373.0	0.83
Acetobacterales	246.0	0.55
Corynebacteriales	245.0	0.55
Legionellales	190.0	0.42
Rhodospirillales	170.0	0.38
Bacillales	155.0	0.35
Thermomicrobiales	143.0	0.32
Pseudomonadales	142.0	0.32
Peptostreptococcales	139.0	0.31
Micrococcales	108.0	0.24
Rhodobacterales	99.0	0.22
mle1	88.0	0.20
Solirubrobacterales	82.0	0.18
Azospirillales	74.0	0.17
Ferrovibrionales	68.0	0.15
uncultured	56.0	0.13
Saccharimonadales	54.0	0.12
Sphingobacteriales	44.0	0.10
Kallotenuales	41.0	0.09
MBA03	37.0	0.08
SJA	31.0	0.07
Bacteroidetes_VC2	31.0	0.07
SAR324_clade	24.0	0.05
Puniceispirillales	19.0	0.04
Anaerolineales	19.0	0.04

Christensenellales	15.0	0.03
Izemoplasmatales	13.0	0.03
Oscillospirales	13.0	0.03
Defluviicoccales	12.0	0.03
Propionibacteriales	12.0	0.03
Clostridia	11.0	0.02
Desulfovibrionales	10.0	0.02
Lachnospirales	7.0	0.02
Micropepsales	6.0	0.01

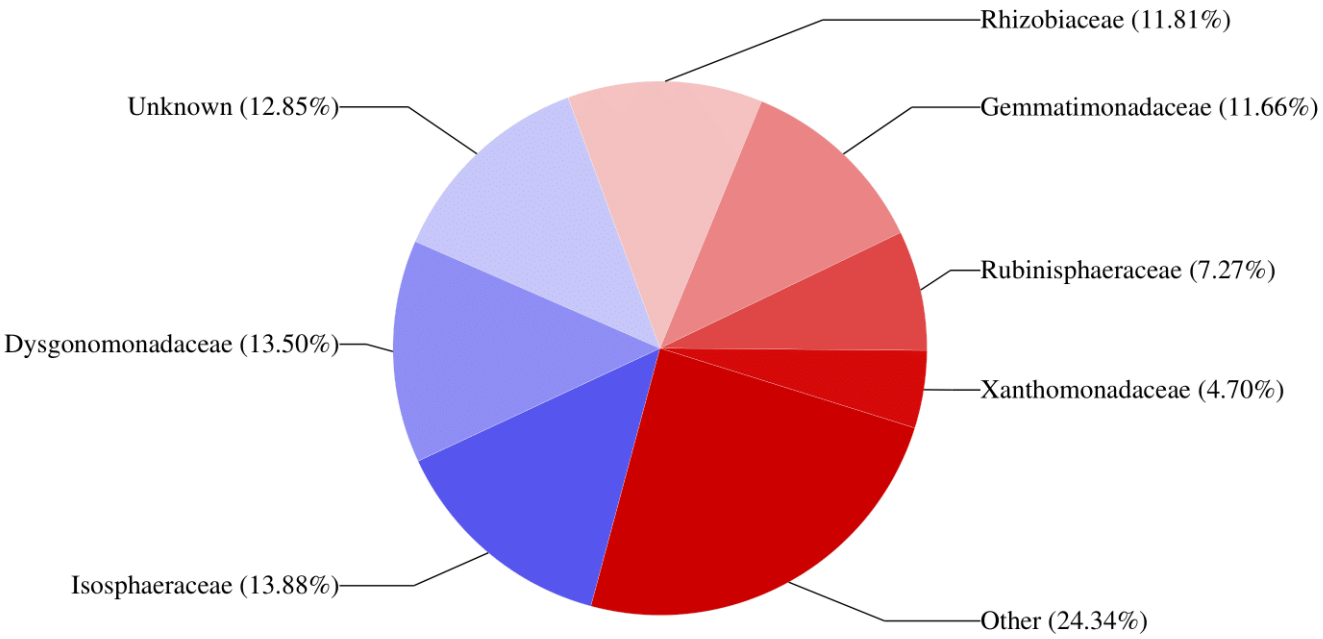


Family Classification

Family	Read Count	%
Isosphaeraceae	6218.0	13.88
Dysgonomonadaceae	6047.0	13.50
Unknown	5757.0	12.85
Rhizobiaceae	5288.0	11.81
Gemmatimonadaceae	5220.0	11.66
Rubinisphaeraceae	3254.0	7.27
Xanthomonadaceae	2103.0	4.70
Comamonadaceae	1719.0	3.84
Xanthobacteraceae	1044.0	2.33
Reyranellaceae	819.0	1.83
Sphingomonadaceae	698.0	1.56
Paenibacillaceae	693.0	1.55
Chitinophagaceae	676.0	1.51
uncultured	476.0	1.06
Schlesneriaceae	466.0	1.04
Caulobacteraceae	442.0	0.99
A4b	359.0	0.80
Microtrichaceae	276.0	0.62
Acetobacteraceae	246.0	0.55
Devosiaceae	244.0	0.54
Beijerinckiaceae	236.0	0.53
Nocardiaceae	235.0	0.52
Legionellaceae	190.0	0.42
Rhodospirillaceae	158.0	0.35
Hyphomicrobiaceae	149.0	0.33
JG30	144.0	0.32
Bacillaceae	121.0	0.27
Alcaligenaceae	119.0	0.27
Moraxellaceae	104.0	0.23
Rhodobacteraceae	99.0	0.22
Microbacteriaceae	96.0	0.21
Rhodanobacteraceae	90.0	0.20
Proteiniboraceae	88.0	0.20
mle1	88.0	0.20
Solirubrobacteraceae	77.0	0.17
Ferrovibrionaceae	68.0	0.15
Azospirillaceae	66.0	0.15
Ilumatobacteraceae	47.0	0.10
Sphingobacteriaceae	44.0	0.10

AKIW781	41.0	0.09
Saccharimonadales	40.0	0.09
Pseudomonadaceae	38.0	0.08
Rhodocyclaceae	38.0	0.08
MBA03	37.0	0.08
Rhizobiales_Incertae_Sedis	32.0	0.07
Planococcaceae	32.0	0.07
SJA	31.0	0.07
Bacteroidetes_VC2	31.0	0.07
Sedimentibacteraceae	25.0	0.06
SAR324_clade	24.0	0.05
Anaerolineaceae	19.0	0.04
Kaistiaceae	15.0	0.03
Christensenellaceae	15.0	0.03
Izemoplasmatales	13.0	0.03
Propionibacteriaceae	12.0	0.03
Anaerovoracaceae	11.0	0.02
Desulfovibrionaceae	10.0	0.02
Peptostreptococcales	9.0	0.02
UCG	8.0	0.02
Azospirillales_Incertae_Sedis	8.0	0.02
Gracilibacteraceae	7.0	0.02
Micropepsaceae	6.0	0.01
Lachnospiraceae	6.0	0.01
SBR1031	5.0	0.01
Burkholderiaceae	5.0	0.01
Mycobacteriaceae	5.0	0.01

Top Family Classification

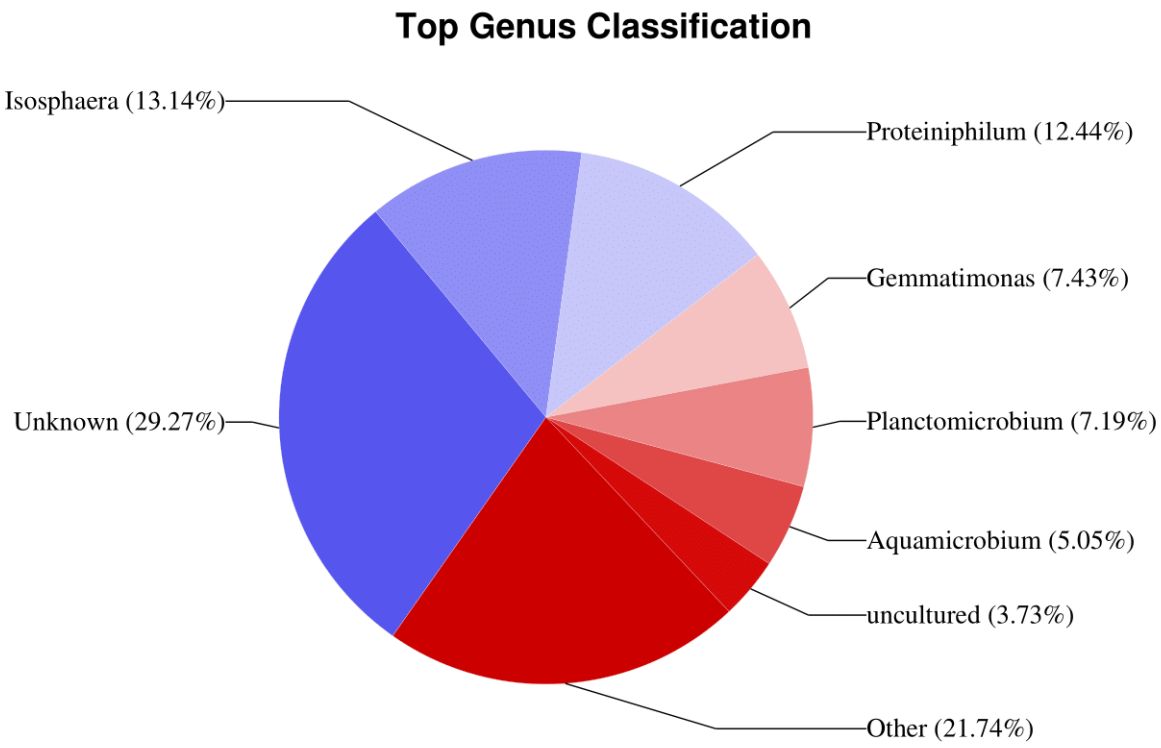


Genus Classification

Genus	Read Count	%
Unknown	13099.0	29.27
Isosphaera	5881.0	13.14
Proteiniphilum	5568.0	12.44
Gemmatimonas	3323.0	7.43
Planctomicrobium	3218.0	7.19
Aquamicrobium	2258.0	5.05
uncultured	1670.0	3.73
Luteimonas	1320.0	2.95
Reyranella	814.0	1.82
Paenibacillus	636.0	1.42
Hydrogenophaga	552.0	1.23
Planctopirus	466.0	1.04
Terrimonas	446.0	1.00
A4b	359.0	0.80
Sphingopyxis	351.0	0.78
Phenylobacterium	310.0	0.69
IMCC26207	275.0	0.61
Roseomonas	229.0	0.51
Shinella	221.0	0.49
Acidovorax	216.0	0.48
Petrimonas	207.0	0.46
Legionella	190.0	0.42
Caenispirillum	157.0	0.35
Bosea	147.0	0.33
JG30	144.0	0.32
Devosia	139.0	0.31
Fermentimonas	126.0	0.28
Rhodococcus	104.0	0.23
Acinetobacter	102.0	0.23
Proteiniborus	88.0	0.20
mle1	88.0	0.20
Stenotrophomonas	81.0	0.18
Bacillus	80.0	0.18
Tahibacter	77.0	0.17
Mesorhizobium	77.0	0.17
Edaphobaculum	76.0	0.17
Pseudorhodoplanes	75.0	0.17
Xanthobacter	74.0	0.17
Hyphomicrobium	70.0	0.16
Ferrovibrio	68.0	0.15
Pseudaminobacter	68.0	0.15

Azospirillum	66.0	0.15
Paracoccus	63.0	0.14
Altererythrobacter	55.0	0.12
Ochrobactrum	47.0	0.11
Microbacterium	47.0	0.11
AKIW781	41.0	0.09
Saccharimonadales	40.0	0.09
Pseudomonas	38.0	0.08
Youhaiella	38.0	0.08
Pedobacter	38.0	0.08
MBA03	37.0	0.08
Brevundimonas	35.0	0.08
SH	33.0	0.07
SJA	31.0	0.07
Comamonas	31.0	0.07
Bacteroidetes_VC2	31.0	0.07
Novosphingobium	30.0	0.07
Pseudolabrys	25.0	0.06
Bradyrhizobium	25.0	0.06
Azospira	25.0	0.06
Sedimentibacter	25.0	0.06
Anoxybacillus	24.0	0.05
Lysinibacillus	24.0	0.05
SAR324_clade	24.0	0.05
Pedomicrobium	24.0	0.05
Pseudorhodoferax	23.0	0.05
Nitratireductor	23.0	0.05
Alicycliphilus	23.0	0.05
Leucobacter	21.0	0.05
Bauldia	19.0	0.04
Lysobacter	19.0	0.04
Pseudoxanthobacter	18.0	0.04
JCM_18997	18.0	0.04
Cohnella	16.0	0.04
Kaistia	15.0	0.03
Xanthobacteraceae	15.0	0.03
alphaI_cluster	14.0	0.03
Christensenellaceae_R	14.0	0.03
Izemoplasmatales	13.0	0.03
Phreatobacter	13.0	0.03
UCG	11.0	0.02
Pseudoxanthomonas	11.0	0.02
Allorhizobium	11.0	0.02

Desulfovibrio	10.0	0.02
Delftia	8.0	0.02
Stella	8.0	0.02
Sphingoaurantiacus	7.0	0.02
Afipia	7.0	0.02
Lutispora	7.0	0.02
Alcaligenes	7.0	0.02
Thauera	6.0	0.01
Sphingomonas	6.0	0.01
Anaerolinea	6.0	0.01
Extensimonas	6.0	0.01
Tepidimonas	5.0	0.01
Amnipila	5.0	0.01
Pseudoflavitalea	5.0	0.01
SBR1031	5.0	0.01
Mycobacterium	5.0	0.01



Species Classification

Species	Read Count	%
Unknown	20327.0	45.52
Isosphaera_uncultured_bacterium	5881.0	13.17
Proteiniphilum_Porphyromonadaceae_bacterium	4716.0	10.56
Gemmatimonas_uncultured_Gemmatimonadetes	3224.0	7.22
Planctomicrobium_uncultured_Planctomyces	3172.0	7.10
Aquamicrobium_uncultured_bacterium	2058.0	4.61
uncultured_uncultured_bacterium	1314.0	2.94
Luteimonas_uncultured_bacterium	1040.0	2.33
Proteiniphilum_Proteiniphilum_saccharofermentans	557.0	1.25
Hydrogenophaga_uncultured_bacterium	402.0	0.90
Petrimonas_Petrimonas_mucosa	196.0	0.44
Caenispirillum_uncultured_Caenispirillum	153.0	0.34
Paenibacillus_uncultured_Firmicutes	118.0	0.26
A4b_uncultured_bacterium	98.0	0.22
mle1_uncultured_bacterium	85.0	0.19
Legionella_Legionella_pneumophila	82.0	0.18
Tahibacter_uncultured_bacterium	77.0	0.17
JG30_uncultured_Chloroflexi	73.0	0.16
uncultured_metagenome	55.0	0.12
Paenibacillus_uncultured_bacterium	49.0	0.11
Stenotrophomonas_Stenotrophomonas_acidaminiphila	49.0	0.11
Luteimonas_Luteimonas_terricola	44.0	0.10
Terrimonas_Hypsibius_dujardini	44.0	0.10
Phenylobacterium_uncultured_bacterium	40.0	0.09
Saccharimonadales_uncultured_bacterium	39.0	0.09
Sphingopyxis_Sphingopyxis_terrae	37.0	0.08
AKIW781_uncultured_Kouleothrix	37.0	0.08
Acinetobacter_Acinetobacter_junii	33.0	0.07
Ferrovibrio_uncultured_bacterium	32.0	0.07
SJA_uncultured_bacterium	31.0	0.07
Proteiniborus_uncultured_bacterium	31.0	0.07
IMCC26207_uncultured_actinobacterium	28.0	0.06
Hyphomicrobium_uncultured_Hyphomicrobiaceae	24.0	0.05
JG30_uncultured_bacterium	21.0	0.05
Fermentimonas_Fermentimonas_caenicola	20.0	0.04
Paracoccus_Paracoccus_versutus	19.0	0.04
Devosia_uncultured_bacterium	19.0	0.04
MBA03_uncultured_bacterium	19.0	0.04
Nitratireductor_Nitratireductor_indicus	19.0	0.04

uncultured_Afipia_sp	18.0	0.04
Pseudorhodoplanes_uncultured_Rhizobiales	18.0	0.04
JG30_uncultured_soil	17.0	0.04
Pseudoxanthobacter_Pseudoxanthobacter_liyangensis	16.0	0.04
Azospirillum_Azospirillum_brasilense	15.0	0.03
Bauldia_metagenome	15.0	0.03
Comamonas_Comamonas_testosteroni	15.0	0.03
Altererythrobacter_uncultured_bacterium	13.0	0.03
Xanthobacteraceae_uncultured_bacterium	13.0	0.03
SAR324_clade_uncultured_bacterium	13.0	0.03
Phenylobacterium_uncultured_Alphaproteobacteria	13.0	0.03
Altererythrobacter_Sphingomonas_sp	12.0	0.03
Desulfovibrio_Desulfovibrio_putealis	10.0	0.02
Anoxybacillus_Anoxybacillus_geothermalis	9.0	0.02
MBA03_uncultured_Clostridiales	9.0	0.02
Sphingopyxis_uncultured_bacterium	9.0	0.02
Pseudolabrys_metagenome	9.0	0.02
Anoxybacillus_Anoxybacillus_sp	9.0	0.02
Pseudomonas_Pseudomonas_aeruginosa	9.0	0.02
SH_uncultured_bacterium	8.0	0.02
Rhodococcus_Rhodococcus_ruber	8.0	0.02
Stella_metagenome	8.0	0.02
Ferrovibrio_metagenome	7.0	0.02
Bosea_uncultured_bacterium	7.0	0.02
Pseudaminobacter_uncultured_bacterium	7.0	0.02
Pseudorhodoplanes_uncultured_Alphaproteobacteria	7.0	0.02
Rhodococcus_Rhodococcus_hoagii	6.0	0.01
Izemoplasmatales_uncultured_bacterium	6.0	0.01
Lysobacter_uncultured_bacterium	6.0	0.01
Acidovorax_uncultured_bacterium	6.0	0.01
Sedimentibacter_iron	6.0	0.01
Sedimentibacter_uncultured_bacterium	6.0	0.01
Reyranella_metagenome	6.0	0.01
Hyphomicrobium_Hyphomicrobium_zavarzinii	6.0	0.01
JCM_18997_Conexibacter_sp	6.0	0.01
Amnipila_uncultured_Anaerovorax	5.0	0.01
Azospirillum_uncultured_Azospirillum	5.0	0.01
UCG_uncultured_bacterium	5.0	0.01
Brevundimonas_uncultured_Caulobacteraceae	5.0	0.01
Leucobacter_Leucobacter_chironomi	5.0	0.01
Christensenellaceae_R_uncultured_bacterium	5.0	0.01
Acinetobacter_uncultured_bacterium	5.0	0.01
Pseudolabrys_Pseudolabrys_taiwanensis	5.0	0.01