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Aerobic Polishing of Liquid Digestate for Preparation of Hydroponic Fertiliser

Lebani Oarabile Joy Mathe, Simira Ramsumer, Hendrik Gideon Brink and Willie Nicol *

Department of Chemical Engineering, University of Pretoria, Pretoria 0002, South Africa; joyoara@gmail.com (L.O.J.M.); u18013580@tuks.co.za (S.R.); deon.brink@up.ac.za (H.G.B.)
* Correspondence: willie.nicol@up.ac.za

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 for promoting 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, this pH condition resulted in decreased microbial longevity and, therefore, increased ammonification due to microbial decay. Therefore, 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 a Hoagland solution. The 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: Mathe, L.O.J.; Ramsumer, S.; Brink, H.G.; Nicol, W. Aerobic Polishing of Liquid Digestate for Preparation of Hydroponic Fertiliser. *Sustainability* **2024**, *16*, 4077. https://doi.org/10.3390/su16104077

Academic Editor: Giovanni Esposito

Received: 3 April 2024
Revised: 7 May 2024
Accepted: 8 May 2024
Published: 13 May 2024

1. Introduction

The circular economy is integral to the future of sustainable practices, especially in the agricultural sector, where the recycling of essential nutrients and minimisation of nutrient spillage are the main concerns. Nitrogen (N) and phosphorus (P) are essential macronutrients in agriculture, and along with other elements, they 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 the water and ultimately reduce biodiversity and harm aquatic life. Agricultural runoff produces leachate rich in N and P, which pollutes groundwater sources. The worldwide aim is to reduce the spillage of N and P by decreasing synthetic production and unsustainable usage while simultaneously enhancing the recycling of these compounds. The treatment of organic waste plays an imperative role in facilitating nutrient recycling.

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 has

applications in the energy sector in addition to the agricultural sector. Anaerobic digestion, which is the process wherein organic matter is broken down by microorganisms in the absence of oxygen [5], is one of the most successful strategies of processing organic waste to produce biogas and fertiliser. Renewable energy and fuel generation from biogas have since become widespread in countries north of the equator, namely, China, Germany, and Sweden, which are members of the IEA Bioenergy Task 37 [6]. Findings from the 2022 IEA report show that China and Germany have the highest number of biogas plants among the IEA members, with 100,000 and 10,000 plants, respectively [6].

The digestate produced from biogas production in European countries, which is approximated to stand at 56 million tonnes per year [7], has primarily been used as a biofertiliser, which has not only allowed the agricultural industry to become more sustainable but also contributed to the EU's commitment to reduce greenhouse gas emissions by 55% by 2030 [8]. Liquid digestate, which accounts for 94% of the digestate produced in Germany, is used as a biofertiliser [7] and is preferred by farmers due to its ease of handling and potential to mix with herbicides. These trends show that the current European practices regarding anaerobic digestion processes have a significant influence on the concept of nutrient recovery, with the added benefit of renewable energy carrier production in the form of methane-rich biogas, which also contributes to achieving SDG 7 [9].

It is evident that the growing biogas industry has played a significant role in facilitating nutrient recycle in soil-based agriculture; however, the increase in the global population has also affected the availability of arable land, and trends suggest that soilless agriculture will become an important supplement in the production of nutritious greens [10,11]. This raises the question of whether digestate, and specifically liquid digestate (LD), can be used as an effective fertiliser in soilless agricultural applications. Soilless agriculture, specifically hydroponics, are suspended growth systems that make use of nutrient-rich liquid mediums like Hoagland's medium. Hydroponic systems have many advantages, including but not limited to high yield and lower water consumption, and they are also favoured for their ability to investigate the effects of nutrients on leaves and fruits [12]. Most hydroponic systems are set up within a controlled environment, which allows for the continued growth of plants irrespective of climate and other external environmental factors [13]. Given the growth of the soilless production industry [10], it is imperative to consider nutrient recycling options to reduce the production and usage of synthetic fertilisers.

A study by Ronga [14] on the use of LD for the cultivation of baby leaf lettuce (*Lactuca sativa* L.) showed the possibility of using digestate to produce high-yielding crops. Another study, conducted by Bergstrand [15], who investigated the cultivation of Pak Choi in a hydroponically controlled climate, also concluded that organic solutions containing LD are valuable for plant growth. The feasibility of digestate as a sole nutrient source for Pak Choi was investigated by Weimers [16], and similar yields to commercial fertilisers were obtained from the digestate. These studies show biogas digestate is a viable biofertiliser in hydroponic systems, and this further supports a circular economy.

Due to its applications in the agricultural sector, it is important to know the composition of 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. Rizziloi et al. [17] state that most digestate produced in Europe contains 2–5 kg/m^3 of N and 0.5–1.5 kg/m^3 of P. Tuszynska et al. conducted a study on the P and N fractions in LD from agricultural biogas plants and found that the fractions varied greatly, with N ranging from 1363 to 3211 mg/L and P from 230.9 to 649.1 mg/L [18]. Compared to Hoagland's solution, which, according to da Silva et al. [19], contains 0.136 g/L of P and 0.505 g/L, in some instances, the stated N and P fractions in LD are slightly lower and would therefore need to be supplemented for effective use in hydroponic systems that use a full-strength Hoagland's medium.

Crude digestate contains a significant amount of organic carbon, with TOC values in the range of 0.5–3 g/L, while the N is mostly in the form of ammonia [20]. Organic carbon being present in hydroponic media typically results in the growth of heterotrophic

bacteria on plant roots, which has the potential to severely inhibit growth [13]; this is also referred to as Pythium root rot [21]. In addition, plants prefer N in the form of nitrate as opposed to ammonia [22,23] and, accordingly, crude digestate requires further processing before being used as an effective hydroponic medium. The first processing step entails the removal of organic carbon and is referred to as the polishing step. This step is aerobic and shares similarities with a conventional biological wastewater treatment system. The main difference between digestate polishing and wastewater purification is that the N and phosphate removal in the digestate polishing should be minimised, unlike biological wastewater treatment, where the complete removal of carbon, N, and P is pursued [24,25]. The second processing step entails the conversion of ammonia to nitrate by chemoautotrophic nitrifiers [26,27]. This processing step is referred to as nitrification, wherein the bacteria use inorganic carbon in the form of CO_2 [28] and typically prefer a low organic carbon content; accordingly, the nitrification step should be performed after the polishing step. It is imperative to minimise the organic carbon present in the LD in order to optimise the nitrification process and prevent microbial growth in closed hydroponic systems [29]. In some cases, the nitrification and hydroponics can be performed in a single unit, like in the work of van Rooyen [22], which focused on managing the use of N in nitrification–hydroponic systems wherein the nitrifiers were grown in the absence of organic carbon.

This study introduces a novel, not previously documented, method for creating a hydroponic growth medium from liquid digestate, incorporating aerobic digestion to streamline the process and improve effluent cleanliness. It explores the use of a biofilm system to polish liquid digestate from anaerobic digestion in order to remove organic carbon while retaining nitrogen and phosphate. Batch experiments were conducted under mesophilic conditions of 35 °C and neutral pH within the range of 6–8 [30], along with investigations into operational modes. The study presents a successful bioreactor concept for achieving these objectives.

2. Materials and Methods

2.1. Medium and Medium Preparation

The liquid digestate (LD) was prepared according to the method described by Gonde [31] so that the solids present in each flask accounted for 5% of the total mass. Cow manure was collected from the experimental farm on the Hillcrest Campus of the University of Pretoria, South Africa (S 25°4′1″, E 28°1′4″). Additionally, IMBO® red split lentils (Pioneer Foods, Cape Town, South Africa) were used, and the distilled water used was sourced from the University of Pretoria's labs. Eight 1L Schott (New York, NY, USA) bottles were filled with 600 mL of the digestion medium, containing 36.59 g of cooked red lentils, 100 g of fresh cow manure, and 463.41 mL of deionised water, which was blended to homogenise the mixture.

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 5 M solution of NaOH and a 3 M 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. An analysis of the digestate indicated an average COD value of 12,000 mg/L and an average ammonium concentration of 845 mg/L.

Next, 1.2 L of the process medium was prepared by diluting the LD by a factor of 4. The digestate was centrifuged for 10 min at 9000 rpm (13,584× *g*), 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 carried out to minimise any suspended solids that would cause blockages in the piping of the reactor. The averages ± standard deviations of the concentrations of the diluted digestate measurements for all runs (pH 6, 7, and 8; batch runs; and continuous operation) are shown in Table 1.

Table 1. Measured averages ± standard deviations of the concentrations for quadruple measurements of the diluted digestate.

Component	Concentration (mg/L)
COD	3627.7 ± 177.4
TOC	631.1 ± 5.4
NH_4^+	235.6 ± 22.7
NO_3^-	24.2 ± 1.2
PO_4^{3-}	24.3 ± 2.5

2.2. Polishing Unit Design and Operation

The polishing unit was modified from the fermenter design used to produce fumaric acid from *Rhizopus oryzae* by Swart [32], 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. In the centre of the reactor, a polypropylene tube, onto which the biofilm was meant to adhere, was positioned. A scalpel was used to create notches in the tube's surface to promote biofilm attachment. Lab-grade air containing 20% oxygen and 80% N_2 was continuously sparged at a rate of 200 mL/min for all batch runs from the bottom of the reactor. The flow rate of the feed gas was controlled using a 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 [33].

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 1 M NaOH and 1 M HCl solutions. The temperature was maintained at 35 °C. The reactor was sampled daily in the same time interval, and the samples were stored at −40 °C to prevent any further digestion.

A schematic representation of the reactor modified from the configuration used by Swart et al. [32] is shown in Figure 1 below.

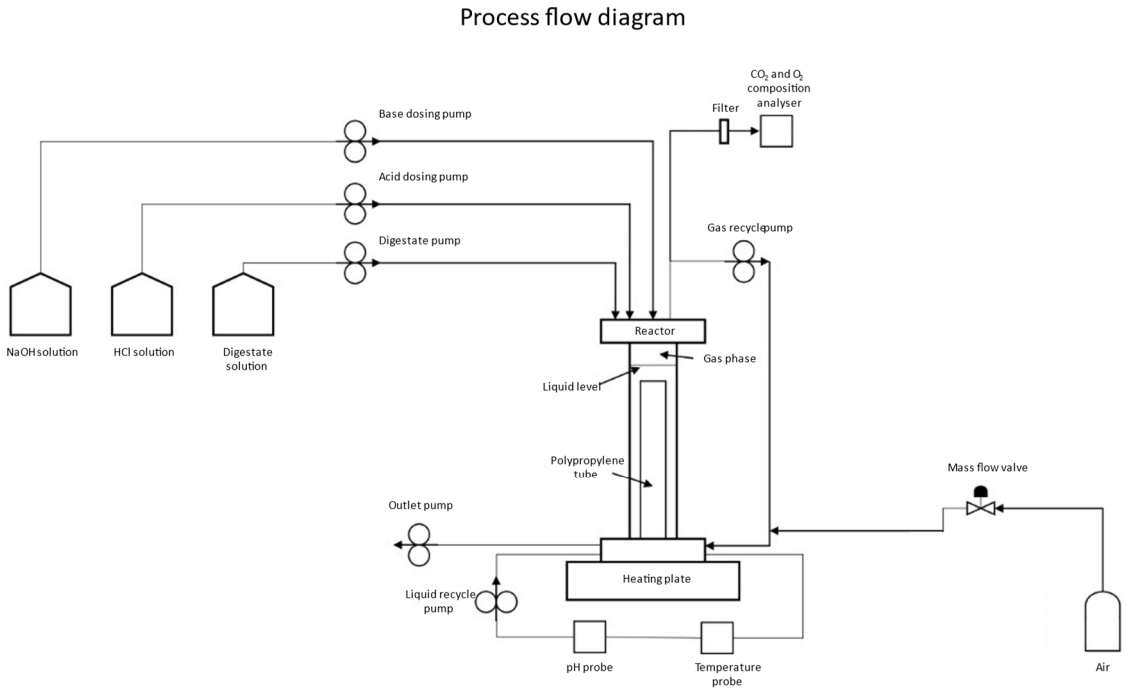

Figure 1. Schematic representation of the aerobic polishing unit showing all equipment and their relevant control mechanisms. This representation has been modified from the work conducted by Swart et al. [32,33].

For continuous operation, an additional 120U Watson-Marlow pump was added to the setup to allow for the continuous addition of the process medium, i.e., the digestate depicted in Figure 1, 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 form at pH 6, and the pH was increased to 7 after four days, at which point the digestate was continuously added. The rate of the continuous addition of the substrate was determined from the results of the repeat batch process.

2.3. Analytical Methods

An analysis of the samples taken from the reactor was performed using the Spectroquant Nitrate Test Kit, Phosphate Test Kit, Ammonium Test Kit, COD Cell Test Kit, and the Total N Cell Test Kit, which were all procured from MERCK KGaA (Darmstadt, Germany). The 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 a 4 mL microcuvette and analysed at a wavelength of 690 nm. For the nitrate test, the wavelength was set to 340 nm. The cell test kits for the COD, TOC, and total N were analysed using the Spectroquant CombiCheck 700 from (MERCK KGaA, Darmstadt, Germany). TOC analysis was conducted using a Shimadzu TOC-V analyser (Kyoto, Japan).

2.4. ngDNA Sequencing

To determine the microbial consortia present in the reactor, a sample of the biofilm in the reactor was sent to Inqaba Biotec (Pretoria, South Africa), who conducted a 16S full-scale gene metagenomic analysis on the sample.

3. Results

This study aimed to remove organic carbon from the system while retaining the nitrogen in the digestate to ultimately improve the efficiency of the proposed downstream process. Organic carbon was quantified by total organic carbon (TOC) and chemical oxygen demand (COD) measurements. The relationship between TOC and COD are shown in Figure A1 and discussed Appendix A. Most of the nitrogen in liquid digestate (LD) is in the form of ammonia [20], while small amounts of nitrate are also detectable. Ultimately the hydroponic medium should contain nitrate as the major nitrogenous component since high concentrations of ammonia can be toxic to plants [30]. This implies that nitrification [13,15,16] should be performed in addition to organic carbon removal. A study conducted by Hu et al. [34] indicates a correlation between increased C/N ratios and increased nitrogen losses during nitrification, which also highlights the importance of minimising the organic carbon prior to nitrification. In this study, the aim was not to convert ammonia to nitrate, although both ammonium and nitrate were measured to holistically characterise the nitrogen in the system; rather, the aim was to elucidate whether nitrification did occur in the aerobic polishing unit. Lastly, phosphate consumption was also quantified.

3.1. Repeat Batch Experiments

Figure 2 illustrates the results of the analysis of the TOC, COD, ammonium, nitrate, and phosphate profiles from the investigation of the effect of pH on the aerobic polishing of LD in a repeat batch process. The process conditions, namely, the temperature and air flow rate, were kept constant throughout the experiments at 35 °C and 200 mL/min, respectively. All runs were performed in duplicate, and the error bars in Figure 2 indicate the variation between the two runs using the standard error [35].

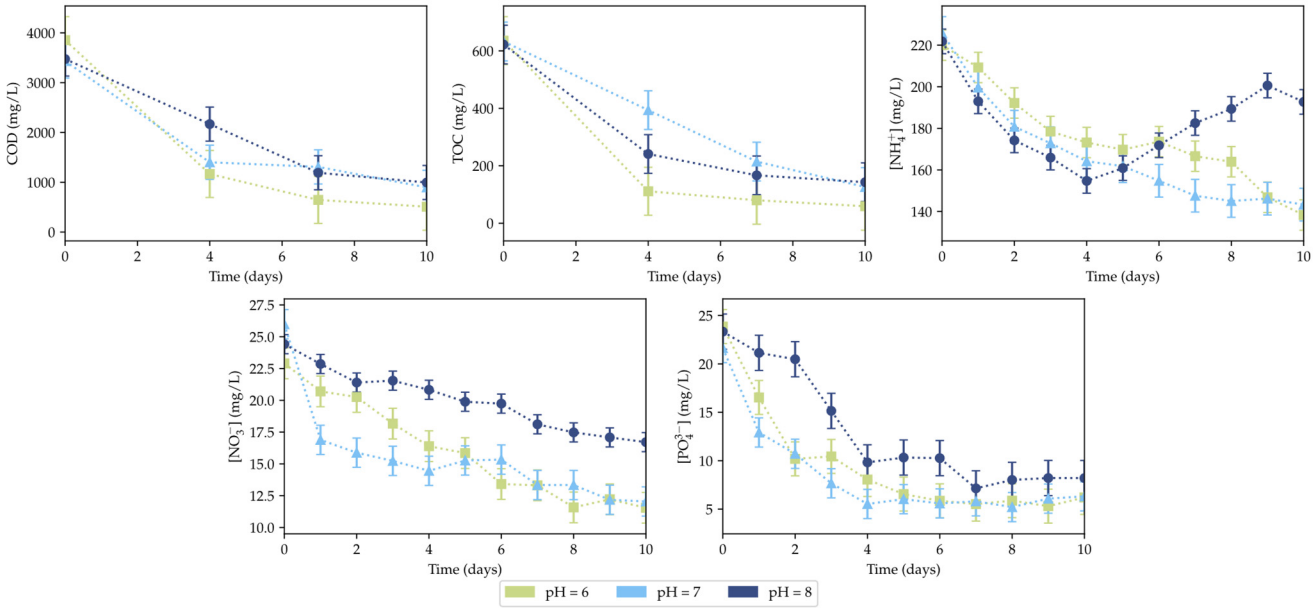

Figure 2. Figure indicating the changes in the TOC, ammonium concentration, nitrate concentration, and phosphate concentration as a function of time due to a change in pH. pH 6 is shown in green with square markers, pH 7 is shown in light blue with triangular markers, and pH 8 is shown in dark blue with circular markers. The averaged results are depicted, with the error bars indicating the standard error.

The data in Figure 2 show a significant conversion of organic carbon over the 10 days of operation, as is shown by the COD and TOC profiles. Minimising the organic carbon is essential to optimise the nitrification process and limit the possibility of bacterial growth, especially within closed hydroponic systems [29]. In addition to the nutrients quantified in Figure 2, organic matter, represented by the TOC and COD profiles, was also consumed to different extents throughout all the experiments. These profiles give an indication of the microbial carbon consumption, and it can also be seen that the lowest pH of 6 displayed the fastest consumption of organic matter in the TOC and COD profiles, and the same was observed at this pH for all other nutrients analysed in this study. This indicates a preference of the microbial consortia established in the reactor for a neutral to slightly acidic pH environment, as the same trends were observed even in the duplicate experiments. The slowest rate of COD consumption was observed at pH 8, whereas in the TOC analysis, this is occurred at pH 7. Li et al. [36] state 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. However, seeing that TOC is only a measure of the organic carbon, it is plausible that at pH 8, the oxidation of non-carbon-containing molecules occurred slower than at pH 6 and pH 7.

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 mineralises N to ammonium [15], which, apart from the organic carbon, is the only other component (of a notable concentration) that can be further oxidised (to NO_3^-) [37]. However, the COD tests made use of potassium dichromate, which is a very strong oxidising agent that does not account for the oxygen demand due to nitrification [38]. The TOC and COD profiles in Figure 2 depict the depletion of most of the organic carbon, and it would therefore follow that bacterial growth and cell maintenance occurred at all the evaluated pH values, and the analysis also shows that pH 6 is optimal for the rapid removal of organic carbon. The majority of the bacteria present in the LD are heterotrophic, as shown by the 16S metagenomic sequencing (discussed further in Section 3.3), since they use organic and inorganic compounds for growth [39].

In terms of quantity, apart from the organic carbon, the ammonium is the largest contributor of nutrients to the system, as its concentration is approximately ten times that of the nitrate. The nitrogenous compounds present will make up the nitrogen content of the hydroponic medium; therefore, it is imperative to minimise nitrogen losses and/or consumption in the reactor. Kirchman [40] states that heterotrophic bacteria frequently account for a large fraction of ammonium uptake in aquatic systems; therefore, it follows that most of the nitrogen loss in the system is attributed to ammonium consumption by the heterotrophs present in the reactor.

However, this is not particularly the case for pH 8, and this anomaly can be attributed to microbial stress. In Figure 2, initially decreasing trends are shown for all experiments with pH 8 showing the fastest ammonium consumption and pH 6 showing the slowest. However, halfway through the established runtime, pH 8 experienced an increase in ammonium concentration, while all other nutrients were still decreasing. This behaviour could be attributed to endogenous respiration, where active cells utilise cell material [41]; however, this only occurs in the complete absence of a carbon substrate. Figure 2 indicates that on day 4, there were still sufficient amounts of all nutrients to allow for continued microbial growth, which nullifies this hypothesis. Ammonification is a step in the N cycle where organic N is converted to ammonia (ammonium), which is sourced from dead microorganisms [42]. The data suggest ammonification occurs after 4 days at pH 8, which is induced by the alkali conditions and is within the optimum pH range for ammonification [42]. It therefore follows that, in this instance, this is the only plausible explanation for the increase in ammonium concentration. From a nitrogen loss perspective, the increase in ammonium after the turning point is promising for recovery; however, since the organic N being converted to ammonium is sourced from decaying microorganisms, long-term operation at this condition may be problematic for the longevity of the microbial consortia in the biofilm.

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 [37]. The decrease in concentration over time is attributed to the assimilation of nitrate by NAB (nitrate-assimilating bacteria) [43] that can then be used for growth, which have also been identified (*Luteimonas* spp.) in metagenomic analyses [44]. The effect of pH on nitrate is documented in a study conducted by Akpor et al. [45], where optimum nitrate uptake was observed at pH 6–7, which is congruent with the findings shown in Figure 2.

Throughout all the experiments, the oxygen consumption should be analysed in tandem with the consumption of the nutrients present in the reactor. As stated by Dodds [46], the continued growth of the bacteria is driven by the mobilisation of organic carbon and the inorganic nutrients (ammonium, nitrate, and phosphate). In an aerobic environment, the bacteria would grow rapidly due to the vast energy produced as adenosine triphosphate (ATP) during oxidative phosphorylation [47].

A total of 288 L/day of lab-grade air (80% nitrogen and 20% oxygen) was sparged at a constant rate to the polishing unit. Thus, 57.6 L/day of O_2 was supplied, and the measured O_2 flow in the off gas is shown in Figure 3. The biofilm was reused throughout the runs, which allowed for the continued growth and accumulation of the microbial population [39]. This is supported by the findings in Figure 3, which indicates that the oxygen content of the off gas continuously decreased throughout all the experiments, with a slower rate in the latter runs. This indicates that per run, more oxygen is being taken up by the microbial colony, which is ever increasing. However, from Run 3 onward, flatter slopes can be observed for the experiments. The steep slopes of Run 1 indicate rapid growth throughout the duration of the respective runs, and flat slopes can be observed for the subsequent runs.

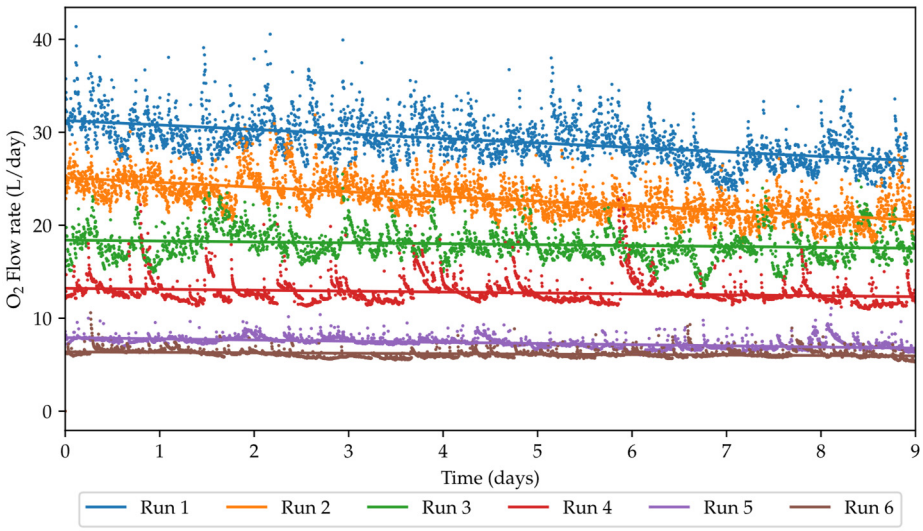

Figure 3. Comparison of oxygen consumption per run in the aerobic polishing unit.

Although variance can be observed in the profiles shown in Figure 3, this is not attributed to a decrease in biomass growth, but rather the reaching of a steady state for the microbial colony with each run. As stated by Garcia-Ochoa [48], the oxygen utilisation rate is limited by the maximum oxygen transfer rate, and the shapes of the latter profiles allude to the approach of a oxygen consumption limit, which, when reached, will hinder the growth of the colony [48]. In further studies, these rates and limits will be determined and discussed in detail.

However, in light of these findings, it is important to note that throughout the continued growth of the biofilm, the observed effects of pH on the consumption of nutrients did not vary by a significant margin other than what can be expected in a repeat batch experiment with a continuously reused biofilm. It should be noted that the oxygen profiles shown are included as supplementary to the information discussed previously to aid the analysis of the findings. In this instance, the oxygen uptake and utilisation rates were not investigated seeing as the aim of the study was to prove the viability of aerobic polishing under the effect of pH.

Ultimately, the findings from the repeat batch experiments clearly indicate that nitrogen and, to a lesser extent, phosphorus retention within the solution is possible while simultaneously removing most of the dissolved organic carbon. These findings are also consistent with documented figures from other studies, such as that of Kirchman et al. [40], who reported a similar ammonium uptake of 36%, which was observed at pH 6–7. The 47% and 54% decreases in nitrate concentration at pH 6 and pH 7, respectively, are consistent with the findings of Akpor et al. [45]. Figure 2 shows a 73% decrease in phosphate at pH 6 and pH 7 and a 64% decrease at pH 8. Based on the data shown in Figure 2, pH 7 is the ideal pH for microbial growth in this system, with the least N and P consumption sans microbial decay. Although pH 6 provides a larger decrease in organic carbon, the rapid growth also comes at the expense of the essential nutrients, which is not ideal for nutrient recovery. Overall, the repeat batch mode of operation is successful in achieving the objective of the polishing unit of removing carbon while retaining fertiliser components.

3.2. Continuous Experiments

For the repeat batch experiments, the matured biofilm was reused from run to run (Figure 4). The repeatability of the repeat batch runs suggests that the biofilm quantity on the supplied support remains more or less constant. It therefore follows that the amount of biofilm growth is approximately equal to the amount of biofilm shedding—where the shedded biomass is removed with the polished effluent. Continuous operation presents as an alternative to repeat batch operation, wherein shedded biofilm is removed continuously

with the polished digestate and separated to prepare the product for downstream use. Through continuous operation, it is possible to reduce microbial growth while observing the maintenance requirements of the biofilm by operating at low carbon substrate concentrations [49]. Carbon limitation initiates starvation, which drives cells into the maintenance regime, where the production of cellular ATP is prioritised [50]; therefore, the requirement for growth nutrients like N and P, which are used in the anabolism for building new cellular material, will be minimised [51]. With the utilisation of N and P being limited while organic carbon is still being removed, the objective of retaining the nutrients within the solution will be met. Thus, a continuous run was also studied to investigate the magnitude of nutrient recovery possible with the strategy.

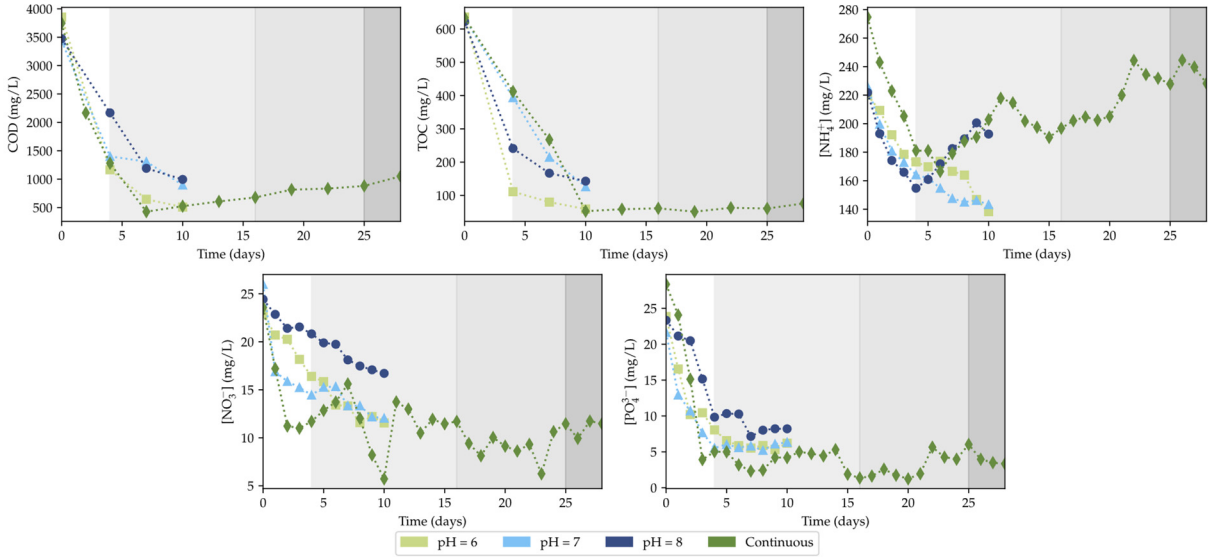

Figure 4. Comparison of the batch and continuous profiles. The shaded regions depict the various hydraulic retention times (τ = 7.38 days, τ = 3.18 days, and τ = 1.63 days) from light to dark, respectively. The values shown are the averaged values of the repeats, and the error bars depict the standard error.

The concentrations shown in Table 2 were calculated by multiplying each HRT with the relevant concentration from Table 1. The continuous run was first conducted in batches at pH 6 to rapidly minimise the COD to approximately 1000 mg/L, and thereafter, the pH was changed to 7 for the rest of the run to minimise the continued loss of nutrients. No other additional pH changes took place. The flow rate for the digestate being added was doubled on two instances to determine the effect of additional nutrients on the microbial uptake of nutrients. This helped us 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 was observed from day 13 onwards, when the concentration began to increase over time. 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 the consumption of organic carbon, which is one of the main purposes of this study. Even with the continuous addition of the substrate, the organic matter readings remained low, leaving little feed for heterotrophic bacteria in further steps.

Table 2. Concentrations of nutrients added at the different hydraulic retention times (HRTs) derived from Table 1.

	Concentration (mg/day)		
Component	**τ = 7.38**	**τ = 3.18**	**τ = 1.63**
COD	511.88	1187.59	2319.42
TOC	87.75	203.59	397.61
NH_4^+	32.18	74.65	145.79
NO_3^-	3.51	8.14	15.9
PO_4^{3-}	3.36	7.8	15.24

As shown in Figure 3, the TOC data for all the runs decrease, which further proves the hypothesis of the consumption or depletion of organic matter in the system, specifically organic carbon. However, compared to the COD, especially for the continuous run, an increase is observable, which is not present in the TOC data. The nutrient content of LD varies depending on feed, manure, and retention time; however, for reference, as discussed by Weimers [16], 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 3 emphasise the presence of non-organic minerals that are chemically oxidisable as proposed by Li et al. [36]—the increase in COD while TOC remains low stems 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 the 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 can be observed for the continuous run, while the organic carbon is sufficiently removed even with increases in the HRT. The N content in the polished product is >220 mg/L NH_4^+—N, which is higher than a standard Hoagland medium, which contains roughly 210 mg/L total N [52]. In addition, with the remaining—NO_3—N, 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, as previous findings show, most hydroponic systems use [53].

However, phosphate losses are significant, with a noted 88% decrease for the continuous run and a maximum of 75% for the batch runs. Yao et al. [1] state that phosphorus is vital for biological information storage and energy transfer, among other crucial systems. *Gemmatimonas* spp., which is present in the biofilm, is a genus that is known to be responsible for the rapid removal of phosphorus [54]. Therefore, it is imperative to maintain the phosphorus levels in the polished product, as phosphate starvation in plants leads to severe damage to the roots while plant immunity is further repressed [55]. A standard Hoagland solution contains 31 mg/L [52] P, which is far greater than the P present in the polished product. Therefore, prior to use in a hydroponic system, the phosphate levels would need to be supplemented.

The data for the continuous run show 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 [52]. The analysis shows that even with the continued addition of LD, the organic carbon is rapidly taken up, as the TOC values remained below 100 mg/L 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. The findings from this run indicate that the continuous run is the superior method for using the aerobic polishing process to prepare a hydroponic medium.

3.3. Bacterial Characterisation Using ngDNA Sequencing

Bacterial identification was outsourced to Inqaba Biotec (Pretoria, South Africa) who conducted a 16S metagenomic analysis on a sample from the biofilm, identifying the prevalent species summarised in Table 3. Most of the species identified in the analysis were found to be aerobic, with some exceptions for facultative anaerobes and species which exist in soil. Furthermore, the analysis identified 83 different bacterial species which are documented in detail in the Supplementary Materials, and it was found that *Proteobacteria* and *Planctomycetota* accounted for over 50% of the phyla classifications in the biofilm. Roopnarain [56] states that mesophilic digestate contains a high microbial diversity, which was further indicated by the metagenomic analysis of the biofilm, which was grown through the aerobic digestion of liquid digestate. Despite being pivotal to the anaerobic digestion process, no methanogenic bacteria were identified in the microbial analysis given that methanogens are obligate anaerobes [57].

The findings in Table 3 indicate that most of the bacteria in the biofilm are 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, nitrogen, and phosphorus for growth [39]. The rapid removal of the TOC is offset by the combined effect of all the heterotrophs in the biofilm. Along with *Gemmatimonas* spp., the rapid phosphate removal observed in Figure 3 is also attributed to *Terrimonas* spp., which has been identified as a phosphorus mobilising bacteria [58]. The optimal pH ranges of the bacteria listed in Table 3 illustrate that most of the microbes thrive at near-neutral pH values [54,59–61]. 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 was observed in the continuous run with the increases in nitrogen concentration at pH 7, which represents the optimal pH for this genus [60]. Table 3 lists the predominant genomes identified in the metagenomic analysis and serves to characterise their effect. While other species are present in the reactor, their relatively low concentrations render their effects negligible compared to the prevalent bacteria. 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 [44]. Although it is difficult to accurately ascertain the specific effect of a bacterial species and its magnitude in a multi-cultural biofilm such as this, the comprehensive analysis of the biofilm does highlight the key species which contributed to the observed trends regarding nutrient consumption.

Table 3. Summary of the prevalent species detected in the 16S metagenomic analysis of the biofilm.

Genus	Heterotrophic	Optimum pH	Applications	Reference
Proteiniphilum	Yes	7.1–7.8	Facultative anaerobe found in anaerobic digesters	[62,63]
Gemmatimonas	Yes	7.0	Aerobic bacteria linked to rapid phosphorus removal in wastewater treatment	[54]
Planctomicrobium	Yes	6.0–6.5	Aquatic aerobe found in wetlands with a preference for organic sugars	[59,64]
Aquamicrobium	Yes	6.5–7.5	Strictly aerobic marine bacteria capable of reducing nitrate	[61]
Luteimonas	Yes	7.0	Aerobic bacteria found in wastewater treatment plants capable of increasing nitrogen acquisition	[44,60]

4. Discussion

The results of this investigation indicate that the aerobic polishing of LD is a viable tool for the minimisation of organic carbon while optimal levels of nutrients are maintained. This adaptation of a well-known wastewater treatment system—the attached growth system—has shown viability for the specialised treatment of LD prior to hydroponic fertiliser use. It has also been observed that pH plays a significant role in influencing the rate at which these processes occur, and it can either hinder or promote nutrient recovery. Previous studies conducted on the use of LD as a hydroponic fertiliser have employed different mechanisms of minimising the organic carbon, such as longer anaerobic digestion times and various separation techniques such as dilution and multiple rounds of centrifugation prior to nitrifying the LD. The data also show that the implementation of a continuous reactor is essential to the goal of nutrient recovery for hydroponic use by minimising the organic carbon while retaining a large fraction of N. In the continuous system, the organic carbon remains below 100 mg/L, while the final N concentration is above 220 mg/L. This approach offers a more efficient method of preparing hydroponic growth mediums by minimising the time between the anaerobic digestion and nitrification step and providing a cleaner effluent, improving nitrification efficacy. The success of the process is also attributed to the microbial colony present in the biofilm, which contains heterotrophic organisms that also contribute to the observed nitrogen recovery. Although the final phosphate concentration is low, it can be supplemented to produce a near-hydroponic-ready medium. Additionally, this method would also entail an additional unit for the aerobic processing of the medium, which may prove more costly and cause complications for existing processes in terms of space. To improve this research, further detailed studies also including mass and energy balances should be conducted on the continuous method and which hydraulic times maximise the removal of organic carbon while high levels of nutrients are maintained. Furthermore, in-depth investigations should be carried out on the microbial population and its oxygen utilisation and uptake rates and the optimisation thereof. The cost implications of the large-scale implementation of this research, which can only be accurately determined once the oxygen usage has been optimised, should also be investigated to assess the feasibility of this novel approach.

5. Conclusions

It is evident from the results that LD polishing for hydroponic use can be successfully achieved with the proposed biofilm system. Continuous operation at a pH of 7 resulted in the best removal of carbon and retainment of nitrogen, with the phosphate retainment being unsatisfactory. The study paves the way for improving the design of LD polishing units, contributing to the concept of enhanced recycling in soilless agricultural systems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su16104077/s1.

Author Contributions: Conceptualisation, L.O.J.M., S.R., H.G.B. and W.N.; methodology, L.O.J.M. and S.R.; software, L.O.J.M. and S.R.; validation, L.O.J.M., H.G.B. and W.N.; formal analysis, L.O.J.M., H.G.B. and W.N.; investigation, L.O.J.M. and S.R.; resources, H.G.B. and W.N.; data curation, L.O.J.M.; writing—original draft preparation, L.O.J.M., H.G.B. and W.N.; writing—review and editing, L.O.J.M., H.G.B. and W.N.; visualisation, L.O.J.M. and S.R.; supervision, H.G.B. and W.N.; project administration W.N.; funding acquisition, H.G.B. and W.N. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Dataset available on request from the authors.

Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

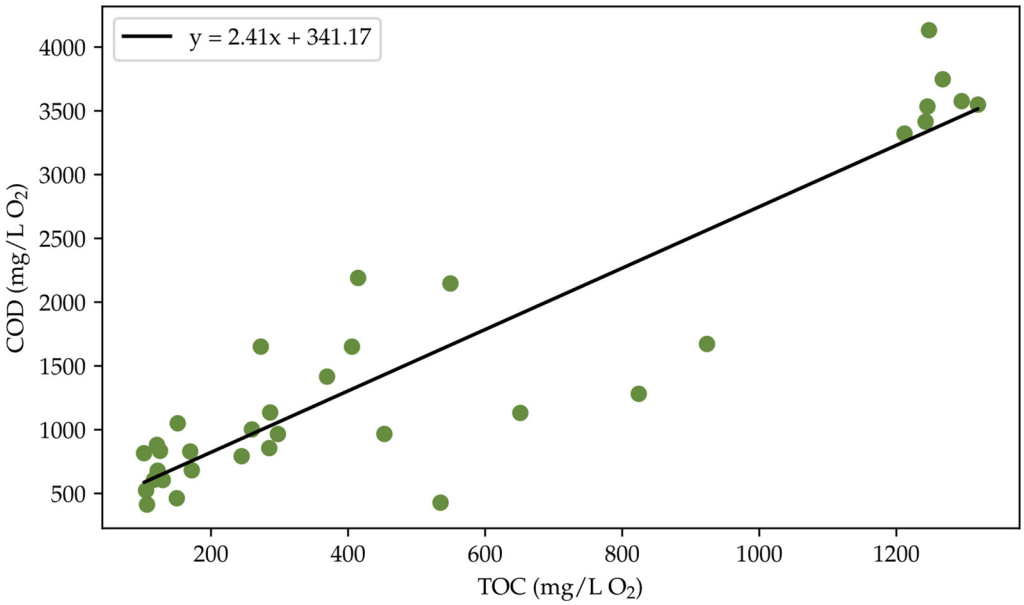

Figure A1. Correlation between TOC and COD for all experimental measurements, with line of best fit indicated in black.

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 mineralises N to ammonium [15], which, apart from the organic carbon, is the only other component (of a notable concentration) that can be further oxidised (to NO_3^-) [37]. However, the COD tests made use of potassium dichromate, which is a very strong oxidising agent that does not account for the oxygen demand due to nitrification [38]. 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 found for n sample points, and in accordance with the equation for the linear regression shown in Figure A1, it was found that $R^2 = 0.77$, which proves the statistically significant relationship between the TOC and COD measurements [65]. 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. These data are consistent with the findings from Dubber's study [66], wherein the proportionality between TOC and COD is reported.

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