applied sciences

MDPI

*Article*

# Biodefoamer-Supported Activated Sludge System for the Treatment of Poultry Slaughterhouse Wastewater

**Cynthia Dlangamandla [1], Seteno K. O. Ntwampe [2], Moses Basitere [3,*], Boredi S. Chidi [1] and Benjamin I. Okeleye [1]**

[1] Bioresource Engineering Research Group (*BioERG*), Cape Peninsula University of Technology, P.O. Box 652, Cape Town 8000, South Africa; dlangamandlac@cput.ac.za (C.D.); chidib@cput.ac.za (B.S.C.); biokeleye@hallmarkuniversity.edu.ng (B.I.O.)
[2] Department of Chemical Engineering Technology, Doornfontein Campus, University of Johannesburg, P.O. Box 524, Auckland Park, Johannesburg 2006, South Africa; karabosntwampe@gmail.com
[3] Academic Support Programme for Engineering in Cape Town (ASPECT), Water Research Group, Department of Civil Engineering, University of Cape Town, P.O. Box 3408, Cape Town 7700, South Africa
* Correspondence: moses.basitere@uct.ac.za; Tel.: +27-21-650-3238

**Abstract:** Poultry slaughterhouse wastewater (PSW) is laden with fats, oil, and grease (FOG), as well as proteins. As such, PSW promotes the proliferation of filamentous organisms, which cause foam formation. In this study, the production of biological defoamers (biodefoamers) uses a consortium with antagonistic properties, i.e., 1.39 L of wastewater/mL defoamers, as reported in our previous study, toward foam formers and their application in the treatment of PSW using a bench-scale activated sludge (AS)-supported treatment system consisting of an aeration and clarification tank. The foam produced was slimy, brown, and thick, suggesting the presence of *Nocardia*, *Microthrix*, and Type 1863 species in the PSW/AS wastewater treatment system. The bio (Bio-AS) and synthetic-defoamers (Syn-AS, positive control) supplementation, i.e., at 4% $v/v$ in the PSW/AS primary treatment stage (aeration tank) operated over ten days, resulted in 94% and 98% FOG and protein removal for the biodefoamers, respectively, when compared to 50% and 92% for a synthetic defoamer, respectively. Similarly, the Bio-AS treatment achieved 85.4% COD removal, while a lowly 51% was observed for the Syn-AS PSW treatment regime. Overall, the biodefoamers performed vehemently compared to synthetic defoamers, improving the PSW/AS system's performance. It was prudent to hypothesize that the biodefoamers might have had FOG solubilization attributes, an assertion that needs further research in future studies. It was concluded that Bio-AS was more efficient in the removal of FOG, proteins, TSS, and COD in comparison to Syn-AS and negative control without supplementation (CAS).

**Keywords:** activated sludge; chemical oxygen demand; defoamers; fats–oil–grease removal; *Nocardia* spp.; poultry slaughterhouse wastewater; protein removal



**Citation:** Dlangamandla, C.; Ntwampe, S.K.O.; Basitere, M.; Chidi, B.S.; Okeleye, B.I. Biodefoamer-Supported Activated Sludge System for the Treatment of Poultry Slaughterhouse Wastewater. *Appl. Sci.* **2023**, *13*, 9225. https://doi.org/10.3390/app13169225

Academic Editor: José Carlos Magalhães Pires

Received: 19 June 2023
Revised: 4 August 2023
Accepted: 10 August 2023
Published: 14 August 2023



## 1. Introduction

Wastewater treatment, management, and reuse are promising solutions to the water crisis, especially in semi-arid countries such as South Africa [1]. To protect the environment, it is necessary to treat wastewater before discharging it into freshwater sources [2]. Various methods, such as physical, mechanical, chemical, and biological, can be used depending on the wastewater type and the constituents to be removed [3]. Poultry slaughterhouse wastewater (PSW) is toxic and decreases dissolved oxygen concentrations, leading to eutrophication if discharged untreated into the environment [4]. It contains high chemical oxygen demand (COD), biochemical oxygen demand (BOD), total nitrogen (TN), total phosphorous (TP), pathogenic microorganisms, blood, fecal matter, soluble proteins, fats, oil, and grease (FOG), including total suspended solids (TSS). These characteristics make PSW

amenable to biological treatment [5]. Overall, biological treatment systems are considered environmentally friendly and are thus preferred over other treatment technologies.

Amongst biological wastewater treatment systems are aerobic, anaerobic, and wetland systems. These systems use biological cells to remediate pollutants from wastewater [6]. The anaerobic systems require the use of simple systems, require less energy, have high BOD removal efficiency, and produce biogas; however, high-strength wastewater (which contains a high concentration of FOG, proteins, TSS, and COD) reduces its treatment efficiency and those of other systems; moreover, it requires an aerobic post-treatment system to enhance microbial community contact time to improve COD and BOD removal by >90%, as well as to remove residual TP, TN, and pathogenic microorganisms [7–9]. Additionally, high concentrations of TP and TN are detrimental to the aquatic ecological system as they result in algal blooms that result in oxygen depletion in the water, which leads to a loss of aquatic life [10]. To decrease the concentration of TN, ammonia oxidizing microorganisms play a huge role. Aerobic ammonia oxidizing microorganisms (AOMs) such as ammonia-oxidizing bacteria (AOB) as well as ammonia-oxidizing archaea (AOA) and complete ammonia oxidizers (Commamox) oxidize $NH_4$ (ammonium) to $NO_2$ (nitrite) in a process called nitrification. Furthermore, anaerobic ammonia-oxidizing bacteria convert $NH_4^+$-N to $NO_2$ and $N_2$ under the anaerobic system in a process called denitrification [11]. Wang et al. [12] discovered a novel *Thauera* sp. strain SND5 that enhances simultaneous nitrification and denitrification as well as the removal of TP under microoxic conditions, and such strains can be used to enhance the existing conventional wastewater treatment systems. FOG in aerobic systems decreases the DO concentration, and the filamentous bacteria use it as their carbon and nitrogen to enhance their growth, and during their growth, they solubilize it to produce biosurfactants that stabilize biofoam [13].

PSW contains a high lipid content that leads to biofoamation and excessive growth of filamentous bacteria, which leads to sludge floatation and biofoam [14]. To treat such wastewater, both aerobic and anaerobic systems can be integrated to improve the performance of a wastewater treatment system. Examples include integrated aerobic/anaerobic sequential batch reactor (IAASBR) and anaerobic fluid bed reactor (AFBR) systems, among others [15,16]. However, aerobic systems such as sequential batch reactors, granular aerobic sludge (GAS), activated sludge (AS), and defoamer-supported dissolved air flotation use aerobic microorganisms to flocculate the microbial cells and improve the flocculation of suspended solids as well as FOG [3,17,18]. The oxygen concentration can be adjusted depending on the wastewater's strength. This system improves ammonia oxidation and reduces TP, TN, COD, BOD, suspended solids (SS), and recycled biomass, increasing microbial density. This enhances the biodegradation of organic matter and is cost-effective and efficient [8]. In South Africa, PSW is disposed of through municipal wastewater treatment systems.

AS systems are primarily used in domestic wastewater, livestock, and PSW treatment [19]. The disadvantage of municipal wastewater treatment systems, especially in PSW treatment, is the prevalence of biofoam, which occurs due to the presence of protein and FOG in the wastewater. To overcome this problem, synthetic defoamers have been used. However, reports have shown that they are toxic to humans and the environment; hence, there is a need to produce biodefoamers [20,21].

To regulate AS and biofoam formation, several techniques are applied, i.e., mechanical (stirring), thermal, chemical (the addition of polymers and chlorination to regulate filamentous microbial growth), and biological [22]. Natural means (biodefoamers) in AS regulation would thus be preferable due to the technique's eco-friendliness and reduced input costs. Overall, AS regulation can improve its dewatering, reduce its bulking [23], and provide sloughing of inactive sludge particles, including filaments, i.e., turning them into soluble substrates, which are reduced to minimize foaming, thus imparting the AS granular formation and maintaining the sludge balance (keeping the mixed liquor TSS within a defined range). Filament sloughing can ensure the AS's health while reducing

foaming and bulking, thus improving PSW treatment efficiency. Preferably, biodefoamers have antagonistic characteristics against biofoam formers for AS regulation.

This study aimed to apply biodefoamers that were produced by a consortium isolated from PSW, as reported elsewhere [21], in an AS-designed bench-scale treatment system, whereby an assessment of the biodefoamer antimicrobial activity in terms of volumetric zones of inhibition (VZI, described by Mewa-Ngongang) [24] against the PSW and mixed liquor suspended solids (MLSS) microbial community in the form of AS and the removal of FOG, TSS, proteins, and COD. A comparison between bio- and synthetic-defoamer-supported activated sludge (Bio/Syn-AS) systems was made for the treatment of PSW. A control system containing no defoamer (CAS) was also used as a control experiment.

## 2. Materials and Methods

### 2.1. Isolation, Identification, and Biodefoamer Production

Four pure strains, namely, *Bacillus subtilis* (GCA_000009045.1), *Aeromonas veronii* (GCA_000204115.1), *Klebsiella grimontii* (UGJQ01000001.1), and *Comamonas testosteroni* (GCA_900461225.1), were isolated as previously reported elsewhere [21], to constitute a consortium for biodefoamer production. These isolates were obtained from the spout of a commercial poultry slaughterhouse (Cape Town, South Africa). Their identification was made by initially extracting the DNA using the Quick-DNA Fungal/Bacterial Miniprep kit (Zymo Research, Irvine, CA, USA, Catalogue No. D6005), following the kit manufacturer's instructions without modification. Subsequently, the purified DNA fragments were analyzed using an Applied Biosystems™ 3500xL Genetic Analyzer (Applied Biosystems, Waltham, MA, USA) for each strain with the instrument producing ab1 files, which were used to identify the individual strains using Basic Local Alignment Search Tool (BLAST) search provided by the National Centre for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov (accessed on 4 December 2022)). To ascertain the efficacy of the isolates' ability to produce biodefoamers individually, a biodefoamer production protocol, as listed in the subsequent paragraph, was followed. Biofoam collapse (decay) and destabilization rates were used to quantify the efficacy of the biodefoamer produced by the individual isolates [21]. The foam generated was generated using an apparatus described in Dlangamandla et al. [21], with the formed biofoam being visualized.

To produce the biodefoamer, a seed culture was first developed by inoculating a volume (50 mL) of sterile nutrient broth in conical flasks (250 mL) with a loop full of the individual pure strains grown overnight (24 h, 37 °C) in agar plates. The inoculated nutrient broth was put into an orbital shaker (120 rpm, Labwit ZWYR-240 shaking incubator, Labwit Scientific, Burwood East, VIC, Australia) for a further 24 h at 37 °C. To assess the seed culture's viability, a volume (1 mL) of the consortium was plated for 24 h at 37 °C. Subsequently, the seed culture (1 mL), i.e., after 48 h of incubation at 37 °C in an orbital shaker, was inoculated in 99 mL of nutrient broth in conical flasks to constitute a 100 mL fermentation broth. The fermentation broth (1.5 mL) was sampled from each flask used daily for 120 h and centrifuged (Hermle-Z233M-2 centrifuge, Labortechnik GmbH, Wasserburg, Germany) at 15,000 rpm for 30 min to recover a biomass-free supernatant. Supernatant samples (from $n = 3$ flasks) were pooled such that a sufficient volume of the crude biodefoamer was obtained. To characterize the crude biodefoamer produced, cold (4 °C) ethanol was mixed with cell-free biodefoamers using a 2:1 ratio (cold ethanol:supernatant) followed by shaking at 4 °C overnight to produce a precipitate, which was collected by initially centrifuging the mixture (15,000 rpm, 30 min) subsequent to pooling the collected precipitates and washing using sterile distilled water. After that, the washed precipitate was vacuum-dried in a desiccator for 24 h, with Fourier transform infrared (FTIR) (Spectrum Two, PerkinElmer, Waltham MA, USA) and $^{1}$H nuclear magnetic resonance (NMR, Bruker 400, Bruker Nano GmbH, Berlin, Germany) spectroscopy analyses being conducted [21].

### 2.2. Biofoamer Inhibition Using Cell-Free Crude Biodefoamers

After 120 h, 1.5 mL samples of the incubated biodefoamer-containing broth were centrifuged at 15,000 rpm for 15 min to obtain cell-free biodefoamer aliquots, which were then assessed for their ability to inhibit AS and PSW microbial community growth by adding 20 µL of the cell-free-biodefoamers to Mueller–Hinton agar plates. These plates were inoculated with AS and PSW microbial communities at 37 °C, and microbial growth was observed every 24 h for 120 h, i.e., assessing the volumetric zone of inhibition (VZI) as described by Mewa-Ngongang et al. [21]. This was to assess whether filaments from biofoamers can be reduced while sterilizing the PSW microbial community.

### 2.3. Activated Sludge (MLSS) System Design

The AS treatment system used in this study comprised a feed tank, an aerobic tank, and a secondary clarifier constructed using a transparent polyvinyl chloride sheet. The feed tank height was 28.3 cm with a diameter of 18 cm. The aerobic tank and secondary clarifier systems had the same height and diameter of 28.5 and 15 cm, respectively; however, the clarification tank had a cone with a height of 11 cm. The feed tank had a carrying capacity of 6 L, the aerobic tank had a carrying capacity of 4.2 L, and the secondary clarifier had a 5 L carrying capacity. The air was sparged using a Resun air pump (Resun Ac 9906, Shenzhen Xing Risheng Industrial Co. Ltd., Baolong, China) through silicon tubing connected to 3 air diffusers into the aerobic tank for efficient air supply. The air was pumped at a flow rate of 7 L/min. A 624 h old AS, which was used, was collected from a municipal wastewater treatment plant (WWTP) in Scottsdene near Cape Town, South Africa. The PSW was pumped into the system using a Gilson® Minipuls Evolution peristaltic pump (Gilson Inc., Middleton, WI, USA) at a flow rate of 3.4 mL/min at a hydraulic retention time (HRT) of 24 h. The AS overflow was recycled from the secondary clarifier back into the AS tank using the Gilson® Minipuls Evolution peristaltic pump at a 3.2 mL/min flow rate, and an AS retention time (SRT) of 10 days was maintained. This experiment was carried out for 240 h. The effluent from the aeration tank was pumped into the secondary clarifier at the same rate as the influent, and the HRT of the secondary clarifier was similar to that of the aerobic tank. The effluent from the secondary clarifier was pumped out at the same rate as the influent to the aeration tank to maintain a steady state of operation for the whole system. Figure 1 diagrammatically illustrates the activated sludge treatment system used in this study.

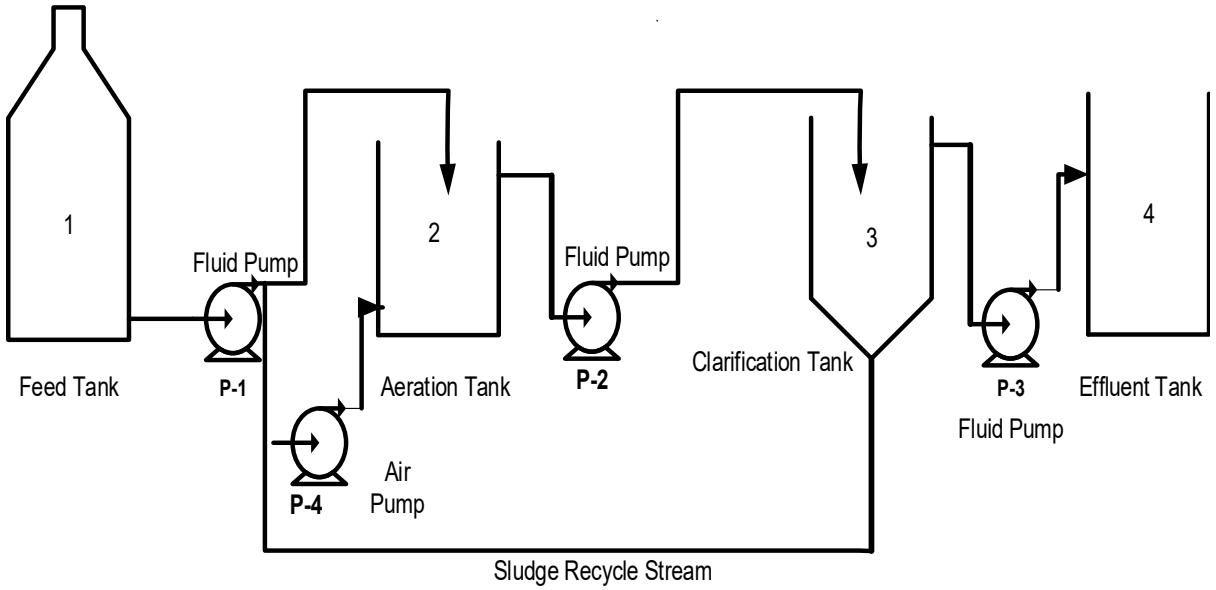


**Figure 1.** Design of the bench-scale activated sludge treatment system.

### 2.4. Activated Sludge-Supported Poultry Slaughterhouse Wastewater Treatment System Start-Up

After the addition of PSW to the AS system, the aeration tank was inoculated with 0.16 L of a 624 h old AS collected from a WWTP near Cape Town, South Africa. For every 5 L of PSW, 4% (*v*/*v*) of biodefoamers, i.e., those produced by a consortium of *Bacillus subtilis*, *Aeromonas veronii*, *Klebsiella grimontii*, and *Comamonas testosteroni*, were added into the AS aeration tank, after which the air pump was switched on. Air supplied by the air pump thoroughly mixed the MLSS, PSW, and defoamers. This system was acclimated for 48 h before the actual experimental runs. The samples from the feed tank, aeration tank, and secondary clarifier were collected periodically at 24 h intervals and immediately analyzed. Flow rates, as elucidated in Section 2.3, were used to maintain a steady state operation. The AS systems were operated using a biodefoamer, synthetic defoamer, i.e., silicone polymer antifoam A/defoamer by Sigma-Aldrich, St. Louis, MO, USA (positive control), and a non-defoamer supplemented system (negative control) to determine the efficiency of individual defoamer-AS systems. The systems were operated under unsteady (0–48), transition (72 h), and steady state (96–240 h).

### 2.5. Analytical Methods for Wastewater Quality Assessment

The 100 L of PSW collected biweekly using sterile 25 L polypropylene bottles from a poultry slaughterhouse near Cape Town, South Africa, was analyzed for pH, temperature, turbidity, TSS, COD, proteins, FOG, and ammonium nitrogen ($NH_4$-N). Temperature and pH were analyzed using a PSCTestr 35 multi-parameter (Wirsam Scientific and Precision Equipment (Pty) Ltd., Johannesburg, South Africa). Turbidity was quantified using an Oakton Turbidmeter TN 100 (Eutech Instruments Pte Ltd., Paisley, UK). In contrast, the TSS was quantified using EPA method 160.2; the COD was analyzed using EPA method 410.4, and FOG was analyzed using EPA method 10056. The $NH_4$-N was analyzed using test kits from Merck SA, a Merck & Co., Rahway, NJ, USA franchise, and the Merck Nova 60 spectroquant was used to quantify its concentration. The Bradford assay determined the protein concentration [25].

## 3. Results and Discussion

### 3.1. Biofoam Visualisation, Description, and Filament Reduction by Biodefoamers

Since PSW has a high FOG and protein content, both of which are sluggishly biodegradable and thus favor filamentous bacteria proliferation [26], *Nocardia* spp., *Microthrix parvicella*, and Type 1863 organisms were inevitably the cause of a thick, brown stable foam—see Figure 2. In our previous study [21], the dominant biofoamers were *Nostocoida limicola* (causes a scum layer), *Gordonia kroppenstedtii*, *Candidatus Microthrix parvicella* (FOG-accumulating bacterium), *Norcardioides insulae,* and *Bacteroides nordii* determined using metagenomic analysis. Several types of biofoam can be observed in WWTPs. These types of foam depend on the characteristics of the wastewater. White or gray foam is generated when there is fresh sludge, and this type of foam is usually unstable. The prevalence of non-biodegradable synthetic defoamers is what causes the frothy foam, and gray pumice-like foam occurs due to recycled fine TSS from other downstream processes. Due to the availability of excess extracellular polysaccharides, a slimy/pasty foam develops. However, the current study also observed the presence of a dense, slimy, brown, stable foam that is produced by the excessive growth of *Nocardia* spp., *Microthrix*, Type 1863 species, and lysis materials from flocs [23]. The secondary consequence of excessive filamentous microorganism growth is bulking, resulting in lousy settling and, thus, excessive solid thickening. This phenomenon can be mitigated by supplementing biodefoamers with antagonistic effects on filament-forming organisms within the AS. Biodefoamers are microbial bioproducts such as Extracellular polymeric substance (EPS). They are of microbial origin, and they bioflocculate the sludge and fix the mycolata within the flocs, which prevents them from getting enough nutrients from the environment and prevents excessive growth. Additionally, these defoamers are readily available in the treated wastewater, meaning that they do

not disrupt the ecosystem. The biodefoamers used in this study were antimicrobial toward filamentous organisms compared to synthetic defoamers, as reported elsewhere [21].

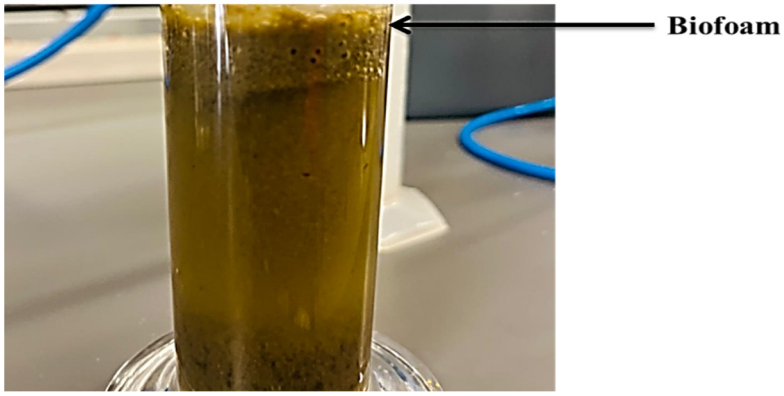


**Figure 2.** Biofoam observed in this study.

Overall, it was of great importance to test the biodefoamers' ability to inhibit the growth of filament-forming organisms in the PSW and AS mixture to assess the compatibility of the biodefoamers with the PSW treatment system to be used. The highest growth inhibition of 1.39 L/mL was observed when the consortium biodefoamer was used—see Figure 3. This revealed that the consortium biodefoamer can withstand the presence of PSW and the AS community and thus cannot be destructed, neutralized, or inactivated [27,28]. The literature reviewed showed that the inhibition of biodefoamers commonly occurs due to the production of other extracellular by-products [29]. The lowest growth inhibition of 1.32 L/mL was noticed when a biodefoamer produced by *Aeromonas veronii* and *Klebsiella grimontii* was used. This demonstrated that the biodefoamers produced by the consortium were functionally superior to those produced by other co-cultures.

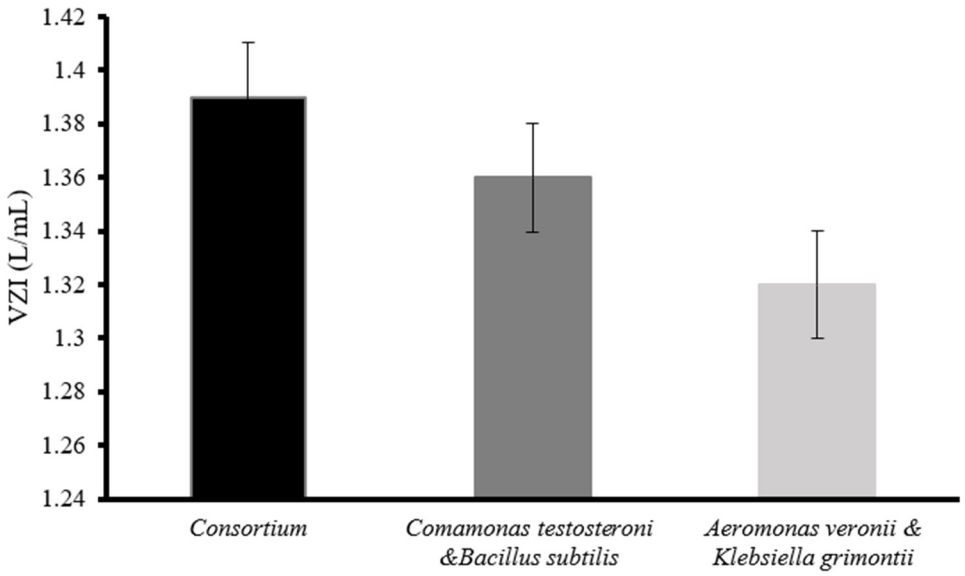


**Figure 3.** Volumetric zones of inhibition of crude biodefoamer produced by the consortium in comparison to biodefoamers produced by co-cultures.

### *3.2. Aeration Tank Performance*

This Bio-AS system was supported by biodefoamers, which are polysaccharide constituents that inhibit foam formation through a bridging mechanism that binds the bioflocculants and the filamentous bacteria through extracellular polymeric structure and embeds the foam formers within the flocs, thereby depriving them of the nutrients that are available

in the environment. This reduces the excessive growth of the filamentous bacteria and prevents biofoaming. Whereas the synthetic defoamer contains a short alkane chain and can diffuse into the liquid–gas interface and break down the lamellae; however, this defoamer leaves oil residues, which favors the proliferation of filamentous bacteria in the activated sludge system and results in weaker floc compaction [21]. Lipids are determined as fats, oil, and grease, which are long-chain fatty acids that are the constituents of the PSW [3]. A high concentration of FOG reduces dissolved oxygen (DO), resulting in pipe blockages and deflocculation of AS into the secondary clarifier. They are degraded at a lower rate than sugars, and their accumulation enhances the growth of filamentous bacteria, resulting in foaming in the AS treatment system [13]. Figure 4A,D,G,J represents the characteristics of the PSW (FOG, protein, TSS, and COD) fed into the AS aeration tank. These attributes fluctuated over time, depicting higher concentrations of FOG, TSS, protein, and COD. These characteristics were influenced by various factors, such as sampling time (slaughtering time) as well as external factors like temperature.

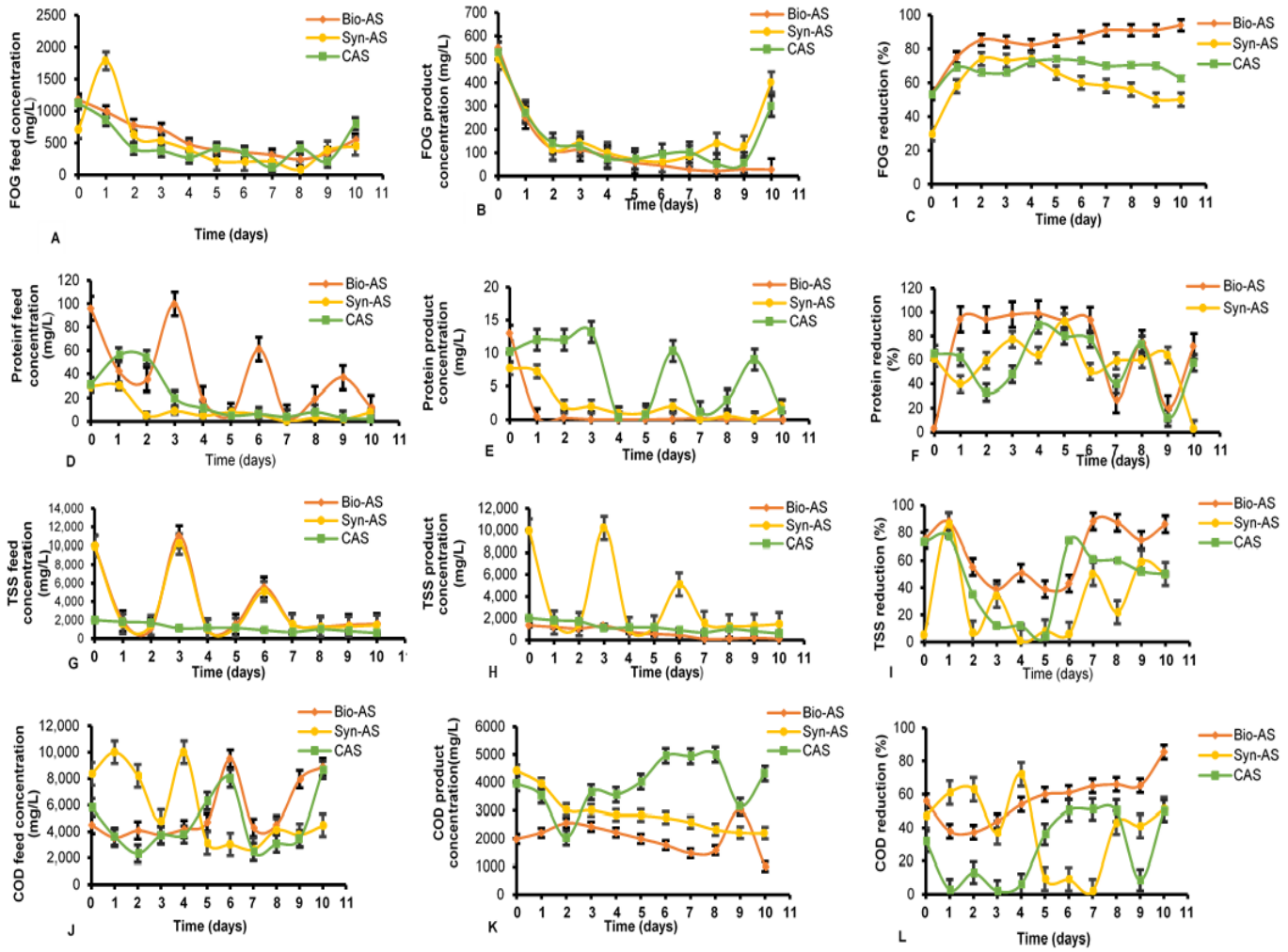


**Figure 4.** Aeration tank FOG feed (**A**); aerobic tank FOG product concentration (**B**); and reduction profiles (**C**). Protein feed concentration (**D**); aerobic tank protein product concentration (**E**); protein concentration reduction profile (**F**); TSS feed (**G**); product concentration (**H**); reduction profile (**I**); COD feed (**J**); product concentration (**K**); and reduction profile (**L**). All of the above profiles were operated under Bio-AS, Syn-AS, and CAS.

Figure 4C illustrates that during an unsteady state, FOG removal was between 53.3% and 85.3% in the Bio-AS compared to 29.6% and 74% achieved in the Syn-AS. During the

transition, state FOG was removed by 84.2% in the Bio-AS, whereas only 73% was removed in the Syn-AS. During the steady state FOG, removal increased from 82.3 to 94% at steady state (day 10) for the Bio-AS, in contrast to the Syn-AS, which acquired 74% and 50% at steady state (on days 4 and 10). Moreover, during the steady state, CAS acquired 72.3% and 62.5% FOG removal (days 4 and 10). The removal of lipids in the Bio-AS was facilitated by the presence of the competitive 48 h acclimated consortium that produced polysaccharide biodefoamers during their growth, which contained multiple charges that attracted the charge of the amphiphilic lipid and facilitated sludge agglomeration. These biopolymers were less affected by environmental conditions; hence, an increase in lipid removal was observed [30].

Furthermore, their presence destabilized the covalent bonds of the lipids and enhanced hydrolyzation [31]. In the Syn-AS system, the FOG removal decreased as the days increased (days 5–10), and this was because the biomass in the AS was still adjusting to the presence of the synthetic defoamer. Additionally, as the days increased, the oily defoamer resulted in sludge bed congestion, which led to an inactive microbial community and a subsequent reduction in the biodegradation of FOG. Furthermore, this revealed that the FOG adsorbed into the sludge, resulting in the overgrowth of saponified hydrophobic microorganisms that cause significant problems downstream, such as sludge deflocculation, foaming, and suspended biomass, which also contributes to increased suspended solids concentration in the reactor [32,33]. FOG also results in an increased organic load that requires additional energy consumption due to increased aeration demand in the clarifier; hence, 35% of COD is in FOG form [13,34].

In this study, the protein removal was quantified to assess the design efficiency of Bio-AS, Syn-AS, and CAS in removing proteins that cause biofoamation. Figure 4D shows that the raw PSW fed into the Bio-AS initially contained 96 mg/L of protein. During days 0 to 2, the Bio-AS aeration tank removed protein by between 50.2% and 51%. During day 3, protein depreciation was 51.5%, and the highest average protein removal obtained from days 4 to 10 was 99%. The PSW fed into the Syn-AS contained 29.4 mg/L of soluble proteins, and the protein removal observed was between 5% and 57% during the unsteady state, while the highest removal during the steady state was 92% on day 5. The CAS was fed with a concentration of 31 mg/L soluble protein, and the highest protein removal achieved during the steady state was 72%. These systems can reduce the soluble proteins; however, Bio-AS achieved the highest protein removal due to the presence of a biodefoamer that also enhanced sludge flocculation in the presence of oxygen.

A high TSS concentration affects the treated wastewater's turbidity and harms water quality and aquatic life [35]. Figure 4H,I graphically illustrates the removal of TSS by Bio-AS, Syn-AS, and CAS at unsteady, transitional, and steady states. At an unsteady state (days 0–2), the Bio-AS removed 55.3% of TSS. During the transition state (day 3), 39% of the TSS was decreased; during the steady state (day 10), the highest TSS reduction was 88%. This indicated the effectiveness of biodefoamers against PSW constituents. The 48 h acclimation defoamer-producing cultures were added to enhance the attachment of the planktonic colloids into the sludge due to the charge neutralization of the surface charge of the solids and the biodefoamer.

The synthetic defoamer (antifoam A concentrate) used in the Syn-As contained active silicon. Solubility and hydrophobicity of a defoamer are essential for the effectiveness of the defoamer; however, these conditions are not favorable for the AS microbial culture [36]. Throughout the unsteady state, TSS removal in Syn-AS ranged between 5.2% and 86%, whereas through the transition state, it increased to 34%; during the steady state, the highest TSS removal obtained was 59% in the aeration tank.

A high concentration of COD in wastewater indicates high oxidizable organic and inorganic reducible substances, which leads to a decrease in DO, creating an anaerobic environment that deteriorates the efficiency of an aerobic treatment system. As depicted in Figure 4K,L, during the steady state (day 10), the Bio-AS achieved the highest COD removal of 60.2% on day 10. Syn-AS attained 24.4% removal during the unsteady state

(day 1) but reached a maximum of 41.2% later during the steady state (day 6). Though the aeration tank can decrease the organic contaminants in the wastewater a secondary clarifier is necessary to further improve the quality of the wastewater by settling down the aggregates and clarifying the wastewater.

### 3.3. Clarification Tank Performance

In the secondary clarifier, the processed raw wastewater from the aeration tank was held in a tank such that the aggregated biological flocs settled at the bottom due to gravity and density dissimilarities that were adequate to subdue suspension due to turbulence. The function of the secondary clarifier is to ensure that the wastewater nutrients such as FOG, TSS, proteins, and COD are removed through sludge sedimentation and ensures that the wastewater that is discharged into the environment has fewer contaminants. Additionally, the efficiency of the secondary clarifier is dependent on the activated sludge bioflocculants. FOG accumulation in the secondary clarifier results in sludge deflocculation [37]. The aeration tank product was fed into the sedimentation tank or clarifier, where further FOG removal occurred. The highest FOG removals observed during the steady state in Figure 5C for Bio-AS, Syn-AS, and CAS were 64.3%, 64%, and 63.3%, respectively. Despite the decrease in Bio-AS FOG removal on day 2, from days 5 to 8, the Bio-AS secondary clarifier efficacy improved. The secondary clarifier FOG influent concentration was relatively low, hence the reduction in FOG concentration.

Figure 5F graphically illustrates the reduction of soluble protein when the AS was operated under the Bio-AS conditions. During the steady state (day 4), the Bio-AS achieved a maximum of 99% protein reduction because the defoamer functional group was a polysaccharide that has multiple charges on its surface, and this allows the proteins (depending on the wastewater's pH, either negatively or positively charged) to attach to any of these sites leading to flocculation of compact flocs that can easily settle at the bottom of the secondary clarifier [21], whereas during the steady state (day 5), the Syn-AS removed a maximum of 92.2%; moreover, the CAS minimized soluble protein concentration by 89.3% during the steady state (day 4). Furthermore, liquid–solid separation was noted due to TSS concentration removal. The highest TSS removal for the Bio-AS of 88% was obtained during steady state (day 7), which shows that the macro flocs with higher density than the presence of the biodefoamer created sludge flocculation, which ensured good solid–liquid separation. During the unsteady state (day 1), the Bio-AS obtained the highest protein reduction of 88%, whereas the Syn-AS achieved the highest removal of 86%, and the CAS attained 77%. This shows that if there is optimum sludge flocculation in the aeration tank and the secondary clarifier, liquid–solid separation will improve.

The Bio-AS removed COD concentrations by 67% during the steady state (day 9). In contrast, the Syn-AS achieved the highest COD removal of 44.1% during the unsteady state (day 1), and the CAS achieved a COD removal of 43.2% during the steady state (day 7). The removal of COD was higher in Bio-AS because organic TSS was removed. It can be observed that the Bio-AS was consistent in the removal of FOG, protein, COD, and TSS, as it removed FOG up to 10 mg FOG/L in the final effluent, which was less than 400 mg FOG/L that is acceptable according to the Cape of Town (CoCT) discharge standard. It also reduced the TSS concentration of the wastewater up to 100 mg TSS/L which was less than the acceptable CoCT discharge standard concentration of 1000 mg TSS/L. It depreciated COD concentration up to 400 mg/L, less than the $\leq$5000 mg COD/L according to the City of Cape Town (CoCT) discharge standards.

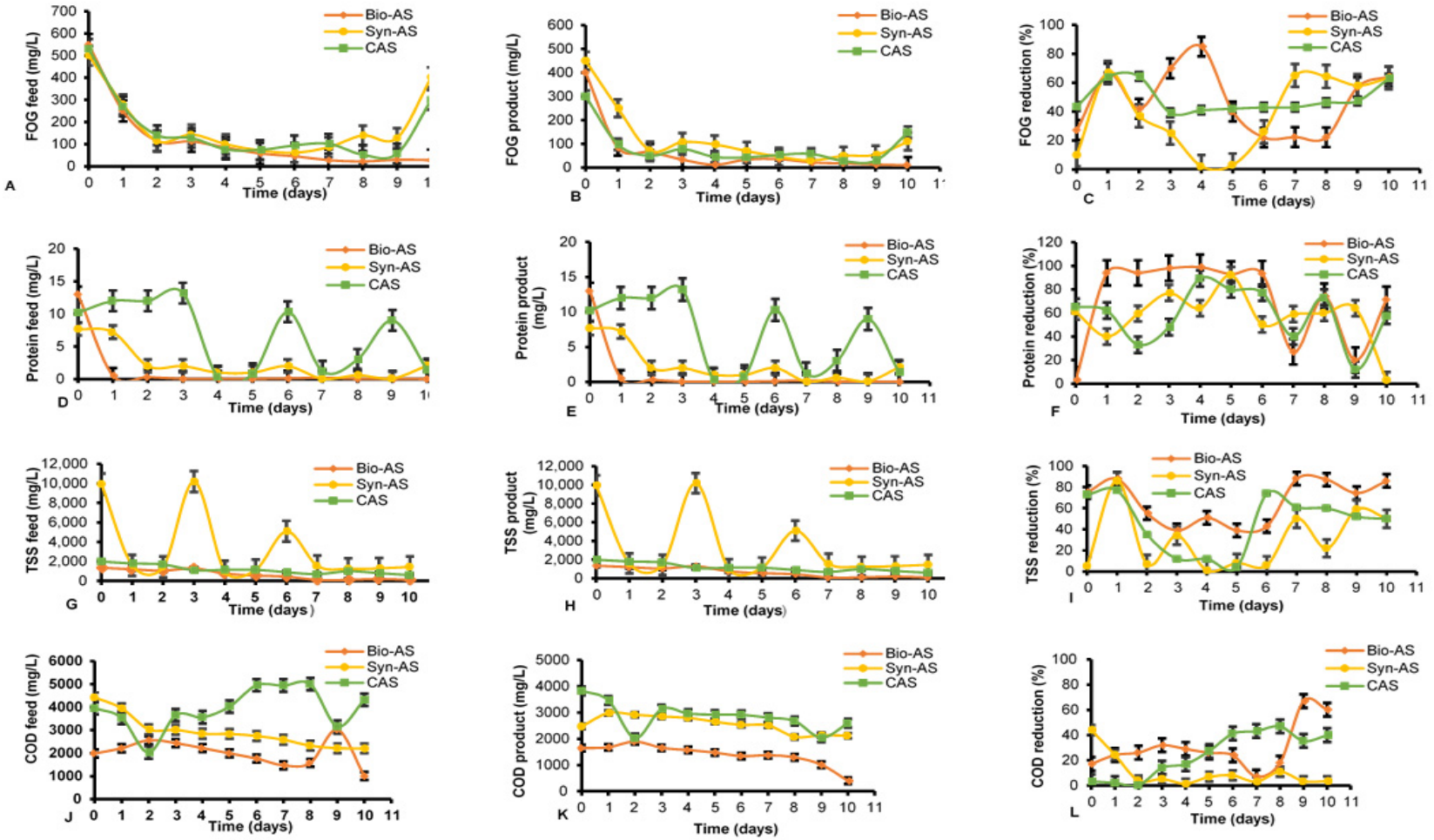


**Figure 5.** Clarification tank FOG (feed: (**A**); product concentration: (**B**), reduction: (**C**)); protein (feed: (**D**); concentration product: (**E**); reduction: (**F**)); TSS (feed: (**G**); product: (**H**); reduction: (**I**)); COD (feed: (**J**); product concentration: (**K**); reduction: (**L**))—profiles carried out under Bio-AS, Syn-AS, and CAS.

## 4. Conclusions

The biodefoamer produced from the microbial culture had antimicrobial activity against filamentous microorganisms that were present in the PSW and AS mixture. The biodefoamers were assessed for their antimicrobial activity in terms of volumetric zones of inhibition. The lowest VZI of 1.32 L/mL was observed when the co-culture of *Aeromonas veronii* and *Klebsiella grimontii* was used. The highest VZI of 1.39 L/mL was achieved when the consortium culture was used, meaning that the microorganisms were more competent as a community than as co-cultures. Furthermore, the results illustrated that the Bio-AS aeration tank could remove up to 94% FOG, 99% of soluble proteins, 93.3% TSS, and 85.4% COD, whereas the Syn-AS removed 74% FOG, 79% soluble proteins, 83.2% TSS, and 61% COD; moreover, the CAS achieved the removal of up to 72.3% FOG, 68% soluble proteins, 87% TSS, and 50.5% COD. The contaminants were further removed in the secondary clarifier, which uses solid–liquid separation by gravity mechanism to separate the sludge from the effluent. The secondary clarifier step of the Bio-AS removed 85% FOG, 99% soluble protein, 86% TSS, and 67% COD. The secondary clarifier for Syn-AS removed 67% FOG, 92.2% soluble proteins, 86% TSS, and 44.1% COD. The CAS secondary clarifier removed 64.3% FOG and 89.3% soluble proteins, 77% TSS, including 47% COD. This indicates that Bio-AS performed better compared to Syn-AS and CAS in terms of the nutrient's removal.

**Author Contributions:** Conceptualization, C.D., S.K.O.N. and M.B.; Data curation, C.D.; Funding acquisition, C.D., S.K.O.N. and M.B.; Investigation, C.D.; Project administration, S.K.O.N.; Resources, S.K.O.N., M.B., B.S.C. and B.I.O.; Supervision, S.K.O.N., M.B., B.S.C. and B.I.O.; Validation (Method), S.K.O.N., M.B., B.S.C., B.I.O. and C.D.; Writing—original draft, C.D.; Writing—review and editing, S.K.O.N., M.B., B.S.C. and B.I.O. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Cape Peninsula University of Technology university research fund (URF RK16). The National Research Foundation of South Africa (NRF) cost center R017.

**Data Availability Statement:** Not applicable.

**Acknowledgments:** The authors would like to thank the Bioresource Engineering Research Group (*BioERG*) students and my supervisors for their unwavering support, and the Chemical Engineering lab Technician Hannelene Jo-Anne Small, Faculty of Applied Sciences, CPUT for their technical support.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Meyo, H.B.; Njoya, M.; Basitere, M.; Ntwampe, S.K.O.; Kaskote, E. Treatment of Poultry Slaughterhouse Wastewater (PSW) Using a Pretreatment Stage, an Expanded Granular Sludge Bed Reactor (EGSB), and a Membrane Bioreactor (MBR). *Membranes* **2021**, *11*, 345. [CrossRef] [PubMed]
2. Rinquest, Z.; Basitere, M.; Ntwampe, S.K.O.; Njoya, M. Poultry Slaughterhouse Wastewater Treatment Using a Static Granular Bed Reactor Coupled with Single Stage Nitrification-Denitrification and Ultrafiltration Systems. *J. Water Process Eng.* **2019**, *29*, 100778. [CrossRef]
3. Dlangamandla, C.; Ntwampe, S.K.O.; Basitere, M. A Bioflocculant-Supported Dissolved Air Flotation System for the Removal of Suspended Solids, Lipids and Protein Matter from Poultry Slaughterhouse Wastewater. *Water Sci. Technol.* **2018**, *78*, 452–458. [CrossRef] [PubMed]
4. Dlangamandla, C.; Dyantyi, S.A.; Mpentshu, Y.P.; Ntwampe, S.K.O.; Basitere, M. Optimisation of Bioflocculant Production by a Biofilm Forming Microorganism from Poultry Slaughterhouse Wastewater for Use in Poultry Wastewater Treatment. *Water Sci. Technol.* **2016**, *73*, 1963–1968. [CrossRef] [PubMed]
5. Basitere, M.; Williams, Y.; Sheldon, M.S.; Ntwampe, S.K.O.; De Jager, D.; Dlangamandla, C. Performance of an Expanded Granular Sludge Bed (EGSB) Reactor Coupled with Anoxic and Aerobic Bioreactors for Treating Poultry Slaughterhouse Wastewater. *Water Pract. Technol.* **2016**, *11*, 86–92. [CrossRef]
6. Kim, K.; Hur, J.W.; Kim, S.; Jung, J.-Y.; Han, H.-S. Biological Wastewater Treatment: Comparison of Heterotrophs (BFT) with Autotrophs (ABFT) in Aquaculture Systems. *Bioresour. Technol.* **2020**, *296*, 122293. [CrossRef] [PubMed]

7. Meiramkulova, K.; Zorpas, A.A.; Orynbekov, D.; Zhumagulov, M.; Saspugayeva, G.; Kydyrbekova, A.; Mkilima, T.; Inglezakis, V.J. The Effect of Scale on the Performance of an Integrated Poultry Slaughterhouse Wastewater Treatment Process. *Sustainability* **2020**, *12*, 4679. [CrossRef]
8. Baker, B.R.; Mohamed, R.; Al-Gheethi, A.; Aziz, H.A. Advanced Technologies for Poultry Slaughterhouse Wastewater Treatment: A Systematic Review. *J. Dispers. Sci. Technol.* **2020**, *42*, 880–899. [CrossRef]
9. Njoya, M.; Basitere, M.; Ntwampe, S.K.O.; Lim, J.W. Performance Evaluation and Kinetic Modeling of Down-Flow High-Rate Anaerobic Bioreactors for Poultry Slaughterhouse Wastewater Treatment. *Environ. Sci. Pollut. Res.* **2020**, *28*, 9529–9541. [CrossRef]
10. Nguyen, T.-T.-D.; Nguyen, T.-T.; An Binh, Q.; Bui, X.-T.; Ngo, H.H.; Vo, H.N.P.; Andrew Lin, K.-Y.; Vo, T.-D.-H.; Guo, W.; Lin, C.; et al. Co-Culture of Microalgae-Activated Sludge for Wastewater Treatment and Biomass Production: Exploring Their Role under Different Inoculation Ratios. *Bioresour. Technol.* **2020**, *314*, 123754. [CrossRef]
11. Zhao, W.; Bi, X.; Bai, M.; Wang, Y. Research Advances of Ammonia Oxidation Microorganisms in Wastewater: Metabolic Characteristics, Microbial Community, Influencing Factors and Process Applications. *Bioprocess Biosyst. Eng.* **2023**, *46*, 621–633. [CrossRef] [PubMed]
12. Wang, Q.; He, J. Complete Nitrogen Removal via Simultaneous Nitrification and Denitrification by a Novel Phosphate Accumulating *Thauera* Sp. Strain SND5. *Water Res.* **2020**, *185*, 116300. [CrossRef] [PubMed]
13. Collin, T.D.; Cunningham, R.; Asghar, M.Q.; Villa, R.; MacAdam, J.; Jefferson, B. Assessing the Potential of Enhanced Primary Clarification to Manage Fats, Oils and Grease (FOG) at Wastewater Treatment Works. *Sci. Total Environ.* **2020**, *728*, 138415. [CrossRef]
14. Rajab, A.R.; Salim, M.R.; Sohaili, J.; Anuar, A.N.; Salmiati; Lakkaboyana, S.K. Performance of Integrated Anaerobic/Aerobic Sequencing Batch Reactor Treating Poultry Slaughterhouse Wastewater. *Chem. Eng. J.* **2017**, *313*, 967–974. [CrossRef]
15. Ardestani, F.; Abbasi, M. Poultry Slaughterhouse Wastewater Treatment Using Anaerobic Fluid Bed Reactor and Aerobic Mobile-Bed Biological Reactor. *Int. J. Eng.* **2019**, *32*, 634–640. [CrossRef]
16. Aziz, H.; Puat, N.; Alazaiza, M.; Hung, Y.-T. Poultry Slaughterhouse Wastewater Treatment Using Submerged Fibers in an Attached Growth Sequential Batch Reactor. *Int. J. Environ. Res. Public Health* **2018**, *15*, 1734. [CrossRef]
17. Septiana, I.; Siami, L.; Tazkiaturrizki, T.; Hadisoebroto, R.; Ratnaningsih, R. Analysis of Load Variation on Chicken Slaughterhouse Waste Water Treatment Using GAS-SBR. *J. Phys. Conf. Ser.* **2019**, *1402*, 022109. [CrossRef]
18. Masoumi, Z.; Shokohi, R.; Atashzaban, Z.; Ghobadi, N.; Rahmani, A.R. Stabilization of Excess Sludge From Poultry Slaughterhouse Wastewater Treatment Plant by the Fenton Process. *Avicenna J. Environ. Health Eng.* **2015**, *2*, 3239. [CrossRef]
19. Mangundu, J. Optimisation of Defoamer in A Bio-Reactor. Master's Thesis, University of the Witwatersrand, Johannesburg, South Africa, 2017.
20. Dlangamandla, C.; Basitere, M.; Okeleye, B.I.; Chidi, B.S.; Karabo Obed Ntwampe, S. Biofoam Formation and Defoamation in Global Wastewater Treatment Systems. *Water Pract. Technol.* **2020**, *16*, 1–18. [CrossRef]
21. Dlangamandla, C.; Ntwampe, S.K.O.; Basitere, M.; Chidi, B.S.; Okeleye, B.I.; Mukandi, M.R. Production, Application, and Efficacy of Biodefoamers from Bacillus, Aeromonas, Klebsiella, Comamonas Spp. Consortium for the Defoamation of Poultry Slaughterhouse Wastewater. *Water* **2023**, *15*, 655. [CrossRef]
22. Pal, P.; Khairnar, K.; Paunikar, W.N. Causes and remedies for filamentous foaming in activated sludge treatment plant. *Glob. NEST J.* **2014**, *16*, 762–772.
23. Marina, A. Filamentous Bacteria—Problems and Solutions. 2018. Available online: https://wtp-operators.thewaternetwork.com/article-FfV/filamentous-bacteria-problems-and-solutions-r94HLW1n20o4ygPMhB7D4A (accessed on 20 March 2023).
24. Mewa-Ngongang, M.; du Plessis, H.; Ntwampe, S.; Chidi, B.; Hutchinson, U.; Mekuto, L.; Jolly, N. Grape Pomace Extracts as Fermentation Medium for the Production of Potential Biopreservation Compounds. *Foods* **2019**, *8*, 51. [CrossRef]
25. Kielkopf, C.L.; Bauer, W.; Urbatsch, I.L. Bradford Assay for Determining Protein Concentration. *Cold Spring Harb. Protoc.* **2020**, *2020*, pdb.prot102269. [CrossRef]
26. Jenkins, D.; Richard, M.G.; Daigger, G.T. *Manual on the Causes and Control of Activated Sludge Bulking and Foaming*, 2nd ed.; Lewis Publishers: Detroit, MI, USA, 1993.
27. Chen, C.; Liu, C.-H.; Cai, J.; Zhang, W.; Qi, W.-L.; Wang, Z.; Liu, Z.-B.; Yang, Y. Broad-Spectrum Antimicrobial Activity, Chemical Composition and Mechanism of Action of Garlic (Allium Sativum) Extracts. *Food Control* **2018**, *86*, 117–125. [CrossRef]
28. Li, B.-B.; Peng, Z.-Y.; Zhi, L.-L.; Li, H.-B.; Zheng, K.-K.; Li, J. Distribution and Diversity of Filamentous Bacteria in Wastewater Treatment Plants Exhibiting Foaming of Taihu Lake Basin, China. *Environ. Pollut.* **2020**, *267*, 115644. [CrossRef] [PubMed]
29. Ejaz, S.; Ihsan, A.; Noor, T.; Shabbir, S.; Imran, M. Mannose Functionalized Chitosan Nanosystems for Enhanced Antimicrobial Activity against Multidrug Resistant Pathogens. *Polym. Test.* **2020**, *91*, 106814. [CrossRef]
30. Pu, L.; Zeng, Y.-J.; Xu, P.; Li, F.-Z.; Zong, M.-H.; Yang, J.-G.; Lou, W.-Y. Using a Novel Polysaccharide BM2 Produced by Bacillus Megaterium Strain PL8 as an Efficient Bioflocculant for Wastewater Treatment. *Int. J. Biol. Macromol.* **2020**, *162*, 374–384. [CrossRef]
31. Mbulawa, S.; Ntwampe, S.K.O.; Basitere, M.; Mpentshu, Y.P. Bio-Delipidation of Dissolved Air Flotation Pre-Treated Poultry Slaughterhouse Wastewater. In Proceedings of the 10th International Conference on Advances in Science, Engineering, Technology & Healthcare (ASETH-18), Cape Town, South Africa, 19–20 November 2018. [CrossRef]
32. Chipasa, K.B.; Mdrzycka, K. Characterization of the Fate of Lipids in Activated Sludge. *J. Environ. Sci.* **2008**, *20*, 536–542. [CrossRef]

33. Damasceno, F.R.C.; Cavalcanti-Oliveira, E.D.; Kookos, I.K.; Koutinas, A.A.; Cammarota, M.C.; Freire, D.M.G. Treatment of wastewater with high fat content employing an enzyme pool and biosurfactant: Technical and economic feasibility. *Braz. J. Chem. Eng.* **2018**, *35*, 531–542. [CrossRef]
34. Dehghani, M.; Sadatjo, H.; Maleknia, H.; Shamsedini, N. A Survey on the Removal Efficiency of Fat, Oil and Grease in Shiraz Municipal Wastewater Treatment Plant. *Jentashapir J. Health Res.* **2014**, *5*, e26651. [CrossRef]
35. Sadeddin, K.; Naser, A.; Firas, A. Removal of Turbidity and Suspended Solids by Electro-Coagulation to Improve Feed Water Quality of Reverse Osmosis Plant. *Desalination* **2011**, *268*, 204–207. [CrossRef]
36. Mohamad Pauzi, S.; Anak Halbert, D.; Azizi, S.; Ahmad, N.A.A.; Ahmad, N.; Marpani, F. Effect of Organic Antifoam's Concentrations on Filtration Performance. *J. Phys. Conf. Ser.* **2019**, *1349*, 012141. [CrossRef]
37. Amanatidou, E.; Samiotis, G.; Trikoilidou, E.; Pekridis, G.; Taousanidis, N. Evaluating Sedimentation Problems in Activated Sludge Treatment Plants Operating at Complete Sludge Retention Time. *Water Res.* **2015**, *69*, 20–29. [CrossRef]