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Article

Bio-delipidation of Dissolved air Flotation Pre-treated Poultry Slaughterhouse Wastewater

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Abstract: Delipidation is a method of defatting that is generally associated with the removal of residual lipids or lipid groups from matrices in which they are present in minute quantities. The bio-delipidation of protein-rich poultry slaughterhouse wastewater (PSW) pre-treated with a dissolved air flotation (DAF) system was developed using microbial lipases from bacterial strains isolated from the PSW. The efficacy of the bio-delipidation system was quantitatively characterised by comparing the quality parameters i.e. fats, oil and grease (FOGs), turbidity, total suspended solutes (TSS), total chemical oxygen demand (tCOD) and protein concentration of the DAF pre-treated PSW and bio-lipidized samples. As hypothesised, the bio-delipidation system was able to effectively reduce the levels of these quality parameters when crude lipases of *Bacillus cereus AB1 (BF3)* and *Bacillus cereus CC-1 (B30)* strains were used. Strain-dependent quality characteristics were also observed in bio-delipidized samples. The study successfully managed to complement physical reduction techniques (DAF) with biological strategies (bio-delipidation) for improved PSW quality, with potential industrial applications.

Keywords: Bio-delipidation; Dissolved air flotation (DAF); Fats, oil and grease (FOG); Poultry slaughterhouse wastewater (PSW)

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1. Introduction

Pre-treatment processes such as dissolved air flotation systems (DAFs) and grease traps are often applied for poultry slaughterhouse wastewater (PSW) containing fats, oil and grease (FOGs) and proteins [1]. However, complications can occur during their utilisation, contributing to the inefficient removal of FOGs which will build-up in the sludge used in anaerobic processes downstream, reducing their effectiveness to treat the PSW. Furthermore, operational damage and clogging to pipe systems due to the building up of FOG culminates in process redundancies. In certain instances, solidified lipids are at low temperatures during anaerobic treatment, a phenomenon that has been reported in numerous studies for slaughterhouse wastewater [2,3,4]. Even after successful primary pre-treatment, further lipid removal might be required in a process, which is environmentally benign. When a DAF system is utilised as a pre-treatment system, 60-85% of lipids can be removed [5,6], with the rest passing down to downstream processes. Clearly, the remaining lipids will thus accumulate in the downstream PSW bioremediation systems, which will effectively reduce the efficiency of such processes overtime. The use of alternative biological methods with the current pre-treatment systems involving enzymes is a promising alternative for further FOG reduction in effluent from pre-treatment processes, a technique suitable for high lipid-containing wastewater such as PSW [7,8]. Enzyme usage can sustainably provide a way for which residual FOG in high lipid-containing effluents can be separated from protein-laden wastewater. These enzymes can be produced by a variety of organisms that are catalysing a wide range of reactions, providing for catalytic conversions and destabilisation of bonds between proteins and lipids, and resulting in the further removal of residual FOG in pre-treated wastewater [9].

In bio-delipidation, a biological process via biological catalysis using macromolecules such as lipases can facilitate the removal or reduction of lipids from matrices. This could involve reactions whereby the covalent bonds attaching lipids are broken or destabilised, with the lipids being hydrolysed and/or semi-hydrolysed. Due to the high insolubility of lipids in liquid media, usually facilitated by their hydrophobicity, the application of suitable enzymes can thus further reduce the effectiveness of the bondage mechanism, which will result in easier delipidation reactions. In most cases, studies on delipidation focus on biological samples of plasma or serum, with minimal research focusing on processes in wastewater treatment. For this research, the removal of lipids from protein-rich PSW prior to downstream processing of the wastewater is a necessary step, since proteins in biological mixtures from slaughterhouse comprises of triglycerides, phospholipids, and amphiphilic constituents. Physical separation systems such as adsorption are often used to remove such constituents and by further incorporating a biological delipidation system, the effluent produced can be suitable for anaerobic treatments. The aim of the current study was therefore; to assess the efficacy of the bio-delipidation systems on the DAF pre-treated PSW quality parameters (FOGs, protein concentration, turbidity and tCOD). Overall, the proposed bio-delipidation system will be valuable for quality improvement of PSW. However, based on our preliminary findings, a more comprehensive investigation with thorough statistical and experimental design is required.

2. Materials and Methods

2.1. Poultry Slaughterhouse Wastewater: Microbial Isolation and Identification, Collection and DAF Pre-Treatment

Microorganisms were isolated from local poultry slaughterhouse wastewater discharge point in Cape Town (South Africa), and tested for their ability to produce lipases using the screening method adapted from [10]. Those that tested positive (lypolytic microorganisms) were gram stained and morphologically characterised by 16S rRNA sequencing as *Bacillus cereus*, with Genebank accession numbers *CP023179.1* (*B30*) and *MF800922.1 (BF3)* [5]. The poultry slaughterhouse wastewater (PSW) was collected from a poultry slaughterhouse in Cape Town, South Africa. The wastewater was stored at 4°C until pre-treated using DAF systems with effluent of the PSW being collected and analyzed for water quality parameters. DAF systems were operated by dispersing pressurized dissolved air into the system, flocculating the lipids and solids from the PSW to the surface, prior to removal using skimming equipment at 60 rpm [5]. The resultant pre-treated PSW was collected and analyzed for water quality parameters. The analysis was then followed by bio-delipidation using crude lipase enzymes produced from the isolated and identified lypolytic *Bacillus cereus AB1 (BF3)* and *Bacillus* cereus CC-1 (*B30*) strains. The schematic diagram illustrating the interconnections for initial set-up DAF pre-treatment systems [5] as well as the current defatting bio-delipidation design are shown in Figure 1.

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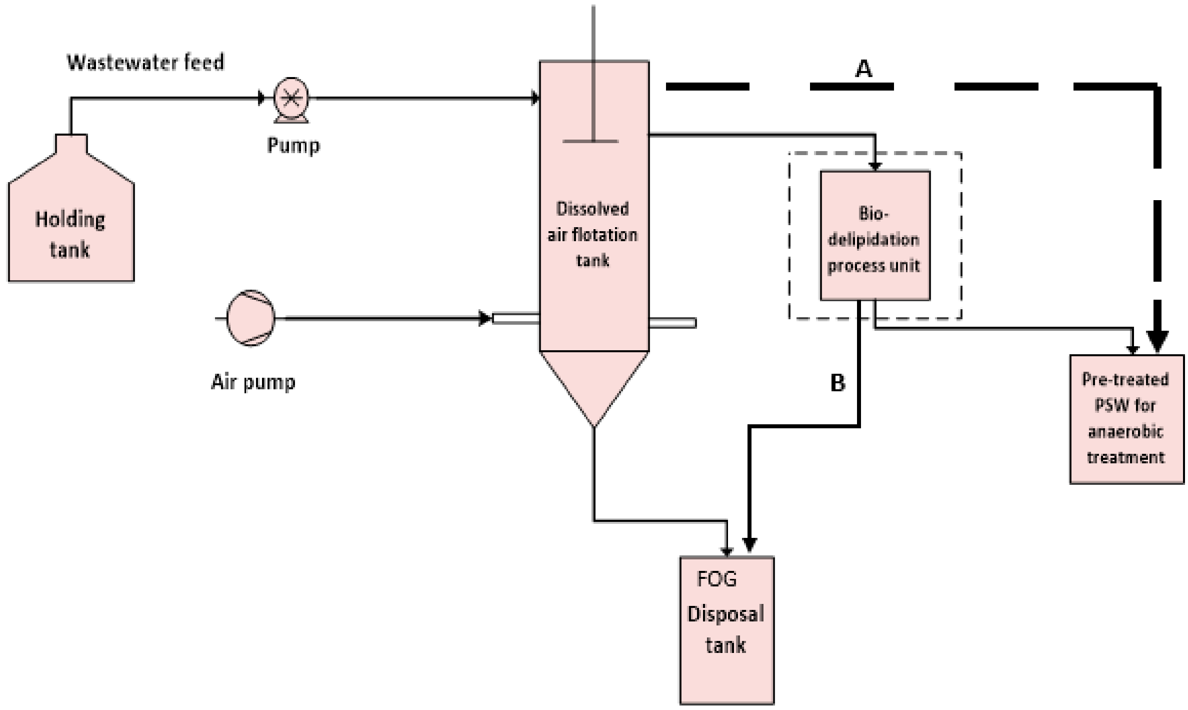

Figure 1. The schematic representation of the DAF pre-treatment system and bio-delipidation process unit. A stream, illustrates the previous approach/initial setup [5] without the bio-delipidation process unit. B stream process design enhancement incorporating the bio-delipidation unit and prosess streams.

2.2. Bio-delipidation Assessment

PSW samples were prepared by mixing PSW (13.5 mL) and crude lipase extracts (1.5 mL) in 15 mL tubes using a vortex mixer. A homogenized PSW/lipase mixture was then allowed to settle for 10 min, which culminated in separate zones being formed after enzyme treatment. PSW without an oily layer was used to prepare delipidation samples, in which 13.5 mL of PSW and 1.5 mL of semi-purified enzyme supernatants were mixed. Lipid layer thickness including differentiation was measured after preparing both bio-delipidation samples and reference experiments. A reference experiments were those samples that did not undergo any bio-delipidation fermentations. The efficiency of the bio-delipidation was observed by comparing PSW quality parameters pre- and post-bio-delipidation process.

2.3. Effect of pH and Temperature on Bio-delipidation

To assess bio-delipidation at different pHs, actual fatty acid content pre- and post- defatting was quantified. To determine the optimal pH for bio-delipidation, different reactions mixtures were used at pH values 3, 4, 5, 6, 7, 8, 9, 10 and 12, which were adjusted using 2M NaOH and 2M HCl. Furthermore, protein concentrations were determined spectrophotometrically (Anthos Xenyth1100 microtitre plate reader) at 450nm, using the Bradford protein assay [11] with Bovine serum albumin (BSA) being used to prepare protein standards. The percentage removal efficiency of fatty acids from the PSW at various pH levels was determined by titration using ethanol to dissolve oil and titrate with a strong base with phenolphthalein as an indicator, in accordance with Equation 1.

$$\frac{RA/[P]A}{RB/[P]B} \times 100\% \quad (1)$$

Where: *A*, *B*, *R*, and *P* refers to post- biodelipidation, pre- bio-delipidation, lipase activity and protein concentration, respectively.

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The optimum temperature for bio-delipidation was also assessed with tubes agitated at 121 rpm in a temperature-controlled water baths set differently between temperatures (20 to 55°C) for 6 h. The residual percentage removal efficiency of fatty acids from the PSW using bio-delipidation at the various temperatures was also determined using Equation 1.

2.4. Analytical Tests and Methods

Quality parameters of the wastewater, i.e. collected samples from the DAF pre-treatment system, were analyzed pre- and post- bio-delipidation. The parameters analyzed were pH, fats, oil and grease (FOGs), turbidity, total suspended solutes (TSS), total chemical oxygen demand (tCOD) and protein concentration**.** All the quality parameters were measured using standard techniques as described in Standard methods for the examination of wastewater [12]. All wastewater quality parameters were conducted in triplicates, with average values. For evaluation purposes, the variations in the quality of the pre-treated PSW was conducted twice (P1 and P2) to confirm removal efficiency and reproducibility.

3. Results and Discussion

3.1. Effect of pH and Temperature on bio-Delipidation

The role of environmental conditions that affect bio-delipidation was investigated in order to elucidate optimal bio-delipidation conditions suitable for industrial bio-delipidation applications. For this purpose, the deffating assessment of pre-treated PSW under varying pH (4, 5, 6, 7, 8, 9, 10, 11 and 12) conditions was conducted at 37°C. Figure 2A shows the impact of pH on residual fatty acid removal efficiency from the DAF pre-treated PSW. Bio-delipidation removal efficiency was higher between pH 7 and 10, with pH values below 7 and above 10 showing less than 50% fatty acids removal efficiency. However, the optimal pH for removal efficiency by *BF3* and *B30* was at 8. Based on these observations, reduction in fatty acid emulsion and lipase activities were viewed as characteristics associated with extreme acidic and alkaline conditions. [13], used a micromethod and observed an improved delipidation of aqueous proteins at extreme pH values (i.e. below 3 and above 12). Our study, therefore, acknowledged that, in addition to pH, many other factors (e.g. wastewater contents and recovery/removal method) impact on deffating. [14] observed greater FOG removal efficiency at pH 5-9 using bacterial bio-delipidation aliquots. However, the authors indicated that the removal of FOG may not have been dependent on the initial pH of the wastewater containing FOG, but the pH of the aliquots containing bio-delipidation enzymes. Apart from pH dependent characteristics, strain variability also played an important role during bio-delipidation. Fatty acid removal efficiency for *BF3* strain was consistently higher than *B30* at most different pH conditions (Figure 2A). These efficiency trends may be genetic and metabolic dependent.

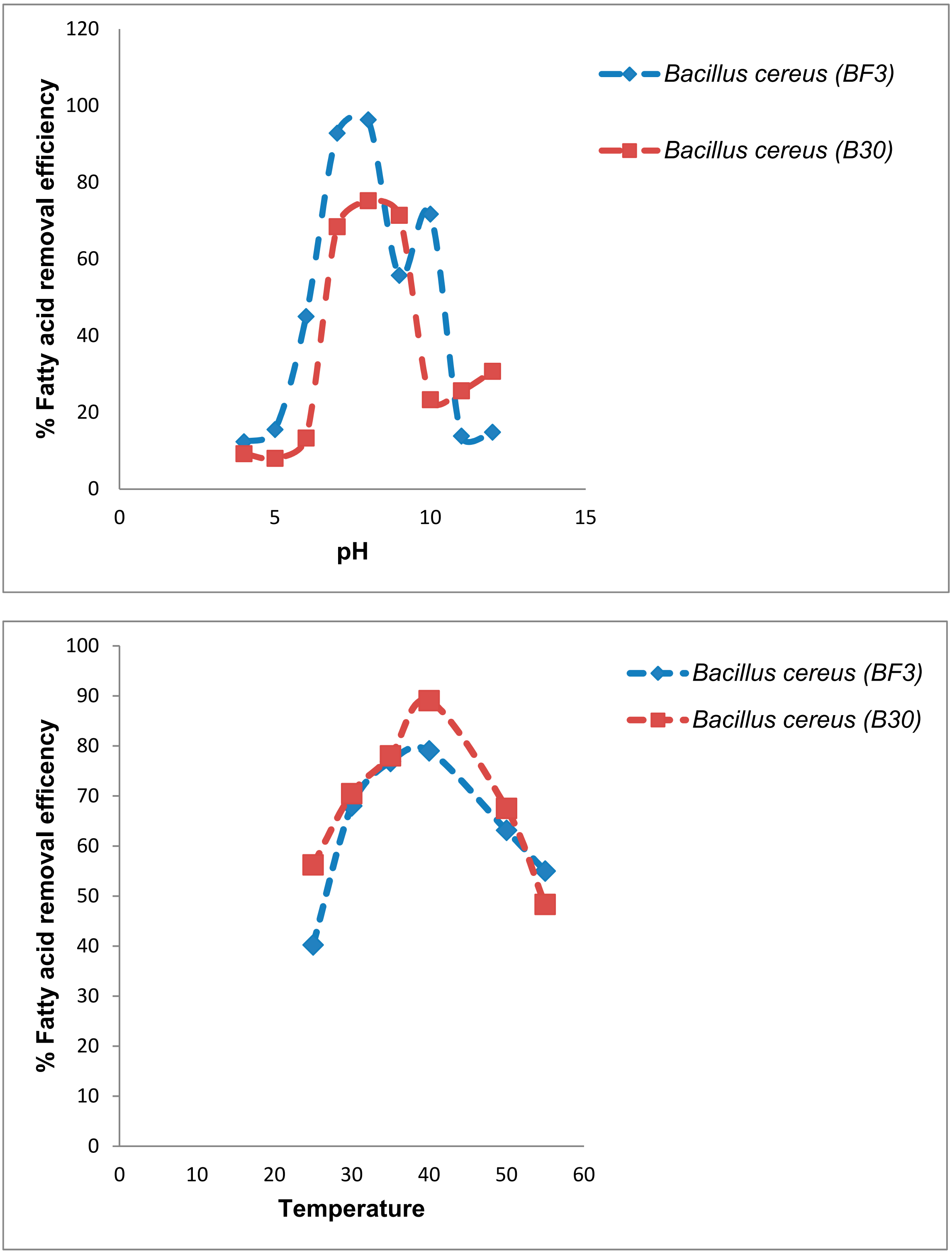

Figure 2. The impact of pH (A) and temperature (B) on the fatty acid removal of DAF pre-treated PSW using crude lipases of *Bacillus cereus AB1 (BF3) and Bacillus cereus CC-1 (B30)* strains.

Similarly, the effect of temperature on PSW bio-delipidation by lipases from isolated organisms was carried out under optimum pH (8) at different temperatures. As shown in Figure 2B, the bio-delipidation efficiency was highest at 45°C. It was an unsuprising observation for these isolates since the stability, activity, functionality and retainment of lipase activity was previously reported at 40-45°C [5]. Additionally, [15] reported a rapid pre-treatment of slaughterhouse wastewater and a faster delipidation rate at 45°C.

3.2. Effect of Bio-delipidation on Pre-treated PSW Quality Parameters

Figure 3 shows the impact of bio-delipidation of DAF pre-treated PSW on tCOD using crude lipases produced by *Bacillus* cereus *AB1 (BF3)* and *Bacillus cereus CC-1 (B30).* Initially, the tCOD of the untreated PSW was 6500 mg/L until, subsequent to DAF treatments, which resulted in 68.0% (P1), and 77.7% (P2) tCOD reduction. Furthermore, the bio-delipidation of pre-treated PSW by crude lipases from *BF3* reduced tCOD by 49% (P1) and 55.7% (P2) while *B30* lipase reduced tCOD by 56.3% (P1) and 66.0% (P2). As in the current study, the improvement of poultry abattoir wastewater quality was previously realised by [16], using enzymatic approaches. Elsewhere, the application of lipases in wastewater treatment processes was reported to reduce organic matter and the tCOD of lipid-rich wastewater [15]. Strain-dependent variations were observed when 49 to 55.7% and 56 to 66% of tCOD was reduced by crude lipases of *BF3* and *B30*, respectively. Since high COD is associated with compromised ecosystem and/or pollution [17], B30 strain was therefore recommended in the current study, for further research.

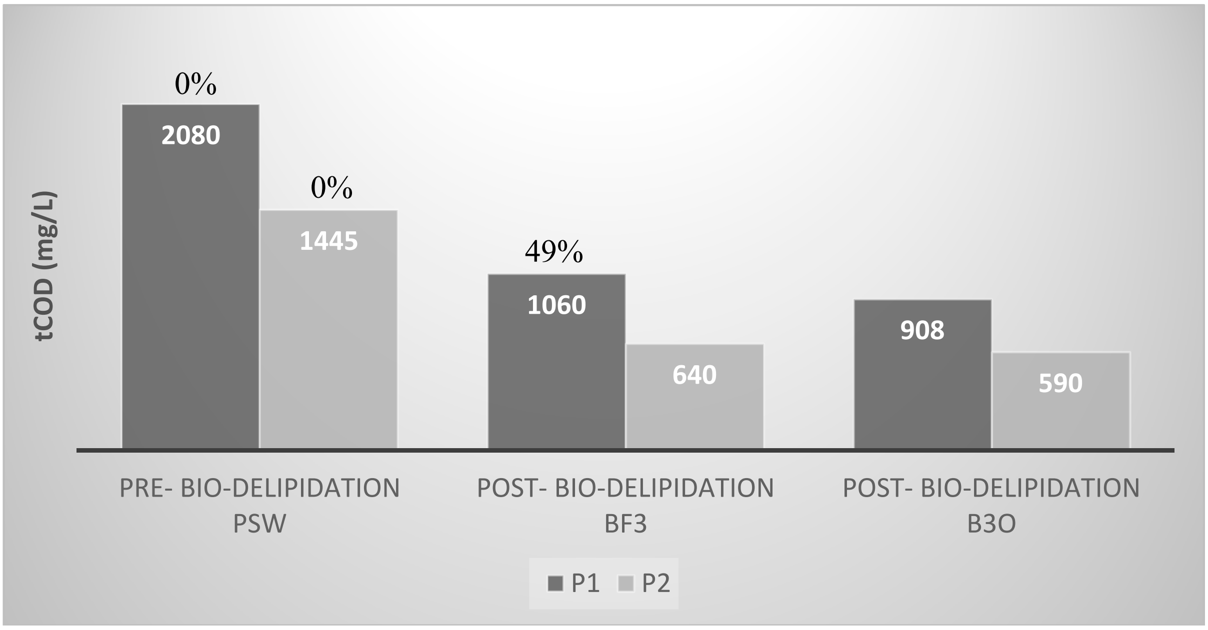

Figure 3. The profiles of (tCOD) pre- and post- bio-delipidation of pre-treated PSW by crude lipases produced by *Bacillus cereus AB1 (BF3)* and *Bacillus cereus CC-1 (B30)* strains.

Similarly, the influence of bio-delipidation on turbidity of the DAF pre-treated PSW was evaluated using crude lipases produced by *BF3* and *B30*. Previously, [5] managed to reduce turbidity of PSW (792 mg/L) by 35.85% (P1) and 56.1% (P2). In order to further, reduce turbidity of the pre-treated PSW samples (P1 and P2), bio-delipidation was done on these samples. *BF3* reduced turbidity by 60.2% (P1) and 68.6% (P2) while *B30* reduced turbidity by 76.2% (P1) and 75.5% (P2). Subsequent to bio-delipidation, these findings were also confirmed by visual inspection of the tubes, which showed less turbid characteristics for the bio-delipidized samples. [7] and [2] also observed similar results, where the application of lipase enzyme in fatty wastewater reduced turbidity.

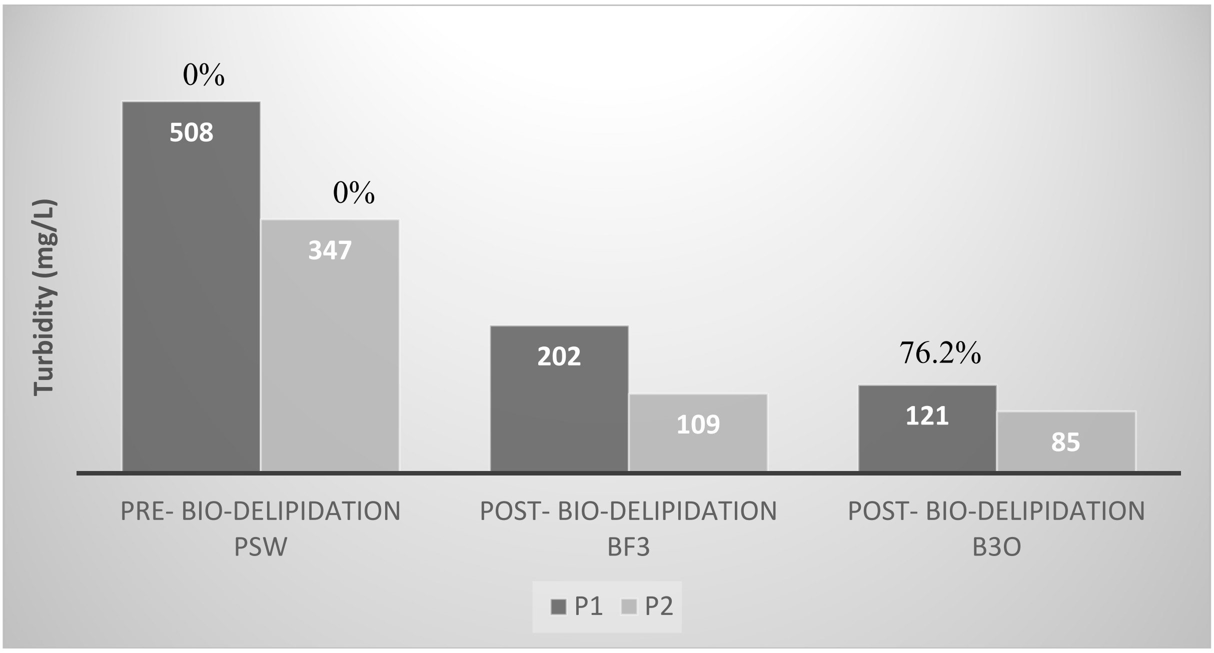

Figure 4. The profiles of turbidity pre- and pot- bio-delipidation of pre-treated PSW by crude lipases produced by *Bacillus cereus AB1 (BF3)* and *Bacillus cereus CC-1 (B30)* strains.

In this study, the comparative analysis for total protein concentration of DAF pre-treated PSW and bio-delipidized samples was necessary because proteins are huge contributors to pollution and are prevalent in PSW [18]. Figure 5 shows the protein removal efficiency of the bio-delipidation unit for pre-treated PSW samples using crude lipases of *B30* and *BF3* strains. After bio-delipidation of DAF pre-treated samples, 68.1% (P2) to 72.3% (P1) and 42.7% (P1) and 44% (P2) protein reduction was obtained for *BF3* and B30, respectively. Unlike other quality parameters such as tCOD and turbidity, *BF3* strain displayed much improved protein reduction, compared to *B30* (Figure 5); a tendency/trend that reconfirms the importance of strain variability and bioproduct influence on treated wastewater.

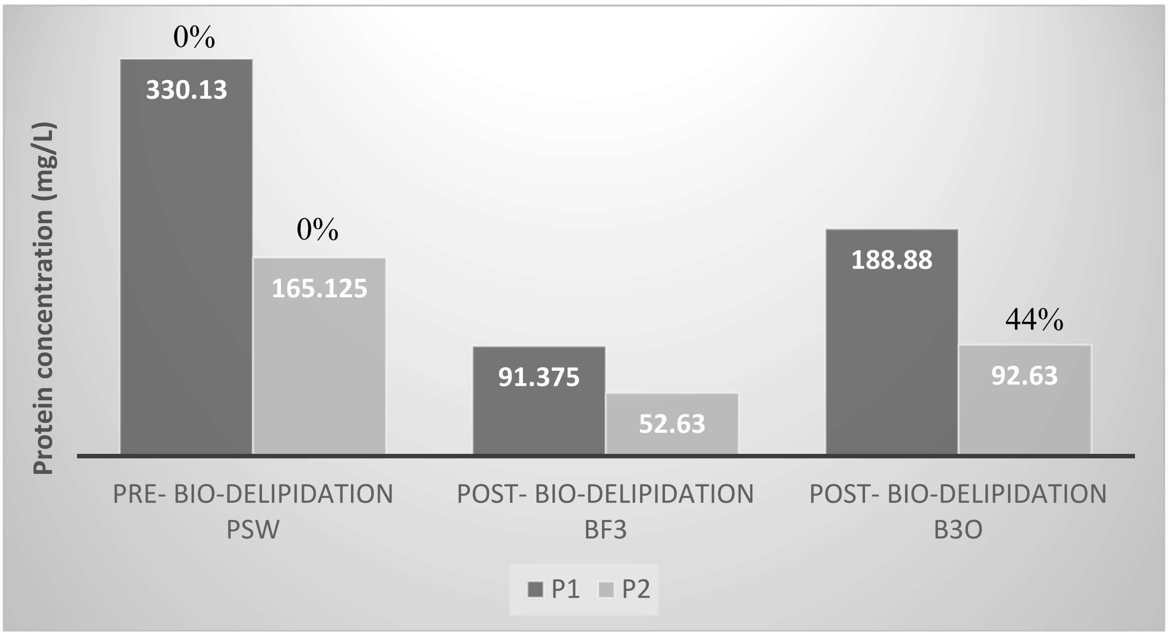

Figure 5. The profiles of protein levels pre- and post- bio-delipidation of pre-treated PSW by crude lipases produced by *Bacillus cereus AB1 (BF3)* and *Bacillus cereus CC-1 (B30)* strains.

TSS evaluations pre- and post- bio-delipidation were done since they are form an important quality parameter to assess wastewater treatments [19]. Figure 6 displays the relative influence of crude lipases of *BF3* and *B30* on the TSS removal/reduction in DAF pre-treated PSW. Prior to DAF treatments, TSS of the untreated PSW was 2400 mg/L. However, partial removal of TSS was achieved at 58% (P1) and 67.5 % (P2) using physical DAF methods reported elsewhere [5]. The treatment of these DAF pre-treated samples with crude lipases resulted in the removal of between 64% (P1) and 74.3% (P2) for *BF3* strain and of between 69.3% (P2) and 80% (P1) for *B30* strain. As with other quality parameters, the potential usage of *Bacillus* species as potential bio-delipidation agents for TSS removal was remarkably evident.

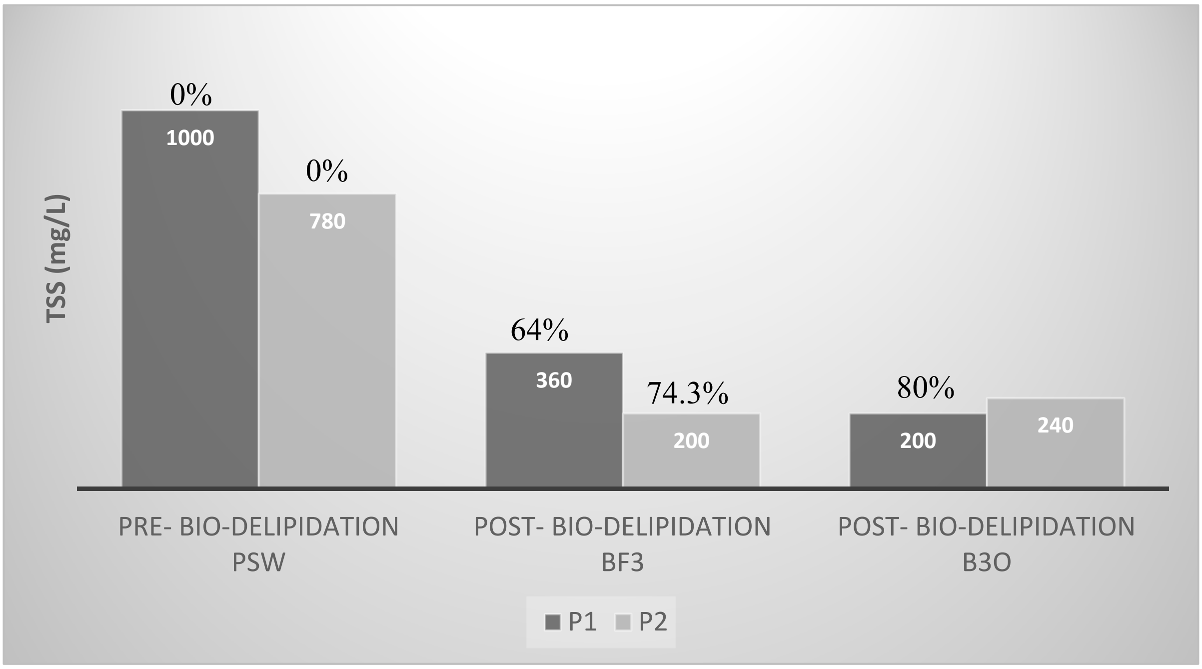

Figure 6. The profiles of TSS pre- and post- bio-delipidation of pre-treated PSW by crude lipases produced by *Bacillus cereus AB1 (BF3)* and *Bacillus cereus CC-1 (B30*) strains.

3.3. Removal of residual FOG by bio-delipidation

The removal of residual FOG from DAF pre-treated samples by bio-delipidation was also investigated in the current study. The complete removal of this quality parameter is essential for efficient downstream anaerobic biological processes. Partial removal of FOGs using a DAF system [5] with 49.8% (P1) and 80.37% (P2) removal being achieved for this study. Furthermore, bio-delipidation treatments were effected on these samples where 70.1% (P1) and 64.3% (P2) FOG reduction by *BF3* crude lipases was obtained. On the other hand, crude lipases from B30 strain displayed 80.4% (P1) and 66.3% (P2) reduction. Similar studies on wastewater treatment of slaughterhouses and dairy industries also showed the efficacy of lipase enzymes on FOG removal and the usefulness of the biological systems in downstream processes [20,7].

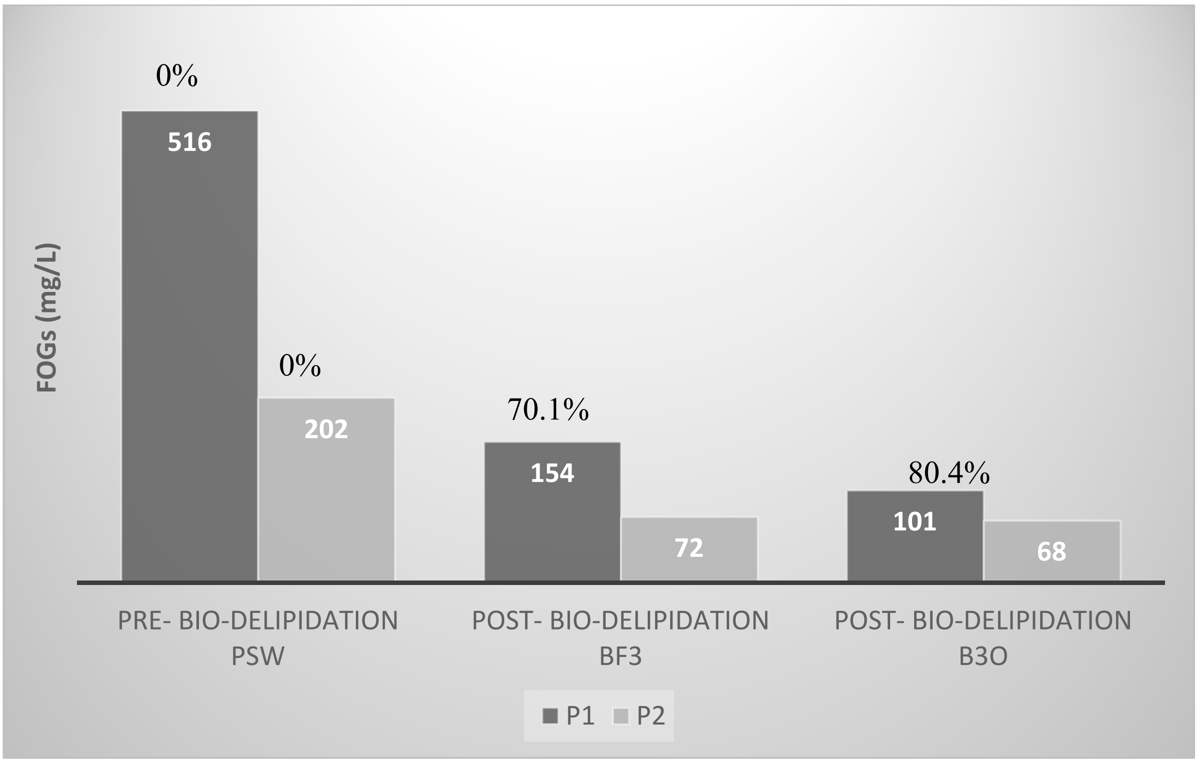

Figure 7. The profiles of FOGs pre- and post- bio-delipidation of pre-treated PSW by crude lipases produced by Bacillus cereus AB1 (*BF3*) and Bacillus cereus CC-1 (*B30*) strains.

4. Conclusions

Bio-delipidation strategies by crude lipases of *Bacillus cereus* strains improved the quality of PSW. The study recommended the complementary applications of both physical and biological delipidation strategies, to obtain improved wastewater quality. The screening of more lipase-producing microorganisms is therefore, necessary for optimal bio-delipidation processes. The study acknowledges that a complete, statistically supported experimental design is necessary for better understanding of the bio-delipidation concept.

Author Contributions: Seteno Karabo Obed Ntwampe and Moses Basitere conceived and designed the methodology, and revised the paper; Siyasanga Mabaluwa, Yolanda Mpentshu and Cynthia Dlangamandla developed the bio-delipidation unit and wrote the paper; Boredi Silas Chidi contributed to the analyses and interpretation of the results.

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Conflict of interest: The authors declare no conflict of interest.

References

1. Tanikawa, D.; Yokote, N.; Nakahara, K.; Yamaguchi, T. Evaluation of Process Performance for Lipid-rich Wastewater Treatment Using a Combination System of an Anaerobic Baffled Reactor and an Aerobic Trickling Filter. *J. Water. Environ Tech.* **2016**, 14(2), 90-95.
2. Meng, Y.; Luan, F.; Yuan, H.; Chen, X; Li, X. Enhancing anaerobic digestion performance of crude lipid in food waste by enzymatic pre-treatment. *Bioresour Technol*. **2017**, 224(1), 48-55.
3. Valladão, A.B.G.; Freire, D.M.G.; Cammarota, M.C. Enzymatic pre-hydrolysis applied to the anaerobic treatment of effluents from poultry slaughterhouses. *Int Biodeter Biodegr*. **2007**, 60(4), 219-225.

4. Cammarota, M.C.; Freire, D.M.G. A review on hydrolytic enzymes in the treatment of wastewater with high oil and grease content. *Bioresour Technol*. **2006**, 97(17), 2195-2210.
5. 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(2), 452-458, DOI. Available online: https://doi.org/10.2166/wst.2018.324 (accessed 14 August 2018).
6. Massé, D.; Masse, L. Characterization of wastewater. *Can Agr Eng*. **2000**, 42(3), 139-146.
7. Mendes, A.A.; Pereira, E.B.; Furigo, A.; De Castro, H.F.D. Anaerobic biodegradability of dairy wastewater pre-treated with porcine pancreas lipase. *Braz. Arch. Biol Technol*. **2010**, 53(6), 1279-1284.
8. Rigo, E.; Rigoni, R. E.; Lodea, P.; De Oliveira, D.; Freire, D. M.; Treiche, L.H.; DI Luccio, M. Comparison of two lipases in the hydrolysis of oil and grease in wastewater of the swine meat industry. *Ind. Eng. Chem Res*. **2008**, 47(6), 1760-1765.
9. Klaucans, E.; Sams, K. Problems with fat, oil, and grease (FOG) in food industry wastewaters and recovered FOG recycling methods using anaerobic co-digestion: A short review. *Key. Eng. Mater*. **2018**, 762, 61-68
10. Ramnath, L.; Sithole, B.; Govinden, R. Identification of lipolytic enzymes isolated from bacteria indigenous to Eucalyptus wood species for application in the pulping industry. *Biotechnol Rep*. **2017**, 15(1), 114-124.
11. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Chem*. **1976**, 72(1-2), 248-254.
12. Kraghhansen, U. A micro-method for delipidation of aqueous proteins. *Anal. Biochem.* **1993**, 210(2), 318-327.
13. Shon, H.K.; Tian, D.; Kwon, D.Y.; Jin, C.S.; Lee, T.J.; Chung, W.J. Degradation of fat, oil, and grease (FOGs) by lipase-producing bacterium Pseudomonas sp. strain D2D3. *J. Microb Biotechnol.* **2002**, 12(4), 583-591.
14. Leal, M.C.; Freire, D.M.; Cammarota, M.C; Sant'Anna, G.L. Effect of enzymatic hydrolysis on anaerobic treatment of dairy wastewater. *Process Biochem*. **2006**, 41(5), 1173-1178.
15. Valladão, A.B.G.; Cammarota, M.C.; Freire, D.M.G. Performance of an anaerobic reactor treating poultry abattoir wastewater with high fat content after enzymatic hydrolysis. *Environ. Eng. Sci.* **2011**, 28, 299–307.
16. Kundu, P.; Debsarkar, A.; Mukherjee, S. Treatment of slaughterhouse wastewater in a sequencing batch reactor: performance evaluation and biodegradation kinetics. *Biomed Res. Int*. **2013**, 1-11.
17. Lo, Y.M.; Cao, D.; Argin-Soysal, S.; Wang, J; Hahm, T.S. Recovery of protein from poultry processing wastewater using membrane ultrafiltration. *Bioresour Technol*. **2005**, 96(6), 687-698.
18. Plumber, H.S; Kiepper, B.H. Impact of poultry processing by-products on wastewater generation, treatment, and discharges. Proceedings of the 2011 Georgia Water Resources Conference, Georgia, Athens, 11-13 April 2011.
19. Rosa, D.R.; Duarte, I.C.; Saavedra, N.K.; Varesche, M.B.; Zaiat, M.; Cammarota, M.C.; Freire, D.M. Performance and molecular evaluation of an anaerobic system with suspended biomass for treating wastewater with high fat content after enzymatic hydrolysis. *Bioresour Technol*. **2009**, 100(24), 6170-6176.