Application of Biofloc technology in furfural residue wastewater treatment: insights from water purification, microbial composition, and animal survival trial

Aplicación de la tecnología de biofloc en el tratamiento de aguas residuales con residuos de furfural: Perspectivas a partir de la purificación del agua, la composición microbiana y un ensayo de supervivencia animal

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Fecha de recepción: 15/11/2025
Fecha de aceptación del artículo: 13/12/2025

DOI: https://doi.org/10.18041/1794-4953/avances.2.13276

Z. Lei & W. Feihong, "*Application of Biofloc technology in furfural residue wastewater treatment: insights from water purification, microbial composition, and animal survival trial*", Avances, vol. 22, no. 2 (julio-diciembre), Dic. 2025, doi: https://doi.org/10.18041/1794-4953/avances.2.13276

Abstract

Biofloc technology (BFT) was utilized to mitigate the risks associated with furfural residue wastewater (FRW). After BFT treatment, the removal efficiencies for COD, TOC, NH_4^+-N, NO_3^--N, and SO_4^{2-} were 94.2%, 94.3%, 100%, 89.5%, and 89.4%, respectively. Results show commercial biofloc inoculation in FRW forms biofloc with a microbial community structure distinct from conventional sludge. The biofloc was dominated by Clavispora lusitaniae (11%), Weissella paramesenteroides (14%), and Mucor lusitanicus (11%)-with functions including single-cell protein production, probiotic activity, and organic matter degradation—and had fewer pathogens than sludge, making it more suitable as animal feed. Additionally, black soldier fly (BSF) larvae exhibited a high survival rate (97.9%) when reared on the biofloc, highlighting its potential in insect farming. This study shows using FRW as the sole substrate plus commercial biofloc inoculation effectively cultivates animal feed-suitable biofloc and substantially reduces pollutants, offering an efficient, resource-sustainable FRW treatment solution.

Keywords: biofloc technology, furfural residue wastewater, microbial community, black soldier fly survival trials.

Resumen

La tecnología biofloc (BFT) se utilizó para mitigar los riesgos asociados con las aguas residuales de residuos de furfural (FRW). Después del tratamiento con BFT, las eficiencias de remoción de DQO, COT, NH_4^+-N, NO_3^--N y SO_4^{2-} fueron del 94,2%, 94,3%, 100%, 89,5% y 89,4%, respectivamente. Los resultados muestran que la inoculación comercial de biofloc en FRW forma biofloc con una estructura de comunidad microbiana distinta a la del lodo convencional. El biofloc estuvo dominado por Clavispora lusitaniae (11%), Weissella paramesenteroides (14%) y Mucor lusitanicus (11%), con funciones que incluyen la producción de proteína unicelular, actividad probiótica y degradación de materia orgánica, y presentó menos patógenos que el lodo, lo que lo hace más adecuado como alimento animal. Además, las larvas de mosca soldado negra (BSF) mostraron una alta tasa de supervivencia (97,9%) cuando fueron alimentadas con biofloc, lo que resalta su potencial en la producción de insectos. Este estudio demuestra que el uso de FRW como único sustrato, combinado con la inoculación comercial de biofloc, permite cultivar biofloc apto para alimentación animal y reducir significativamente los contaminantes, ofreciendo una solución eficiente y sostenible para el tratamiento de FRW.

Palabras clave:: Tecnología biofloc, aguas residuales de residuos de furfural, comunidad microbiana, ensayos de supervivencia de la mosca soldado negra.

1. Introduction

Furfural residue (FR), a by-product of furfural production, is characterized by the presence of strong acids and chemical residues[1] The indiscriminate discharge of FR poses significant environmental risks. Although FR has proven to be cost-effective in biomass refining, the efficient and sustainable treatment of the wastewater it generates remains a considerable challenge. Furfural residue wastewater (FRW) is notable for its high concentrations of chemical oxygen demand (COD), total organic carbon (TOC), ammonia nitrogen, and organic compounds. Traditional FRW treatment methods, such as filtration, flocculation, sedimentation, and advanced oxidation, are costly and have limited potential for organic matter recovery. In contrast, biofloc technology (BFT)—a wastewater treatment and biomass recovery strategy—offers a promising alternative [2].

BFT-based systems typically require supplemental carbon sources to maintain high biofloc levels. However, organic compounds in FRW, including cellulose, hemicellulose, organic acids, and small molecules, can serve as carbon sources in these systems. For example, in aquaculture wastewater treatment, Ghonimy[3] used BFT to generate biofloc from carbon and nitrogen sources, which was subsequently utilized as shrimp feed, achieving both water purification and waste reuse. Additionally, bioflocs contain cellular polysaccharides that stimulate non-specific immunity in animals, thereby enhancing their immune responses and survival[4]. BFT has also been identified as a viable method for single-cell protein production[5]. Single-cell proteins, known for their high protein content, are an effective feed option in animal husbandry, including poultry farming. The increase in protein and polysaccharide content enhances flocculation, further improving wastewater purification efficiency. By converting organic matter in wastewater into valuable single-cell proteins and polysaccharides, BFT optimizes resource utilization and aligns with sustainable development goals, addressing the issue of inadequate traditional protein sources.

Biofloc has also proven effective in treating aquaculture wastewater with COD levels ranging from 50 to 1200 mg/L, achieving removal rates between 40% and 90% [6,7]. Microorganisms play a critical role as the primary agents of wastewater purification, and the specific composition of the wastewater shapes the microbial structure of bioflocs. Common microbial communities in biofloc systems include *Bacillus spp., Roseobacter sp., Cytophaga sp., Rhodobacteraceae, Flavobacteriaceae, Tetraedron, Coelastrella, and Selenastrum*[8]. BFT has been shown to optimize microbial community structures and reduce harmful microorganisms and pathogens, ensuring the safety of biofloc use.

The aim of this study was to evaluate the potential of BFT for the simultaneous recovery of biomass and treatment of wastewater. The first objective was to assess BFT's capacity to treat FRW. Next, the microbial structure and characteristics of bioflocs were analyzed to elucidate the underlying purification mechanisms. Finally, the feasibility and safety of using bioflocs as animal feed were examined.

2. Materials and methods

2.1. Microbial source and contamination

Commercial biofloc, comprising Lactobacillus（30%）, yeast（25%）, photosynthetic bacteria（20%）, Bacillus strains（20%）, and actinomycetes（5%）, was procured from Nissan Industries Co., Ltd. FR was obtained from Bayan County Yuanda Furfural Co., Ltd. 1.Furfural is produced via corncob acid hydrolysis (sulfuric acid as catalyst, reaction temperature 170–180 °C, reaction time 2–3 h). The furfural residue generated after distillation was used as the raw material in this experiment. In addition, the preservation process of samples is specified as: After sampling, the FRW samples were immediately transported to the laboratory in an ice box (4 °C) and stored at 4 °C. The FR underwent a washing process with water at a 1:10 weight ratio. The washing water exhibited an acidic pH of 2.5. To adjust the pH to a range of 6.0 to 7.0, calcium oxide was added at a dosage of 5–6% (w/v). Prior to inoculation with biofloc, the FR washing wastewater was filtered through 0.1 mm filter paper. The commercial biofloc was then added at a dosage of 0.1% (1 g per 1000 ml of washing water), and the solution was placed in an Erlenmeyer flask.

2.2. Experimental conditions

The experiment was conducted in March 2024 at Taizhou Focusing Biotechnology Co., Ltd. in Taizhou City, Jiangsu Province, China. All experiments were performed in triplicate for each treatment group (biofloc group) and the control group (CK, un-inoculated FRW sludge), and all data were expressed as mean ± standard deviation (SD). The culture flasks were incubated under natural sunlight with continuous aeration at a flow rate of 1.5 L/min. The ambient temperature ranged from 5 to 18 degrees Celsius. The Erlenmeyer flasks underwent oscillatory motion at a frequency of 100 rpm for three hours each day until the conclusion of the experiment. Samples of 5 ml were collected for COD and TOC analysis at four-day intervals. On day 21, the solids were collected, and some samples were frozen using dry ice for subsequent microbial diversity analysis. The remaining samples were utilized in an animal survival trial.

2.3. Water quality detection

The TOC-5000A from Shimadzu Corporation (Japan) was utilized to determine the total organic carbon (TOC) content. The DR1010 COD Rapid Detector from HACH Corporation (United States) was employed to measure chemical oxygen demand (COD). Prior to analyzing the wastewater sample, it was necessary to dilute the sample by a factor of 10 to 500. The concentrations of ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), and sulfate (SO_4^{2-}) were quantified using Nessler's reagent spectrophotometry, ultraviolet spectrophotometry, and sulfate-barium chromate spectrophotometry, respectively. Flocculation performance: The water-absorbed bioflocs were observed under a microscope. Dry weight (DW) concentration: 100 mL of the culture solution was filtered through a pre-dried (105 °C, 2 h) and pre-weighed glass fiber filter membrane (0.45 μm). The filter membrane with bioflocs was then dried at 70 °C to a constant weight, and the biofloc DW concentration was calculated and expressed as g/L.

2.4. Black soldier fly survival trial

The black solid fly (BSF) was incubated in 100-mesh bran and demonstrated survival for a period of 4 days. A total of 100 larvae were collected with tweezers and transferred to a trough for weighing. The control group (CK) received 5 grams of sludge, while the biofloc group received 5 grams of biofloc. All specimens were produced specifically for this study. The troughs were then covered with a breathable membrane and placed in a shaded area. After one week, the larvae were collected, counted, and weighed. 6.Black soldier fly (BSF) eggs were purchased from Nanjing Green Insect Technology Co., Ltd. (Jiangsu, China) and hatched in an artificial climate incubator (temperature 28 ± 1 °C, relative humidity 70 ± 5%) to obtain neonate larvae (1st instar). The neonate larvae were pre-fed with sterile bran (100-mesh) for 4 days to reach the uniform 2nd instar stage (larval weight ~1.6 mg/individual) before the formal experiment, ensuring consistent initial growth status of the larvae.

2.5. DNA extraction and microbial diversity analysis

Total microbial DNA from the samples was extracted using a TIANamp DNA Kit (Tiangen Biotech Co., Ltd., China). The 338F-806R primer pairs were employed to amplify the 16S rRNA gene after purification and fragmentation via sonication. DNA sequencing was subsequently performed by Novogene (Tianjin, China) using the Illumina MiSeq PE300 platform. The sequence data were analyzed using UPARSE, and sequencing libraries were generated with QIIME software following the manufacturer's instructions. The functional composition of the microbial community was predicted using PICRUSt2.

2.6. Analytical methods

All experimental data were expressed as mean ± standard deviation (SD) with triplicate repeats for each treatment group and control group. Statistical analysis of the data was performed using standard statistical software, and the differences between groups were compared to ensure the reliability and statistical significance of the experimental results.

3. Results and discussions

3.1. Morphology analysis

Figure 1 illustrates the morphology of bioflocs cultivated in furfural residue wastewater (FRW). As shown in Figure 1a, BFT can efficiently generate bioflocs in FRW, predominantly ranging from 1 to 10 mm in diameter. For comparison, Figures 1b and 1c present data from other sources of bioflocs and flocculated sludge, respectively[9,10]. Previous studies have demonstrated that aquatic animal excreta in aquaculture effluents serve as suitable carriers for bioflocs, resulting in larger biofloc diameters, as observed in Figure 1b. Conversely, the flocculated sludge in Figure 1c exhibited significantly smaller diameters than the bioflocs, which may be attributed to the higher concentration of extracellular polymeric substances (EPS), including polysaccharides and proteins, that enrich bioflocs and provide additional adsorption sites. While aquatic animal feces are commonly used as carriers for bioflocs, this study found that bioflocs could form in FRW even without a carrier. This confirms the potential of BFT for effective FRW treatment.

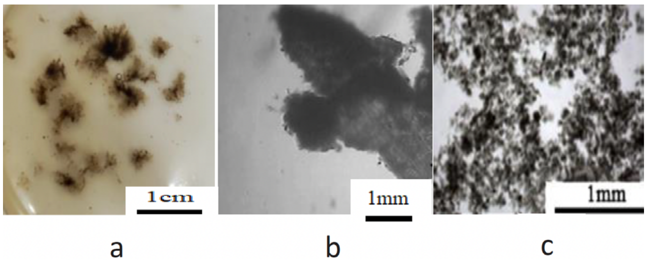

Figure 1. Photo of (a) biofloc from this research, (b) biofloc (image from reference [9]), (c) flocculent sludge (image from reference [10])

3.2. The performance of BFT in FRW treatment

In comparison to the organic contaminants found in FRW (TOC: 8310 mg/L, COD: 5670 mg/L), those present in aquaculture wastewater are significantly lower (COD: 50–1200 mg/L) [6,7]. As shown in Table 1, the biofloc system demonstrated a remarkable capacity for organic matter degradation. Over time, there was a significant reduction in the TOC and COD levels of the wastewater, achieving removal rates exceeding 94.3% and 94.2%, respectively, by day 16. These efficiencies surpassed the performance of bioflocs in treating aquaculture wastewater.

Microalgae-based wastewater treatment (MBWT) has also been utilized for treating FRW, with the addition of nitrates shown to enhance oil content in Chlorella pyrenoidosa, reaching a maximum of 18.53%. However, at a COD concentration of 5000 mg/L, the COD removal rate was only 73.29%, which was lower than that observed with BFT [11].

It is noteworthy that ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) concentrations in FRW were 104 ± 2.4 mg/L and 578 ± 41 mg/L, respectively, which are more than 100 times higher than those typically found in aquaculture wastewater (Deswati Deswati et al., 2020). However, following BFT treatment, nitrogen removal was highly effective, with NH_4^+-N levels dropping below the detection limit and NO_3^--N content reduced to 61 ± 17 mg/L, resulting in an 89.5% removal efficiency. The sulfuric acid present in FR was transferred to the wastewater, resulting in a reduction of sulfate content from 1800 mg/L to 470 mg/L through calcium precipitation. After further biofloc treatment, sulfate levels were further reduced to 50 mg/L. In conclusion, BFT represents an efficient tool for treating multi-contaminated FRW.

Table 1. Water treatment effect of BFT to FR wastewater.

Treatment Time/ day	TOC content / (mg/L)	COD content / (mg/L)	NH4+-N content / (mg/L)	NO3--N content / (mg/L)	SO42- content / (mg/L)
0	8310±1180	5670±520	104±2.4	578±41	470±42
4	4900±620	3380±420	37.2±3.9	455±37	381±22
8	2750±770	1870±170	12.8±2.2	304±16	240±37
12	1110±180	760±60	n.d.	172±28	116±13
16	480±60	330±20	n.d.	61±17	50±8

n.d.: not detected

3.3. Analysis of microbial communities in bioflocs

3.3.1. *Microbial diversity equations*

As shown in Figure 2a, the bacterial domain dominated the CK sample, comprising 88% of the microbial community. Other domains were detected, with archaea representing 0.008%ofthecommunity.IntheCKsample,the predominant phylum was Pseudomonadota, accounting for 81% of the total sequences, with Gammaproteobacteria constituting 79%. Additionally, Xanthomonadales (38%), Enterobacterales (23%), Pseudomonadales (8%), Burkholderiales (1%), and Mucorales (1%) were present. The most dominant microbes, ranked by prevalence, were Stenotrophomonas (37%), Stenotrophomonas maltophilia (15%), Pseudomonas (8%), Pantoea (3%), Cronobacter sakazakii (2%), Cronobacter dublinensis (1%), Burkholderiales (1%), and Rhizopus arrhizus (1%).

In contrast, biofloc showed a high prevalence of both the Bacteria and Eukaryota domains, with abundances of 46% and 35%, respectively (Figure 2b). Further analysis indicated that Bacillota (27%) and Pseudomonadota (18%) were part of the Bacteria domain, while Mucoromycota (20%) and Ascomycota (15%) belonged to the Eukaryota domain. The microbial composition of biofloc included Weissella paramesenteroides (14%), Mucor lusitanicus (11%), Clavispora lusitaniae (11%), Pediococcus (8%), Pantoea (5%), Pediococcus pentosaceus (3%), and Rhizopus arrhizus (2%).

Xanthomonadales, Pseudomonadales, and Burkholderiales collectively represented 47% of the microbial population, making them prevalent in both water treatment and natural aquatic environments [13,14]. Additionally, Enterobacterales were readily reproducible in untreated organic water, especially in environments containing straw [15,16] The abundances of Bacillota

(27%) and Pseudomonadota (18%) in biofloc were comparable to those in the ATS system, used for treating wastewater from municipal sources, manure, and biogas plant effluent [17]. These findings indicate that BFT effectively optimizes the microbial community structure in FRW, enhancing its suitability for wastewater treatment.

3.3.2. Function of the dominant microorganisms

Table 2 presents the five most important characteristics of biofloc, which include single-cell protein (SCP) production, probiotic activity, food fermentation, organic matter degradation, and pathogen degradation. The first four functions collectively represent a category of edible microorganisms, indicating that the biofloc cultivated in this study meets these criteria. In contrast, only two functions—SCP production and organic matter degradation—were observed in the CK group. Pseudomonas has been reported to convert waste motor oil into SCP, yielding a crude protein content of 27.3% and a crude fat content of 10% [18], although it is also known as an opportunistic pathogen responsible for various infections [19]. Clavispora lusitaniae, a yeast species, has been widely used as a source of SCP [20]. Pediococcus and Pediococcus pentosaceus are well-recognized probiotics [21,22], and Pediococcus species can utilize residual xylose found in furfural residue [23]. Mucor circinelloides is commonly employed in the fermentation of straw and food products [24]. Notably, the biofloc associated with FRW contained dominant microbial populations linked to lignocellulose degradation and pentose conversion. Compared to the CK sample, the biofloc more closely aligns with a combination of edible microorganisms. In conclusion, BFT optimized the microbial community structure in FRW, enhancing its functional composition and expanding the potential applications of bioflocs.

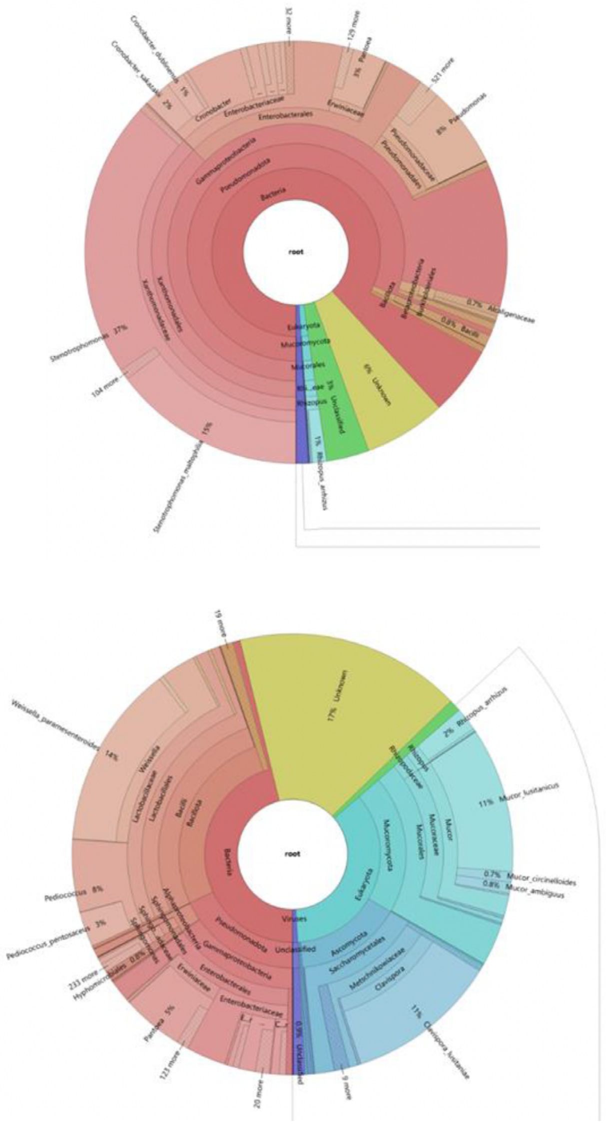

Figure 2. (A)Microbial community and relative abundance in CK;(B) Microbial community and relative abundance in biofloc

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Table 2. Function of the dominant microorganisms in CK and Biofloc.

Group		SCP Production	Probiotics	Food fermentation	Pathogen	Degradation of organic matter	References
CK							
Stenotrophomonas	37%					+	[25]
Stenotrophomonas_maltophilia	15%				+		[25]
Pseudomonas	8%	+				+	[12][19]
Pantoea	3%				+		[15]
Cronobacter_sakazakii	2%				+		[27]
Cronobacter_dublinensis	1%				+		[28]
Rhizopus_arrhizus	1%					+	[29]
Biofloc							
Weissella_paramesenteroides	14%			+			[22]
Mucor_lusitanicus	11%			+	+	+	[24][31]
Clavispora_lusitaniae	11%	+		+			[22][32]
Pediococcus	8%		+				[30]
Pantoea	5%				+		[26]
Pediococcus_pentosaceus	3%		+				[21]
Rhizopus_arrhizus	2%					+	[29]

3.3.4. Pathogenic microorganisms

Numerous studies have demonstrated that many pathogenic microorganisms (e.g., Pseudomonas aeruginosa, Cronobacter sakazakii, and Mucor ruditani) exhibit opportunistic pathogenicity. Analyzing the pathogenic microorganisms in biofloc provides insights into their environmental risks and safety. To enable a more detailed comparison of pathogenic bacterial composition, metagenomic data were imported into the PHI database. As shown in Figure 3, the analysis identified ten major pathogenic microorganisms. The application of BFT led to a significant reduction in the abundance of pathogenic microorganisms in the biofloc group compared to the CK group. Notably, there was a clear decline in the abundance of Salmonella enterica, Xanthomonas campestris, Xanthomonas oryzae, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, and Erwinia amylovora.

Salmonella enterica is a common bacterium responsible for food poisoning in humans. Xanthomonas campestris and Xanthomonas oryzae can persist for long periods in plant residues, posing a risk to the effective utilization of biofloc. Although Escherichia coli is a normal intestinal flora in humans and animals and is generally harmless, specific strains can cause foodborne illness. Klebsiella pneumoniae can cause disease through the production of E. coli enterotoxins and other virulence factors and resists the host's immune response and antibiotics due to its protective capsules.

It is noteworthy that the abundance of Fusarium graminearum, Staphylococcus aureus, and Candida albicans increased. While primarily considered pathogenic, these microorganisms also have potential benefits in ecology, industry, immunology, and biotechnology, including biocontrol applications and the maintenance of microecological balance.

In conclusion, the relative abundance of pathogenic microorganisms in bioflocs was reduced by 47.91% compared to FRW, significantly decreasing the environmental risk of BFT. These results indicate that BFT has the potential to reduce the pathogenicity of microorganisms in FRW, thereby enhancing the safety of its applications.

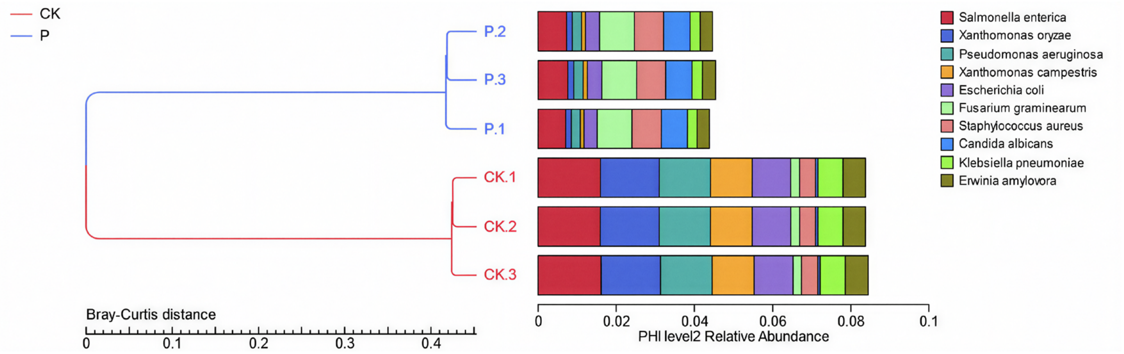

Figure 3. Pathogenic microorganisms in biofloc (P) and FR wastewater (CK) in level 2.

3.3.5. Black soldier fly survival trial

The black soldier fly (BSF) has proven to be an effective treatment for various solid wastes, including food waste, swine manure, and chicken manure. Attempts have also been made to use sludge, a bulk solid organic waste, as feedstock for BSF. However, these efforts were unsuccessful due to low survival rates caused by the high heavy metal content in the sludge [33 , 34] . This study aimed to explore the use of FRW bioflocs as feedstock for BSF to enable efficient biofloc recovery and biomass production.

Table 3 shows three key indicators: survival rate, weight of 100 larvae, and weight gain rate. The results indicated that the CK group's survival rate after one week was 58.6%, lower than previously reported[35]. This lower survival rate may be attributed to the larvae's inability to synthesize sufficient protein and fat to thrive in the challenging sludge environment. In contrast, the biofloc group exhibited a survival rate of 97.9%, meeting production requirements. The biofloc provided ample proteins, lipids, and minerals, creating a nutrient-rich and favorable environment for BSF growth.

Table 3. Animal trial.

Group	Survival rate for 1 week (%)	Initial average weight (100pcs) / mg	Average weight after 1 week (100pcs) / mg	Weight gain rate (%)
CK	58.6 ± 3.7	157 ± 9	209 ± 17	33.12
Biofloc	97.9 ± 0.5	164 ± 11	509 ± 29	210.37

Moreover, the average body weight of BSF after one week in the biofloc group surpassed that of larvae fed with municipal sewage sludge but was slightly lower than those fed with chicken manure [35]. The BSF in the biofloc group showed a body weight gain rate approximately seven times higher than in the CK group, demonstrating effective biomass recycling. Overall, the comparison of BSF growth in bioflocs suggests that biofloc utilization for BSF cultivation can support the ecological reuse of bioflocs and offers a novel solution for addressing the biofloc challenge in FRW treatment.

4. Conclusions

The results of our investigation demonstrate that inoculating commercial biofloc into furfural residual wastewater (FRW) leads to the formation of discrete biofloc. The removal efficiencies for chemical oxygen demand (COD), total organic carbon (TOC), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), and sulfate (SO_4^{2-}) by biofloc technology (BFT) were found to be 94.2%, 94.3%, 100%, 89.5%, and 89.4%, respectively. In addition to the commonly observed yeasts, this study identified significant proportions of Clavispora lusitaniae (11%), Weissella paramesenteroides (14%), and Mucor lusitanicus (11%) within the biofloc. Conversely, the natural microorganisms present in FRW exhibited a markedly different composition, with Stenotrophomonas (37%), Stenotrophomonas maltophilia (15%), Pseudomonas (8%), Pantoea (3%), and Cronobacter sakazakii (2%), among others, compared to the biofloc community, which comprised over 90% beneficial microorganisms. Functionally, the biofloc studied is more suitable for the feed industry than the naturally occurring sludge microorganisms in FRW. This is due to the lower abundance of pathogenic bacteria and the higher prevalence of yeasts, lactic acid bacteria, and other beneficial bacteria suitable for food production. An animal trial with black soldier flies (BSF) demonstrated that biofloc is safe for BSF, achieving a survival rate of 97.9%. These findings indicate that biofloc is an effective method for improving water quality. Therefore, it is proposed that treated FRW undergo BFT, followed by the biofloc treatment cycle, facilitating the realization of clean production and resource utilization objectives.

5. Declarations

The authors declare no competing interests.

6. References

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