International Journal of Hydrological and Environmental for Sustainability
Volume 5, Issue 3, 276-286.
e_ISSN: 2828-5050
https://www.journal.foundae.com/index.php/ijhes

Valorization of Tofu-Wastewater Anaerobic Digestate into Bacterial Cellulose Using *Acetobacter Xylinum*

Laras Andria Wardani*
Environmental Engineering Department,
Faculty of Engineering, Bhayangkara Jakarta
Raya University,
INDONESIA

Andi Nuraliyah
Environmental Engineering Department,
Faculty of Engineering, Bhayangkara Jakarta
Raya University,
INDONESIA

***Correspondence: E-mail:** laras.andria.wardani@dsn.ubharajaya.ac.id

Article Info

Article history:

Received: March 15, 2026
Revised: June 29, 2026
Accepted: August 26, 2026

Abstract

Anaerobic digestate generated from tofu-industry wastewater treatment frequently contains residual organic matter exceeding wastewater quality standards, necessitating further treatment or valorization. This study aimed to evaluate the effects of sugar, NPK supplementation on bacterial-cellulose formation from tofu-wastewater anaerobic digestate, determine the treatment producing the highest pellicle thickness, dried bacterial cellulose sheet; and preliminary supplementary-material cost estimation. A 2×3 factorial design comprising two sugar concentrations (15%; 30%w/v), three NPK concentrations (0.3%; 0.5%; 0.7%w/v) was applied, with three replicates each. Anaerobic digestate (500 mL) was inoculated with 10%(v/v) Acetobacter xylinum and incubated at approximately 30°C (14 days). The greatest bacterial-cellulose thickness was obtained in 15% sugar, 0.3% NPK, reaching 1.13cm, while 30% sugar supplementation substantially reduced pellicle formation. The corresponding dried sheet thickness was 0.075cm. Preliminary supplementary-material costs were estimated at IDR1,386,- for consumable inputs. These findings demonstrate the potential of tofu-wastewater anaerobic digestate as an alternative substrate for bacterial-cellulose production.

Keywords: anaerobic digestate, tofu wastewater, bacterial cellulose, acetobacter xylinum, waste valorization

To cite this article: Wardani, L, A. and Nuraliyah, A. (2026). Valorization of Tofu-Wastewater Anaerobic Digestate into Bacterial Cellulose Using *Acetobacter Xylinum*. *International Journal of Hydrological and Environmental for Sustainability, 5*(3), 276-286. https://doi.org/10.58524/ijhes.v5i3.1458

INTRODUCTION

The number of tofu industries in Indonesia is around 84,000 (Ginting *et al.,* 2024). Data shows that 80% of these industries are located on the island of Java (Prasetyadi *et al.,* 2018). Despite its contribution to food security and the local economy, the tofu production process produces liquid waste with a high organic content, potentially polluting the environment if not managed properly. Typical characteristics of tofu wastewater include COD concentrations of 8,000–11,400 mg/L (Sahaq, 2019), BOD of 1,000–11,880 mg/L (Nostia and Kurniawan, 2023; Permana, 2019), and TSS exceeding 1,000 mg/L (Adisasmito *et al.,* 2018; Satar and Permadi, 2022).

Several tofu industries in Indonesia have implemented Wastewater Treatment Plants (WWTPs) to reduce the environmental impact associated with tofu wastewater discharge. Anaerobic treatment systems are widely adopted due to their ability to degrade organic matter while simultaneously producing biogas as a renewable energy source. Nevertheless, the anaerobic digestate generated from these treatment processes often still contains residual organic pollutants at concentrations exceeding the wastewater quality standards (*Baku Mutu Air Limbah*/BMAL) stipulated in the Minister of Environment Regulation No. 5 of 2014, indicating the need for further treatment or valorization strategies. One example is Proma Tofu Industry, located in Probolinggo, Indonesia, which has implemented an anaerobic wastewater treatment system using a fixed-bed digester. The system has been operated to treat tofu processing wastewater and generate anaerobic digestate as a by-product of the treatment process. To distinguish between the characteristics of the influent and the post-digestion effluent, analyses of Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), Total Suspended Solids (TSS), and pH were conducted for both the raw tofu wastewater and the resulting anaerobic digestate. The measured characteristics of these two substrates are presented in **Table 1**.

Table 1. Characteristics of the raw tofu wastewater and digestate

No	Parameter	Raw tofu wastewater (mg/l)	Digestate concentrations (mg/l)	efficiency of concentration reduction	MoE No.5/2014.	Information
1	COD	7471,47	1250,22	83%	300 mg/l	Exceeding quality standards
2	BOD	1853,99	1268,61	27%	150 mg/l	Exceeding quality standards
3	TSS	723,43	193,12	71%	200 mg/l	According to quality standards
4	pH	4,86	6,94		6-9	According to quality standards

These concentrations remain above the wastewater quality standards stipulated in the Indonesian Ministry of Environment Regulation No. 5/2014 (Minister of Environment, 2014). This situation indicates that existing wastewater treatment is not fully effective in reducing pollutant loads, requiring further treatment to produce digestate that meets environmental quality standards.

Although several studies have reported the production of bacterial cellulose (nata) from raw tofu wastewater (Srikandace *et al.*, 2022; Margono *et al.*, 2023; Laksono *et al.*, 2025), the utilization of anaerobically treated tofu wastewater (digestate) remains largely unexplored. Anaerobic digestion significantly alters the physicochemical characteristics of wastewater, resulting in a substrate composition that differs from untreated tofu effluent and may influence bacterial cellulose production. In contrast, anaerobic digestate has predominantly been valorized for agricultural applications, such as compost, soil amendments, and nutrient recovery (Czekała *et al.*, 2023; Wang *et al.*, 2023; Agyeman-Duah *et al.*, 2023; Asp and Bergstrand, 2024; Villada *et al.*, 2024; Borja *et al.*, 2025; Diawara *et al.*, 2025; Gabler *et al.*, 2025; Letho and Järvelä, 2025; Boarino *et al.*, 2026; Esperanço *et al.*, 2026; Hou *et al.*, 2026; Lin *et al.*, 2026; Kulichkova *et al.*, 2026; Kushwaha and Dhoble, 2026; Luo *et al.*, 2026; Racho *et al.*, 2026; Radkevich *et al.*, 2026; Ramalho *et al.*, 2026; Xu *et al.*, 2026), while its utilization as a substrate for high-value biomaterial production remains largely unexplored. This research gap highlights the opportunity to utilize digestate beyond conventional agricultural uses by converting it into cellulosic products. Therefore, this study aimed to evaluate the effects of sugar and NPK supplementation on bacterial-cellulose formation from tofu-wastewater anaerobic digestate, determine the treatment producing the highest pellicle thickness and dried bacterial cellulose sheet; and preliminary supplementary-material cost estimation.

METHOD

Experimental Substrate

In this study, digestate refers to the liquid effluent discharged from an anaerobic digester treating tofu-industry wastewater after completion of the anaerobic digestion process. The anaerobic digestate used in this study was obtained from Proma Tofu Industry, located in Probolinggo, Indonesia. Samples were collected in 2026 from the outlet of the company's wastewater treatment facility, which employs an anaerobic wastewater treatment system equipped with a fixed-bed digester. The hydraulic retention time (HRT) of the wastewater in the anaerobic reactor was 4.5 days, and the digester was operated under ambient temperature conditions. Prior to anaerobic treatment, the raw tofu wastewater had an acidic pH of 4.86. Following anaerobic digestion, the pH of the resulting liquid digestate increased to 6.94, indicating a substantial change in the physicochemical characteristics of the substrate. Characterization of the anaerobic digestate showed a Chemical Oxygen Demand (COD) of 1,250.22 mg/L, Biological Oxygen Demand (BOD) of 1,268.61 mg/L, and Total Suspended Solids (TSS) of 193.12 mg/L.

The digestate used in the experiments consisted exclusively of the liquid effluent collected from the outlet of the fixed-bed anaerobic digester. Following collection, the digestate was not subjected to prolonged storage and was immediately used as the fermentation medium for bacterial cellulose production to minimize potential changes in its physicochemical composition prior to experimentation.

Experimental Design

The materials used in this study consisted of anaerobic digestate from tofu-industry wastewater treatment, granulated sugar as the primary carbon source, commercial NPK fertilizer as a supplementary nutrient source, acetic acid for pH adjustment, and Acetobacter xylinum as the bacterial inoculum. The principal equipment included a heating vessel for boiling the digestate, plastic fermentation trays, clean newspaper sheets and rubber bands for covering the trays, and a pH meter. A 2 × 3 factorial experimental design was employed, consisting of two sugar concentrations (15% and 30%, w/v) and three NPK fertilizer concentrations (0.3%, 0.5%, and 0.7%, w/v), resulting in six treatment combinations. Each treatment was performed in triplicate (n = 3), resulting in a total of 18 experimental units. The experimental units were randomly positioned during incubation to minimize potential positional bias. Each fermentation tray measured 20 × 15 × 5 cm and contained a working volume of 500 mL of digestate-based fermentation medium. The six treatment combinations were as follows:

1. C1N1: 15% (w/v) sugar + 0.3% (w/v) NPK fertilizer
2. C1N2: 15% (w/v) sugar + 0.5% (w/v) NPK fertilizer
3. C1N3: 15% (w/v) sugar + 0.7% (w/v) NPK fertilizer
4. C2N1: 30% (w/v) sugar + 0.3% (w/v) NPK fertilizer
5. C2N2: 30% (w/v) sugar + 0.5% (w/v) NPK fertilizer
6. C2N3: 30% (w/v) sugar + 0.7% (w/v) NPK fertilizer

No negative or positive control treatment was included in the present experiment. This preliminary study was specifically designed to evaluate the performance of different combinations of sugar and NPK supplementation in digestate-based media. Sugar-only and NPK-only control treatments were not included because of limitations in materials, experimental time, and available resources. Therefore, the results of this study are interpreted as comparisons among the six nutrient-supplementation treatments rather than against an unsupplemented control medium.

Carbon and Nutrient Supplementation

Anaerobic treatment converts a substantial proportion of biodegradable organic matter in tofu wastewater into biogas and other metabolic products. Consequently, the resulting digestate may contain insufficient readily available carbon and nutrients to support optimal growth of Acetobacter xylinum and bacterial cellulose biosynthesis. Supplementation with additional carbon and nutrient sources was therefore applied to improve the suitability of the digestate as a bacterial cellulose fermentation medium.

Sugar was added as the principal carbon and energy source for bacterial growth and cellulose formation. Carbon availability is important not only for cellular energy metabolism but also for the synthesis and accumulation of extracellular cellulose (Sinskey *et al.,* 1986). Acetobacter xylinum produces enzymes involved in the conversion and polymerization of sugars into cellulose chains and fibers (Nugroho *et al.,* 2015). During fermentation, available sugars can therefore contribute to microbial metabolism and the formation of cellulose fibrils, which progressively accumulate to form a pellicle at the liquid-air interface.

In addition to a carbon source, an adequate nitrogen supply is required to support bacterial growth because nitrogen is essential for the biosynthesis of amino acids, proteins, nucleic acids, and other cellular components. Nitrogen sources commonly used for bacterial cellulose production can be classified as organic or inorganic. Organic nitrogen sources typically include yeast extract and peptone, whereas commonly used inorganic nitrogen sources include ammonium dihydrogen phosphate ($NH_4H_2PO_4$) and ammonium sulfate ($(NH_4)_2SO_4$). The formation and thickness of bacterial cellulose can be enhanced by supplementation with appropriate nitrogen sources, including $NH_4H_2PO_4$ and $(NH_4)_2SO_4$ (Krieg and Holt, 1984). Previous studies have also reported that yeast extract and peptone are among the most frequently employed nitrogen supplements for bacterial cellulose production because they can support microbial growth and cellulose biosynthesis. Microorganisms require nitrogen for the synthesis of amino acids and nucleic acids (Catarino *et al.*, 2025).

In the present study, commercial NPK fertilizer was evaluated as an alternative, low-cost nutrient supplement for bacterial cellulose production. The use of commercial NPK fertilizer in bacterial cellulose fermentation has rarely been reported in the literature, providing a rationale for evaluating its potential application in a digestate-based medium. The experimental design primarily considered the nitrogen contribution of the NPK fertilizer in relation to bacterial cellulose production. However, because NPK fertilizer also contains phosphorus and potassium, the individual contributions of these nutrients could not be separated experimentally. Phosphorus and potassium were therefore not treated as independent experimental variables because the primary objective was to evaluate bacterial cellulose formation under different levels of NPK supplementation, with particular emphasis on nitrogen availability. Nevertheless, possible synergistic contributions of phosphorus and potassium to microbial metabolism and cellulose biosynthesis cannot be excluded and should be considered when interpreting the treatment effects.

pH Adjustment and Inoculum Preparation

In addition to carbon and nutrient supplementation, pH is an important factor controlling bacterial cellulose production by Acetobacter xylinum. Acetic acid was therefore used to increase the acidity of the fermentation medium and adjust the pH before inoculation. Santoso (1996) reported that favorable bacterial cellulose thickness can be obtained at approximately pH 5.0–5.5 (Santoso, 1996). The general cultivation principle for Acetobacter xylinum is comparable to conventional bacterial cellulose production using coconut-water-based media. In such procedures, coconut water is boiled, cooled, supplemented with sugar, ZA fertilizer, and acetic acid, and subsequently inoculated with a pure culture of Acetobacter xylinum. The fermentation vessel is then covered with newspaper secured with a rubber band to reduce contamination while permitting exposure to air, and the culture is maintained indoors away from direct sunlight (Salim, 2015). The present study adapted this general cultivation approach by replacing the conventional coconut-water substrate with anaerobic tofu-wastewater digestate and systematically varying the concentrations of sugar and NPK fertilizer.

Preparation and Fermentation of Bacterial Cellulose

Bacterial cellulose production from the anaerobic tofu-wastewater digestate was performed according to the following procedure.

1. The freshly collected digestate was initially filtered through a double-layer muslin cloth with an approximate pore size of 100–150 μm to remove coarse suspended solids and obtain a more homogeneous liquid fermentation substrate.
2. The filtered digestate was heated to approximately 100°C and maintained at boiling temperature for 10–15 min to reduce the indigenous microbial load and minimize interference from undesirable microorganisms during subsequent fermentation.
3. After heating, the digestate was allowed to cool to room temperature, approximately 30°C, before the addition of the inoculum.

4. A total working volume of 500 mL of digestate-based fermentation medium was prepared for each experimental unit and transferred into a clean fermentation tray measuring 20 × 15 × 5 cm.
5. Granulated sugar was added according to the designated treatment concentration, either 15% or 30% (w/v), as the supplementary carbon source.
6. Commercial NPK fertilizer was subsequently added at a concentration of 0.3%, 0.5%, or 0.7% (w/v), according to the corresponding experimental treatment.
7. Acetic acid was added to the supplemented digestate medium until a pH of 3.0–3.5 was obtained, as measured using a pH meter.
8. After the medium had cooled to approximately 30°C and the required pH had been achieved, Acetobacter xylinum inoculum was added at 10% (v/v) relative to the volume of the digestate-based fermentation medium.
9. Each fermentation tray was immediately covered with a clean newspaper sheet and secured with a rubber band. This covering was used to minimize contamination while allowing sufficient gas exchange required for the aerobic production of bacterial cellulose.
10. The fermentation trays were incubated under static conditions in a clean laboratory environment at an ambient temperature of approximately 30°C for 14 days. The trays were maintained away from direct sunlight and were not disturbed during incubation to facilitate the formation of bacterial cellulose pellicles at the air-liquid interface.
11. After 14 days of fermentation, the bacterial cellulose pellicles were carefully harvested from the fermentation trays.
12. The harvested pellicles were subjected to pressing using a standardized flat weight and subsequently dried under ambient air conditions without additional energy input.
13. The thickness of both the wet bacterial cellulose pellicles and the resulting dried bacterial cellulose sheets was measured using calipers. Measurements were performed consistently for all treatment combinations to enable comparison of bacterial cellulose formation among the experimental treatments.

Data Analysis and Preliminary Material Cost Estimation

The thickness measurements obtained from the three replicates of each treatment were analyzed using descriptive statistics and summarized in tabular form to facilitate comparison among the six combinations of sugar and NPK fertilizer concentrations. The treatment producing the greatest bacterial cellulose thickness was identified based on the observed experimental results. In addition, a preliminary supplementary-material cost estimation was conducted for the treatment that produced the highest bacterial cellulose thickness. The estimation focused on the additional consumable materials required for bacterial cellulose production, particularly the supplementary carbon and nutrient inputs, to provide an initial assessment of the material requirements associated with the selected treatment.

RESULTS AND DISCUSSION

Nutrient supplementation was performed using various concentrations of sugar and NPK fertilizer to optimize the composition of the fermentation medium and identify the most effective conditions for bacterial cellulose production. The different nutrient combinations were evaluated based on their ability to support the growth and metabolic activity of *Acetobacter xylinum*, thereby enhancing bacterial cellulose biosynthesis as reflected by increased pellicle thickness. The resulting bacterial cellulose sheets were harvested after 14 days of incubation, and their thicknesses were subsequently measured and recorded (**Table 2**).

Based on the experimental results, it can be shown statistically that the thickness of cellulose solids changes according to variations in nutrient addition. Based on the data from the measurement results of the thickness of cellulose solids, it can be seen that when the variation of 15% sugar addition can form perfect cellulose solids with a thickness of approximately 1 cm, while the addition of 30% sugar nutrients only has an average thickness of cellulose solids of <0.1 cm. The results of the study showed that the best thickness of bacterial cellulose was 1.13 cm, obtained from the C1N1 treatment with the addition of 15% granulated sugar and 0.3% NPK. Next, the harvested bacterial cellulose was dried.

Table 2. Bacterial Cellulose thickness data for each variation of nutrient addition

Repetitive	Unit	C1N1	C1N2	C1N3	C2N1	C2N2	C2N3
1	cm	1.10	1.00	0.80	0.15	0.14	0.14
2	cm	1.10	1.10	0.80	0.26	0.22	0.13
3	cm	1.20	1.00	0.80	0.37	0.19	0.11
Average	**cm**	**1.10**	**1.00**	**0.80**	**0.26**	**0.18**	**0.12**
Standard deviations		**0.058**	**0.058**	**0.000**	**0.110**	**0.040**	**0.015**

Drying was performed by placing a flat weighted object on the cellulose pellicles to provide a uniform compressive load across all samples. This procedure was applied consistently to minimise variations in thickness caused by differences in moisture content and to obtain a more representative measurement of the final cellulose product. The results indicate that the drying process substantially reduced the thickness of bacterial cellulose, particularly in treatments with higher sugar concentrations, further confirming that the addition of 15% sugar was more favourable for producing thicker and structurally stable bacterial cellulose than the 30% sugar treatment. Thickness of bacterial cellulose after the drying process, can be seen in **Table 3**.

Table 3. Dried Bacterial Cellulose thickness data for each variation of nutrient addition

Repetitive	Unit	C1N1	C1N2	C1N3	C2N1	C2N2	C2N3
1	cm	0.073	0.067	0.054	0.010	0.010	0.010
2	cm	0.075	0.069	0.055	0.018	0.015	0.010
3	cm	0.077	0.068	0.056	0.025	0.013	0.010
Average	**cm**	**0.075**	**0.068**	**0.055**	**0.017**	**0.012**	**0.010**
Standard deviations		**0.0020**	**0.0010**	**0.0010**	**0.0075**	**0.0025**	**0.0000**

The reduced pellicle formation observed at 30% sugar supplementation (<0.1 cm) may be associated with osmotic inhibition or substrate-related stress affecting *Acetobacter xylinum* activity; however, these mechanisms were not directly evaluated in the present study. This result contrasts with the findings of Santoso (1999), who reported that bacterial cellulose thickness generally increased with increasing sugar concentrations. The formed bacterial cellulose are then pressed and dried in the open air until they form thin sheets. Representative bacterial cellulose sheets produced from anaerobic tofu wastewater digestate (C1N1) are presented in **Figure 1**.

Figure 1. Transformation of anaerobic digestate into dried bacterial cellulose sheet

On the other hand, Residual liquid volume was recorded at the end of the fermentation period. Beginning with an initial digestate volume of 500 mL per treatment, the remaining free liquid ranged from 40 to 100 mL after 14 days of incubation. Consequently, only approximately 8–20% of the initial digestate remained as free liquid, corresponding to an apparent reduction in liquid volume of 80–92%, depending on the concentrations of the supplemented carbon and nutrient sources. This reduction in volume is likely attributable to a combination of water retention within the wet bacterial cellulose pellicle, evaporation during incubation, and microbial utilization of the substrate, rather than complete conversion into bacterial cellulose. This is consistent with research by Nugroho and Aji (2015) and Zhang *et al.* (2017) indicates that the volume of digestate from bacterial cellulose solids production consistently decreases daily and stabilizes after day 6. Research by Zhang *et al.* (2017) also stated that the lactic and acetic acid content increased during pre-fermentation. The production of lactic and acetic acid would create a favourable acidic environment for further fermentation by *Acetobacter* (Verschuren *et al.,* 2000; Almeida *et al.,* 2013).

The residual liquid remaining after bacterial cellulose production may have potential for recirculation as influent to anaerobic digesters, as it contains acetic acid and residual biodegradable organic compounds that could serve as substrates for anaerobic microorganisms. In the present study, the substrate originated from tofu wastewater, which is inherently acidic, and the source anaerobic fixed-bed digester has been demonstrated to operate effectively under these wastewater characteristics. Furthermore, residual sugar and organic compounds present in the post-fermentation liquid may serve as additional substrates for anaerobic microorganisms and could potentially contribute to methane generation through subsequent anaerobic degradation pathways. However, biochemical methane potential (BMP) and anaerobic treatability were not evaluated in the present study. Therefore, the integration of bacterial cellulose production with anaerobic wastewater treatment systems should be considered a potential resource recovery strategy within a circular bioeconomy framework that warrants further investigation.

Based on the treatment that produced the greatest bacterial cellulose thickness (C1N1), a preliminary supplementary material cost estimation was performed to evaluate the additional consumable inputs required for bacterial cellulose production. Since this study was conducted as a laboratory-scale feasibility assessment, several cost components typically included in a comprehensive techno-economic analysis were considered negligible or were not quantified. Specifically, the bacterial starter culture was obtained at no cost, energy consumption associated with boiling was not estimated because a household stove was used, and labour costs were excluded as all experimental procedures were performed by the author. In addition, no supplementary water was required during the fermentation process. Equipment-related costs, including tray and instrument depreciation, were not considered, while pressing and drying were carried out using a flat weighted object to provide a uniform compressive load and ambient air, respectively, without additional energy input. Therefore, the cost assessment presented in this study solely as a preliminary supplementary material cost estimation, limited to consumable materials used during fermentation, namely sugar, NPK fertilizer, and acetic acid, as seen in **Table 3**. **Table 3** explains that processing 500 ml of digestate from the tofu industry into cellulose solids requires additional materials such as sugar, NPK fertilizer, and acetic acid water, with a total cost of IDR 1,386.

Table 3. Preliminary supplementary material cost estimation of bacterial cellulose with 500 ml of anaerobic digestate tofu industry

Consumable Materials	Price (Rp)	unit price	nutrient needs			IDR
Sugar	17000	Per-kg	15% % w/v =	75	gram	1275
NPK	27000	Per-kg	0,3% % w/v =	1,5	gram	41
Vinegar	3500	Per-100 ml	± 2 ml		litter	70
					Total	**1386**

CONCLUSION

Under the experimental conditions evaluated in this study, the combination of 15% (w/v) sugar and 0.3% (w/v) NPK (C1N1) produced the highest wet bacterial-cellulose pellicle thickness after 14 days of fermentation, with a mean thickness of 1.13cm. In contrast, increasing the sugar concentration to 30% substantially reduced pellicle formation across all NPK treatments. These findings indicate that carbon and nutrient supplementation play important roles in influencing bacterial-cellulose production from tofu-wastewater anaerobic digestate. A preliminary supplementary-material cost estimation indicated that the production of bacterial cellulose from 500 mL of anaerobic digestate required approximately IDR 1,386 for consumable inputs (sugar, NPK fertilizer, and acetic acid).

These findings demonstrate the potential of tofu-wastewater anaerobic digestate as an alternative substrate for bacterial cellulose production and provide preliminary information regarding the effects of carbon and nutrient supplementation on pellicle formation.

AUTHOR CONTRIBUTIONS

Conceptualization, LAW and AN; methodology, LAW and AN; software, LAW; validation, LAW and AN; formal analysis, LAW and AN; investigation, LAW; resources, LAW and AN; data curation, LAW; writing—original draft preparation, LAW; writing—review and editing, LAW and AN; visualization, LAW; supervision, LAW and AN; project administration.

ACKNOWLEDGMENT

The authors sincerely thank the reviewers for their valuable comments and constructive suggestions, which have significantly improved the quality and clarity of this manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest concerning the publication of this article. The authors also confirm that the data and the article are free of plagiarism.

REFERENCES

Adisasmito, S., Rasrendra, C.B., Chandra, H., Gunartono, M.A., (2018). Anaerobic reactor for Indonesian tofu wastewater treatment. Int. J. Eng. Technol. 7 (3), 30–32. https://app.amanote.com/v4.5.18/research/note-taking?resourceId=FJK_13MBKQvf0Bhic4jy

Agyeman-Duah, E., Okonkwo, C. C., & Ujor, V. C. (2023). *Microbial Removal of Nutrients from Anaerobic Digestate: Assessing Product-Coupled and Non-Product-Coupled Approaches*. Frontiers in Microbiology, 14, 1299402. https://doi.org/10.3389/fmicb.2023.1299402

Almeida, D. M., Prestes, R. A., da Fonseca, A. F., Woiciechowski, A. L., & Wosiacki, G. (2013). Minerals consumption by *Acetobacter xylinum* on cultivation medium on coconut water. Brazilian Journal of Microbiology, 44(1), 197e206. https://doi.org/10.1590/S1517-83822013005000012

Asp, H., & Bergstrand, K. J. (2024). Nutrient utilization and growth of tomato crops fertilized with solid anaerobic digestate. *Scientia Horticulturae*, *329*, 112986. https://doi.org/10.1016/j.scienta.2024.112986

Boarino, A., Demichelis, F., Ertani, A., Bulgari, R., Iannicelli, M., Nicola, S., ... & Padoan, E. (2026). Technical, environmental, and economic assessment of anaerobic digestion of pre-treated municipal organic waste for energy production and reuse of liquid digestate. *Bioresource Technology*, 134957. https://doi.org/10.1016/j.biortech.2026.134957

Borja, R., de la Lama-Calvente, D., Mancilla-Leyton, J. M., & Fernández-Rodríguez, M. J. (2025). Valorization of digestate as a biofertilizer and its energy recovery using thermochemical conversion. *Biogas in the Circular Economy*, 193-219. https://doi.org/10.1016/B978-0-443-29230-9.00008-5

Catarino, R. P. F., Mascareli, V. A. B., Leite da Costa, V. L., Pavanello, A. C. L., & Spinosa, W. A. (2025). Sustainability and influencing factors in bacterial cellulose production: A review of the impact of microorganisms, culture media and cultivation methods. *Food Technology and Biotechnology*, *63*(3), 332-350. https://doi.org/10.17113/ftb.63.03.25.8746

Czekała, W., Nowak, M., & Piechota, G. (2023). *Sustainable Management and Recycling of Anaerobic Digestate Solid Fraction by Composting: A Review*. Bioresource Technology, 375, 128813. https://doi.org/10.1016/j.biortech.2023.128813

Diawara, M., Bah, H., Sangare, M., & Bamba, B. (2025). Evaluation de la valeur fertilisante et amendante des pellets de digestat séché de volaille. *Int. J. Biol. Chem. Sci*, *19*(1), 69-86. https://dx.doi.org/10.4314/ijbcs.v19i1.6

Esperanço, P., Ferreira, A., & Ferreira, J. (2026). A Comparative Systematic Review of Life-Cycle Assessments of Treatment Strategies for Swine Slurry with a Focus on Anaerobic Co-Digestion. *Processes*, *14*(3), 573. https://doi.org/10.3390/pr14030573

Gabler, F., Cheng, G., Pizzul, L., Schnürer, A., & Nordberg, Å. (2025). Comparative evaluation of digestate and reject water as nutrient media for syngas biomethanation in thermophilic trickle-bed reactors. *Bioresource Technology*, *435*, 132893. https://doi.org/10.1016/j.biortech.2025.132893

Ginting, E., Elisabeth, D. A. A., Khamidah, A., Rinaldi, J., Ambarsari, I., & Antarlina, S. S. (2024). The nutritional and economic potential of tofu dreg (okara) and its utilization for high protein food products in Indonesia. *Journal of Agriculture and Food Research*, *16*, 101175. https://doi.org/10.1016/j.jafr.2024.101175

Hou, X., Huang, G., Zhang, Y., Wu, B., Zhang, Y., & Li, Y. (2026). Bio-drying coupled with RDF, fertilizer, and thermal energy production: Orthogonal optimization and economic analysis of multi-path resource utilization for food waste digestate. *Bioresource Technology Reports*, 102692. https://doi.org/10.1016/j.biteb.2026.102692

Krieg, N.R., Holt. J. G. (1984). *Bergey Manual of Systematic Bacteriology*. Vol. 1. The Williams & Wilkins Co., Baltimore: xxvii: 964 hlm

Kulichkova, H., Ivanova, T., Samarin, V., Vovk, E., & Tsygankov, S. (2026). МОДЕЛЮВАННЯ ПОЧАТКОВОЇ СТАДІЇ МЕТАНОВОЇ ФЕРМЕНТАЦІЇ ЗЕРНОВОЇ БАРДИ В ЛАБОРАТОРНОМУ РЕАКТОРІ. *Vidnovluvana energetika*, (1 (84)), 330-344. https://doi.org/10.36296/1819-8058.2026.1(84).330-344

Kushwaha, J., & Dhoble, A. S. (2026). Evaluating digestate and raw manure in floral waste composting: physicochemical properties and microbiome dynamics. *Environment, Development and Sustainability*, *28*(3), 7491-7509. https://doi.org/10.1007/s10668-024-05291-0

Lehto, J., & Järvelä, E. (2025). Valorisation of anaerobic digestate to nutrients and humic substances. *Waste Management*, *192*, 39-46. https://doi.org/10.1016/j.wasman.2024.11.033

Lin, Y. I., Aranha, D. J., Negi, S., Pan, S. Y., & Fan, L. S. (2026). Life-cycle greenhouse gas mitigation of biomass technologies for various bioproducts. *Industrial Crops and Products*, *248*, 123556. https://doi.org/10.1016/j.indcrop.2026.123556

Luo, J., Wang, J., Huang, W., Wang, F., Zhao, Y., Fang, S., & Cao, J. (2026). Microbial-driven mechanisms of methylisothiazolinone-interfered anaerobic digestate liquid in enhancing soil fertility and nutrient cycling. *Bioresource Technology*, 134698. https://doi.org/10.1016/j.biortech.2026.134698

Margono, M., Isnaeni, N., Amelia, V., & Shohih, E. N. (2023). Process of bacterial cellulose production from tofu wastewater without pretreatment using Acetobacter xylinum. Jurnal Bahan Alam Terbarukan, 12(2), 129-136.

Nostia, R., Kurniawan, A., 2023. Analysis of solid and liquid waste characteristics of tofu industry in Bancar Village, Bungkal District, Ponorogo Regency. Indones. J. Environ. Sustain. Develop. 14 (1), 1–5. https://doi.org/10.21776/ub.jpal.2023.014.01.01

Nugroho, Darmawan Ari., Aji, Pradipta. (2015). Characterization of Nata de Coco Produced by Fermentation of Immobilized *Acetobacter xylinum.* Faculty of Agricultural Technology, Gadjah Mada University. The 2014 International Conference on Agro-industry (ICoA): Competitive and sustainable Agro-industry for Human Welfare. Science Direct. Agriculture and Agricultural Science Procedia 3 (2015) 278 – 282. https://doi.org/10.1016/j.aaspro.2015.01.053

Permana, D., (2019). Performance of single chamber microbial fuel cell (SCMFC) for biological treatment of tofu wastewater. In: IOP Conference Series: Earth and Environmental Science. IOP Publishing, 012008. https://doi.org/10.1088/1755-1315/277/1/012008

Prasetyadi., Wardani, L. A, & Kusnoputranto, H. (2018). Evaluasi Kinerja Operasi Sistem Anaerobik Tipe Fixed Bed untuk Pengolahan Limbah Cair Industri Tahu menjadi Biogas di Kota Probolinggo Operation of Fixed Bed Anarobic System for Tofu Liquid Waste Treatment in Probolinggo City. Jurnal Teknologi Lingkungan Vol, 19(1), 61-70. https://garuda.kemdiktisaintek.go.id/documents/detail/1568533

Racho, P., Nammana, B., Tantemsapya, N., Wichitsathian, B., Riewklang, K., & Tantrakarnapa, K. (2026). Valorization of recycled paper mill sludge via mass–energy integration for sustainable onsite power generation: A case study. *Waste Management*, *212*, 115354. https://doi.org/10.1016/j.wasman.2026.115354

Radkevich, M., Choriev, M., Umarova, N., Gapirov, A., & Madrakhimova, Z. (2026). Assessment of the potential for using industrial waste in agriculture in the Tashkent region of Uzbekistan. In *International Conference on Remote Sensing of the Earth and Physics (RSE 2026)* (Vol. 14167, pp. 224-239). SPIE. https://doi.org/10.1117/12.3114493

Ramalho, T. P., Bunchek, J. M., Schubert, D., Kerzenmacher, S., Verseux, C., & Pillot, G. (2026). Sustainable Mars agriculture: Fertilizer production from cyanobacterial biomass via anaerobic digestion. *Chemical Engineering Journal*, 174922. https://doi.org/10.1016/j.cej.2026.174922

Regulation of the Minister of Environment Number 5 of 2014 concerning Wastewater Quality Standards (BMAL). https://peraturan.bpk.go.id/Details/322442/permen-lh-no-5-tahun-2014

Salim, Emil. (2015). Modul Pelatihan membuat nata de coco. Agrotekno Consultant Pusat Pelatihan Agroindustri. Yogyakarta.

Santoso, I. (1996). Pengaruh pH awal terhadap pertumbuhan 3 strain bakteri *Acetobacter xylinum* UICC pada limbah tahu. Laporan Penelitian LP-UI.

Santoso, I. (1999). Pemanfaatan limbah cair tahu untuk produksi nata de soya menggunakan *acetobacter xylinum* P1007. Fakultas Matematika dan Ilmu Pengetahuan Alam. Departemen Biologi. Universitas Indonesia

Satar, I., Permadi, A., (2022). Treating the tofu wastewater (TWW) using a green technology of microbial fuel cell (MFC) system. Indones. J. Environ. Manage. Sustain. 6 (1), 1–6. https://doi.org/10.26554/ijems.2022.6.1.162-167

Sinskey, A., S. Jamas., D. Easson., C. Rha. (1986). *Biopolymers and modified polysaccharides.* Biotechnology in food processing. Noyes Publ., New Yersey: 73-114

Srikandace, Y., Apriyana, A. Y., Zahrad, S. A., Ramdhani, W., Asri, P. P. P., Andriani, D., ... & Karina, M. (2022). Bacterial cellulose production by Komagataeibacter xylinus using rice-washed water and tofu processing wastewater with the addition of sodium glutamate. *Fibers and Polymers*, *23*(5), 1190-1196. https://doi.org/10.1007/s12221-022-4729-4

Verschuren, P. G., Cardona, T. D., Nout, M. J., De Gooijer, K. D., & Van den Heuvel, J. C. (2000). Location and limitation of cellulose production by *Acetobacter xylinum* established from oxygen profiles. Journal of Bioscience and Bioengineering, 89(5), 414e419. https://doi.org/10.1016/S1389-1723(00)89089-1

Villada, E., Velasquez, M., Gómez, A. M., Correa, J. D., Saldarriaga, J. F., López, J. E., & Tamayo, A. (2024). Combining anaerobic digestion slurry and different biochars to develop a biochar-based slow-release NPK fertilizer. *Science of The Total Environment*, *927*, 171982. https://doi.org/10.1016/j.scitotenv.2024.171982

Wang, W., Chang, J.-S., & Lee, D.-J. (2023). *Anaerobic Digestate Valorization Beyond Agricultural Application: Current Status and Prospects*. Bioresource Technology, 373, 128742. https://doi.org/10.1016/j.biortech.2023.128742

Xu, Q., Zhang, H., Dong, Q., Li, D., Liang, J., Pan, M., ... & Wong, J. W. (2026). Hydrochar improves the dewaterability of digestate from anaerobic co-digestion of food waste and waste activated sludge: A neglected benefit. *Journal of Environmental Management*, *401*, 128822. https://doi.org/10.1016/j.jenvman.2026.128822

Zhang, Jiachao., Yang, Yichong., Deng, Jian., Wang, Yanmei., Hu, Qisong., Li, Congfa., Liu, Sixin. (2017). Dynamic profile of the microbiota during coconut water pre- fermentation for nata de coco production. Hainan University, Haikou China. Elsevier. LWT - Food Science and Technology 81 (2017) 87e93. https://doi.org/10.1016/j.lwt.2017.03.036