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RESEARCH PAPER

Optimization of solid fermentation conditions of organic fertilizer from fish and tobacco wastes and its effects on crop production and soil quality

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Abstract

Microbial fermentation fertilizer from agricultural organic wastes represents a novel, environmentally friendly, and safe type of bio-organic fertilizer. However, the development and application of solid fermentation using fish by-products and plant waste straw is still rare. In this study, the fermentation substrate and conditions were optimized by single factor test and orthogonal test using fish waste and tobacco waste as raw materials, and its effects on plant growth and soil quality were investigated. The results showed that the addition of 0.5% sodium chloride and 1% ammonium nitrate to the fish waste fermentation substrate, along with a ratio of *Bacillus subtilis*: *Rhodotorula rubrosaensis*: *Rhizobium oryzae* at 2:1:3, a 30% addition of tobacco waste, an inoculation amount of 4×10^{10} CFU/g, and a fermentation temperature of 34 °C, resulted in the best fermentation conditions. Pot experiment demonstrated that application of fish waste fermentation fertilizer significantly increased average fresh weight, dry weight, and chlorophyll content of *Brassica rapa* by 131.6, 60.0, and 18.5% respectively, and *Capsicum annuum* by 74.3, 46.7, and 195.4% respectively, compared with no fertilizer treatment. The activity of urease, invertase and catalase in soils with fish waste fermentation fertilizer application were significantly higher than no fertilizer application. Moreover, the relative abundances of nitrogen-fixing and denitrifying bacteria were improved after application of fish waste fermentation fertilizer. Fish waste fermentation fertilizer significantly affected the functional genes related to carbohydrate metabolism and increased the utilization rate of organic fertilizer. This study provides a theoretical basis for the future fermentation processes of fish waste and its application in crop production.

Keywords

Agricultural products, fermentation, fish waste, soil fertility, soil microorganisms, tobacco waste

Introduction

As global attention increasingly shifts towards green agriculture, the conversion of a agricultural organic waste into effective organic fertilizers presents a promising approach for the sustainable development of modern agriculture. Sustainable agriculture ensures global food security and helps meet rising demands while prioritizing environmental conservation (Bagga et al. 2024). Solid fermentation of low-value organic waste by microorganisms can produce bio-organic fertilizers that effectively improve soil quality and promote plant growth (Zhang et al. 2023; Liu et al. 2024).

During the processing of aquatic products, unused fish by-products account for 40–55%. These by-products, which include head, bones, tails, skin, scales, fins, and viscera, vary in proportion (Thirukumaran et al. 2022; Hassoun et al. 2023). Rich in nutrients, fish by-products can serve as a significant source of green ecological organic fertilizers (Jung and Kim 2016; Ahuja et al. 2020). For instance, Muscolo et al. (2022) showed that the solid residue milled anchovy left-

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overs after fish oil extraction with bio-based limonene is an excellent organic fertilizer that promotes the growth of Tropea's red onion. Moreover, tobacco waste contains high levels of nutrients like potassium and phosphorus, which are easily absorbed by plants, making it a cost-effective and environmentally friendly resource for preparing seedling substrates (Shen et al. 2024). However, the direct application of tobacco waste to the field can negatively affect plant growth and causes environmental pollution due to the presence of nicotine. Microbial fermentation technology can effectively reduce harmful components in tobacco waste, improve soil structure, and provide nutrient supplementation (Mandi et al. 2022). Organic fertilizer based on tobacco waste liquid exhibit water retention properties, insect pest prevention, and promote healthy plant growth (Wang et al. 2022).

Microbial fermentation is a safe and sustainable process widely employed in the production of chemicals, materials, biofuels, food, and drugs (Chen and Liu 2021). This biocomposite process utilizes the biological metabolism of microbial flora to convert proteins, esters, and sugars into cellular energy and other metabolic by-products (Daccache et al. 2020; Zhang et al. 2023). The selection of microbes and substrates is the most important in the fermentation process. Probiotic strains such as *Bacillus*, *Rhodotorula* and *Rhizopus* have demonstrated success in fermentation processes (Bagga et al. 2024). Yu et al. (2020) successfully prepared a seafood-flavored seasoning with antiseptic properties from fermented tuna waste. Currently, research on using fish by-products mainly focuses on producing liquid fish protein fertilizers for agriculture (Kim et al. 2010; Aranganathan and Rajasree 2016; Khiari et al. 2019). However, studies exploring the degradation and transformation of fish by-products into high value fermentation products, particularly in combination with plant waste straw, remain limited (Zhang 2015).

This study evaluates the fermentation efficacy of a mixed bacterial agent based on soluble peptide content, using fish waste and tobacco waste as primary raw materials. Pot experiments were conducted to assess the application of fermentation fertilizer derived from fish waste. The objective is to utilize microbial solid-state fermentation to produce organic fertilizer that enhances plant growth and offers a novel solution for managing aquatic products and tobacco waste, thereby addressing the environmental disposal challenges.

Materials and methods

Experimental materials

The experimental soils were collected from the campus field of Anhui Normal University, Wuhu, China. The brown loam soil was air dried, and sieved by a 2 mm sieve to remove rocks. The characteristics of the soil were 2.26% of soil organic matter, 5 mg/kg ammonium nitrogen, 0.751 mg/kg available phosphorus, and 12 mg/kg available potassium.

Fish waste, comprising mixed fish heads, scales, gills and tails, was obtained from the West Huangshan Road wet market in Wuhu City, China. Tobacco waste, including tobacco leaves and stems, was collected from a cigarette factory in Wuhu City. The fish and tobacco wastes were sterilized at 121 °C for 2 hours, then crushed and dried. The physical and chemical properties of fish waste included pH 6.16, 73.31% organic matter, 42.8% total nitrogen, 48.5% fat, 0.019% total sugar, and 0.87% NaCl. The physical and chemical properties of tobacco waste includes pH 4.90, 56.81% organic matter, 2.01% total nitrogen, 0% fat, 0.14% total sugar, and 1.97% NaCl.

Strain activation

The strains *Bacillus subtilis*, *Rhodotorula taiwanensis* and *Rhizopus oryzae* were isolated and preserved in the microbiology laboratory, Anhui Normal University. A single colony of each strain was obtained by plate scribing, and the activated culture was prepared by inoculating the single colony into an expanded culture medium.

Optimization of fermentation substrate

Using fish and tobacco wastes as fermentation substrate, various inorganic nitrogen sources ($(NH_4)_2SO_4$, NH_4NO_3, NH_4Cl, and $NaNO_3$) and inorganic salts (K_2HPO_4, KH_2PO_4, $MgSO_4$, and NaCl) with the same nitrogen content were added, and the addition amounts were set as 0, 0.5%, 1%, 1.5% and 2%. The same proportion of mixed strains (the ratio of *B. subtilis*: *Rho. taiwanensis*: *Rhi. oryzae* was 2:1:3) were inoculated, and the inoculum amount was 4×10^{10} CFU/g. Fermentation was conducted at 34 °C for 3 days. The total microbial count was determined using the plate counting method to identify the most suitable inorganic nitrogen source and inorganic salt for optimal strain growth (Zhang 2015).

Single factor experiment

Effect of strain ratio on fermentation efficiency

The ratios of *B. subtilis*: *Rho. taiwanensis*: *Rhi. oryzae* were set to 1:1:1, 1:2:1, 1:3:1, 2:1:1, and 3:1:1, and incubated at 34 °C for 3 days. Subsequent experiments were based on the optimal ratio of *B. subtilis* and yeast, adjusting the ratios to 2:1:1, 2:1:2, 2:1:3, 2:1:4, and 2:1:5, with fermentation at 34 °C for 3 days. The fermentation efficiency was assessed according to the content of soluble peptides in the fermentation broth. Soluble peptides were determined by the Lowry method (Zhuang 2021). The fermentation substrate was added into distilled water, shook for 2 min, and filtering. The soluble peptide content in the supernatant was detected using the Lowry protein concentration determination kit, and a standard curve was established using tyrosine as a substrate. The optimum strain ratio was selected for following experiments.

Effect of tobacco addition on fermentation effect

Fermentation substrates were prepared with 20 g, with tobacco added at concentrations of 5%, 10%, 20%, 30%, and 40%. Fermentation was carried out in 50 ml Erlenmeyer flasks, sterilized and inoculated with the mixed strains (*B. subtilis*: *Rho. taiwanensis*: *Rhi. oryzae* at a ratio of 2:1:3) at a final concentration of 4×10^{10} CFU/g. The fermentation was incubated at 34 °C for 3 days. The soluble peptide content in the fermentation broth was used to evaluate the fermentation effect.

Influence of inoculum amount on fermentation effect

A sterilized 20 g fermentation substrate with 30% tobacco was inoculated with the mixed strains (*B. subtilis*: *Rho. taiwanensis*: *Rhi. oryzae* at a ratio of 2:1:3) at final concentrations of 1, 2, 3, 4, and 5×10^{10} CFU/g, followed by fermentation at 34 °C for 3 days.

Effect of temperature on fermentation effect

The optimal growth temperature for *B. subtilis*, *Rho. taiwanensis*, and *Rhi. oryzae* range from 30–37 °C, 27–36 °C, and 28–37 °C, respectively (Huang et al. 2011; Londoño-Hernández et al. 2017; Vehapi et al. 2023). Therefore, temperature of 26, 28, 30, 32, 34, and 36 °C were selected for the single-factor experiment.

Orthogonal experiment

Based on the single factor experiment, an orthogonal experiment with three factors and three levels was designed to obtain the most suitable fermentation conditions by: A, tobacco addition amount (20%, 30%, and 40%), B, bacterial inoculation amount (3, 4, and 5×10^{10} CFU/g), and C, temperature (26, 30, and 34 °C) as experimental factors. The soluble peptide content in the fermentation broth was used to evaluate the fermentation effect.

Optimal fermentation broth samples were collected every 2 days and stored at 4 °C for physicochemical properties testing. Fermented fertilizer quality project testing method refers to “organic fertilizer” (NY525–2021). Ammonium nitrogen, available phosphorus and available potassium were determined by a TFC soil and fertilizer rapid measuring instrument. Cellulose content was measured using the anthrone method (Li et al. 2008). The sample was treated with 60% cold H_2SO_4, digested in an ice bath for 30 min, and filtered. The filtrate was mixed with 2% anthrone (anthrone dissolved in ethyl acetate) and H_2SO_4, followed by incubation in a boiling water bath for 10 min. Absorbance was subsequently measured at 620 nm.

Fertilization efficiency experiment of fermented organic fertilizer

The fish waste fermented fertilizer was produced from solid fermentation products that were subsequently dried and granulated. The physical and chemical properties of the fish waste fermented by the same method (without adding tobacco) were 57.73% organic matter, 1.53% available phosphorus, 2.94% available potassium, and 11.12% ammonium nitrogen.

The experiment consisted of five fertilizer treatments (CK, experimental soil without fertilizer; F0, add 20 g/kg fish waste fermented fertilizer in soil without planting crops; F1, add 20 g/kg fish waste fermented fertilizer in soil; F2, add 20 g/kg fermented fish waste in soil without tobacco waste; and F3, add 20 g/kg fermented fish waste + unfermented tobacco waste) and two crop treatments (*Brassica rapa* and *Capsicum annuum*). The experiment was a complete randomized blocks design with three replicates. For each treatment, twenty seeds of *B. rapa* or *C. annuum* were sown and grown in a light-controlled incubator. The conditions of the light incubator were light intensity of 5000 lx, light-dark ratio of 12 h:12 h, and constant temperature of 25 °C.

After 15 days of incubation, seedling height, fresh weight, dry weight and chlorophyll content of the vegetables, and the soil physicochemical properties and enzyme activities were determined. Total chlorophyll content was determined according to the method of Hu et al. (2024). Leaf sample was shredded and extracted in 80% acetone solution for 12 hours. The extract was collected and read at 663 and 646 nm with a spectrophotometer.

Standard test methods were adopted to measure soil enzyme activities and chemical properties (Li et al. 2008; Zhang et al. 2024). Soil ammonium nitrogen, available phosphorus and available potassium were determined using the TFC soil and fertilizer rapid measuring instrument. Soil urease activity was determined by phenol-sodium hypochlorite colorimetric method, Soil catalase activity was determined by potassium permanganate titration method, and soil invertase activity was determined by DNS colorimetric method. Heavy metal and amino acid contents were measured by Suzhou Panomic biomedical technology company.

Soil DNA extraction and PCR amplification were performed according to Zhang et al. (2024). Purified DNA samples were integrated for sequencing on the Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA) and NovaSeq 6000 S4 Reagent Kit V1.5 (paired-end, 300 cycles, 2×250 bp) at Beijing Bamac Biotechnology Co., Ltd., China. The sequenced data were analysed using QIIME 2 2019.4 (Bolyen et al. 2019) after some modifications. Briefly, raw sequence data were demultiplexed, filtered, denoised, merged and chimera removed using the DADA2 plugin. Non-singleton amplicon sequence variants (ASVs) were aligned with mafft and used to construct a phylogeny with fasttree2. Taxonomy was assigned to ASVs using the classify-sklearn naïve Bayes taxonomy classifier in feature-classifier plugin against the Silva v132 99% operational taxonomic units reference sequences (Quast et al. 2013). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were enriched by PICRUSt2 method (Langille et al. 2013).

Statistical analysis

Data analysis and plotting were conducted using Excel 2019 (Microsoft, Redmond, WA, USA) and Origin 8.1 (OriginLab Corporation, Northampton, MA, USA). Data statistics and analysis of variance using SPSS22.0 (SPSS Inc., Armonk, NY, USA). The Data were subjected to one-way ANOVA. Duncan’s multiple range test was used to compare the means ($P < 0.05$).

Results and discussion

Preliminary optimization of fermentation substrate

Among the four nitrogen sources tested, the application of NH_4NO_3 in the fermentation substrate had the highest total number of viable bacteria (Fig. 1a). In the four inorganic salt, NaCl had the highest total number of viable bacteria (Fig. 1b). Consequently, NH_4NO_3 and NaCl were selected as the preferred nitrogen source and inorganic salt, respectively. However, Zhang et al. (2015) reported that $(NH_4)_2SO_4$ and $MgSO_4$ as the optimal nitrogen source and inorganic salt for fish waste fermentation. The inconsistent results may be due to the variations in experimental materials. Moreover, the optimal concentrations of NH_4NO_3 and NaCl were explored. The highest total number of viable bacteria (3.42×10^8 CFU/mL) was observed with the addition of 0.5% NaCl (Fig. 1c), while the maximum total number of viable bacteria of 4.2×10^8 CFU/mL occurred with 1% NH_4NO_3. It was concluded that the optimal concentrations for the fermentation substrate are 0.5% NaCl and 1% NH_4NO_3.

Single factor test results of solid-state fermentation

In the solid-state fermentation of mixed strains, the content of soluble peptides initially increased with the increase of tobacco addition (Fig. 2a), but decreased to the lowest level when the tobacco content reached 40%. This decline is likely due to the inhibitory effects of nicotine on strain

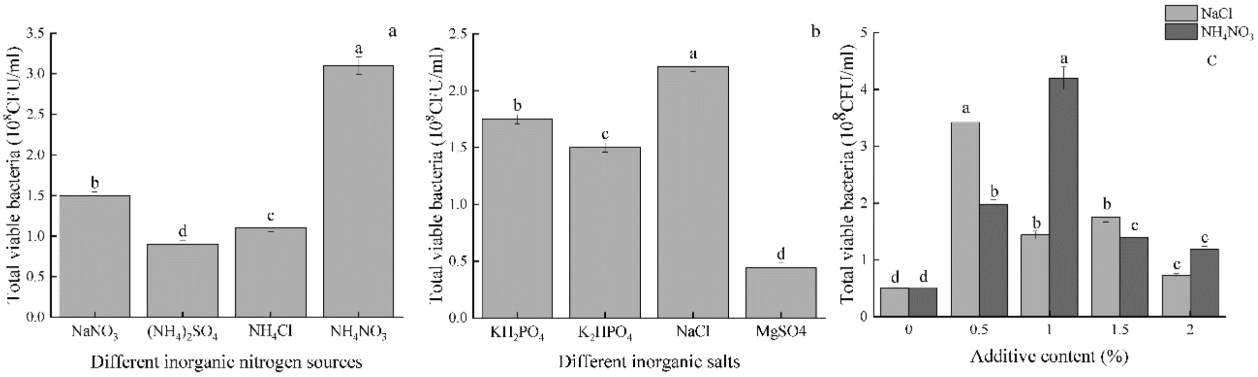

Figure 1. Effect of different inorganic nitrogen sources (**a**), inorganic salts (**b**), and added content of sodium chloride and ammonium nitrate (**c**) on the total number of viable bacteria. Vertical bars represent the means ± standard error. Significant differences among the treatments are indicated by different lower case letters ($p < 0.05$, Duncan's test).

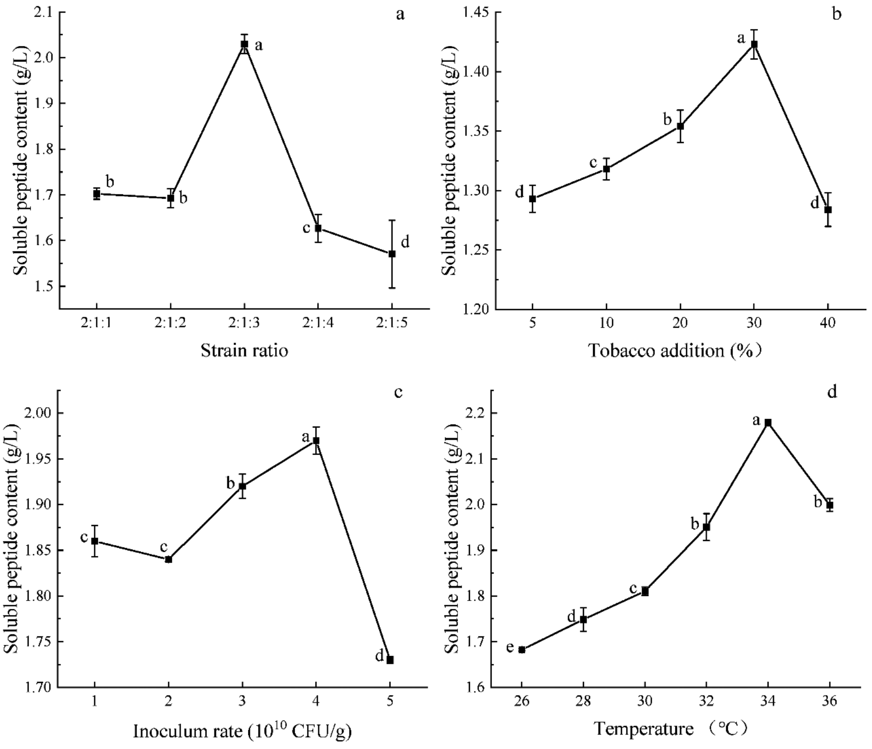

Figure 2. Effect of different fermentation conditions on fermentation substrate. Vertical bars represent the means ± standard error. Significant differences among the treatments are indicated by different lower case letters ($p < 0.05$, Duncan's test).

growth and enzyme activity (Ye et al. 2023). The highest soluble peptide content was recorded at an inoculum level of 4×10^{10} CFU/g (Fig. 2b). Beyond this inoculum level, competition among strains for resources and resultant nutrient depletion led to a decrease in soluble peptide content. The optimum ratio of mixed strains *B. subtilis*: *Rho. taiwanensis*: *Rhi. oryzae* was 2:1:3 (Fig. 2c). Soluble peptide content reached the highest peak at 34 °C (Fig. 2d). Appropriate temperature conditions foster the metabolic activities of microorganisms, while deviations from this range can adversely affect microbial growth and enzyme metabolism (Zhao et al. 2022; Liu et al. 2024).

Table 1. Results of orthogonal experimental design for fermentation process optimization.

Experimental groups	A (%)	B (10^{10} CFU/g)	C (°C)	Soluble peptide content (g/L)
1	20	3	26	1.777 ± 0.001
2	20	4	34	2.042 ± 0.002
3	20	5	30	2.028 ± 0.003
4	30	3	30	1.935 ± 0.002
5	30	4	26	1.962 ± 0.006
6	30	5	34	1.949 ± 0.002
7	40	3	34	2.111 ± 0.004
8	40	4	30	1.901 ± 0.004
9	40	5	26	1.975 ± 0.002
K1	5.95	5.90	5.86	
K2	5.97	6.10	5.79	
K3	5.75	5.67	6.03	
Range	0.07	0.14	0.08	

A, amount of tobacco addition; B, bacterial inoculum amount; C, fermentation temperature; K1, the level of each factor in the experiment; K2, the square of the level ; K3, the cube of the number of levels. Values are means ± s.e.

Results of orthogonal tests for fermentation process optimization

From the results of the range method analysis in Table 1, it can be seen that at the optimal mixed strain ratio of 2:1:3, the primary and secondary influencing factors on soluble peptide content were in the order of B > C > A. The optimal combination of factors consisted of 30% tobacco waste addition, 4×10^{10} CFU/g of bacterial inoculum, and a fermentation temperature of 34 °C. It was proved that the combination was the optimal.

Material changes during solid fermentation of fish waste

During the solid fermentation of fish waste, the organic matter content showed a gradual decreasing trend (Fig. 3a), which is consistent with the results of Zhu et al. (2024). The reduction in organic matter indicates effective fermentation and decomposition of organic waste (Du et al. 2019). Both available phosphorus and available potassium contents significantly increased by 137.2 and 193.63%, respectively (Fig. 3b, c), indicates that the fermentation products achieved good maturity and quality (Zhu et al. 2024). Moreover, cellulose in the fermentation products was degraded by 65.15% during the fermentation process (Fig. 3d), which is agreement with the study on the decomposition of lignocellulose caused by microbial activation (Sun et al. 2024).

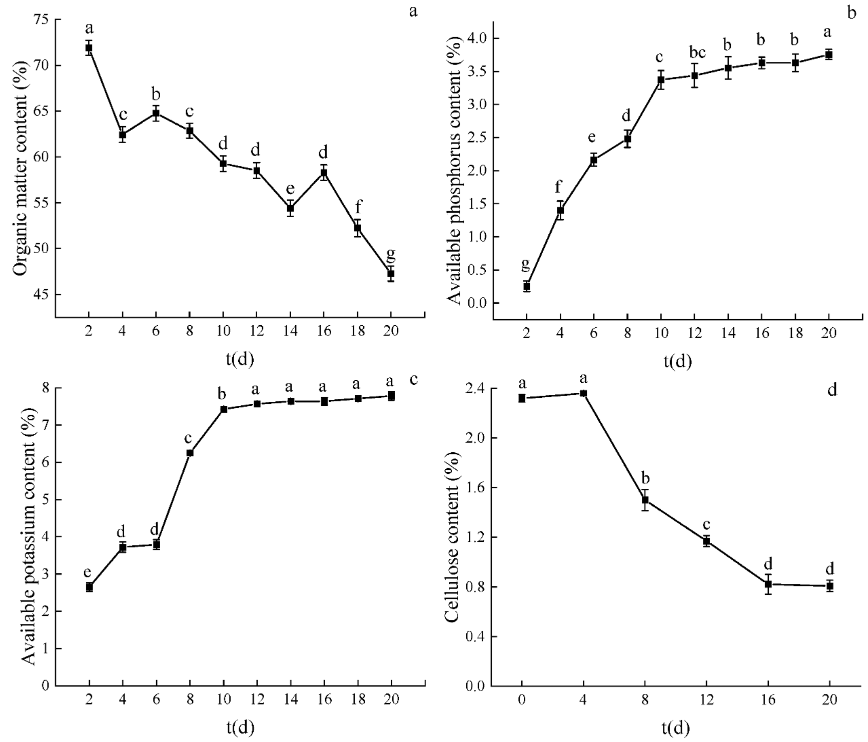

Figure 3. Trend of organic matter (**a**), available phosphorus (**b**), available potassium (**c**) and cellulose (**d**) content during fermentation. Vertical bars represent the means ± standard error. Significant differences among the treatments are indicated by different lower case letters (p < 0.05, Duncan's test).

Table 2. Test results of fish waste fermented organic fertilizer.

Inspection projects	Inspection results	Specification requirements in NY525–2021	Inspection projects	Inspection results	Specification requirements in NY525–2021
Organic matter, %	47.26	≥30	Total mercury, mg/kg	0.023	≤2
Total nutrients (N + P_2O_5 + K_2O), %	26.018	≥ 4.0	Total cadmium, mg/kg	1.53	≤3
Moisture, %	7.26	≤ 30	Total chromium, mg/kg	15.8	≤150
pH (acidity and alkalinity)	5.6	5.5–5.8	Cu^{2+}	0.028%	–
Seed germination index %	82.7	≥70	Mn^{2+}	0.02%	–
Total arsenic, mg/kg	6.68	≤15	Mg^{2+}	0.729%	–
Total lead, mg/kg	2.61	≤50	Fe^{2+}	0.038%	–

Test results of physical and chemical properties of fish waste fermented fertilizer

Quality and safety evaluations are essential for both fermentation process and the resultant biofertilizer products (Bagga et al. 2024). Table 2 presents the physical and chemical properties of fish waste fermented fertilizer. The results of the tests met the specification requirements of the standard of “Organic Fertilizer” (NY/T525–2021). Notably, the levels of organic matter and total nutrients were significantly higher than the standard, while the contents of heavy metals were lower than the standard. Moreover, the fish waste fermented fertilizer contained essential trace elements, including copper, magnesium, manganese, and iron, as well as 19 amino acids required for plant growth (Table 3).

Table 3. Amino acid test results of fish waste fermented organic fertilizer.

Amino acid type	Content (μg/g)	Amino acid type	Content (μg/g)
Glycine	254.66	Aspartic Acid	352.32
Alanine	567.80	Lysine	301.76
Gamma amino butyric acid	93.03	Glutamic acid	364.07
Serine	117.88	Methionine	10.14
Proline	303.82	Histidine	27.68
Valine	378.58	Phenylalanine	239.75
Threonine	176.20	Arginine	131.24
Isoleucine	320.60	Tyrosine	148.96
Leucine	491.19	Tryptophan	21.25
Ornithine	149.75		

Fertility test of fish waste fermented fertilizer

Effect of different fertilization treatments on vegetable growth

Compared to the no fertilizer application, the fish waste fermented fertilizer (F1) demonstrated the most significant overall improvement. The seedling height, fresh weight, dry weight, and chlorophyll contents of *B. rapa* and pepper were increased by 95.7 and 32.5%, 131.6 and 74.3%, 60.0 and 46.7%, and 18.5 and 195.4%, respectively (Table 4), which indicates that vegetable growth was better than the soil substrate after applying fish waste fermented fertilizer. Muscolo et al. (2022) also found that the solid residue comprised of milled anchovy leftovers significant increased the bulb weight, diameter and plant height of Tropea’s red onion. Ahuja et al. (2020) summarized the growth effects of fish-based fertilizer on agricultural and horticultural plants, trees and berries. Conversely, the application of fermented fish waste + unfermented tobacco waste (F3) did not significantly improve seed germination rate, likely due to the high content of nicotine in the unfermented tobacco, which can inhibit seed germination. After mixing with fermented fish, the nicotine content decreased, due to incomplete degradation by *B. subtilis* and red yeast, allowing for seedling growth (Zou et al. 2016; Dai et al. 2023).

Effect of different fertilization treatments on soil N, P and K content

The results of soil nutrient measurements for different treatment groups are shown in Table 5. Compared to the CK, the F1, F2 and F3 treatment groups significantly increased the content of ammonium nitrogen, available

Table 4. Effect of different fertilization treatments on stem length, fresh weight and dry weight of vegetables.

Seeds	Processing group	Germination rate	Seedling height/cm	Fresh weight/g	Dry weight/g	Chlorophyll content (mg/g)
Brassica rapa	CK	35%	2.30 ± 0.91	0.0980 ± 0.014	0.0100 ± 0.001	14.479 ± 0.11
	F1	80%	4.5 ± 0.65*	0.2270 ± 0.044*	0.0160 ± 0.002*	17.124 ± 0.30*
	F2	70%	5.31 ± 0.43*	0.2145 ± 0.022*	0.0160 ± 0.0002*	14.160 ± 0.39
	F3	55%	4.30 ± 0.87*	0.2742 ± 0.031*	0.0164 ± 0.001*	13.607 ± 0.19
Pepper	CK	30%	4.00 ± 0.17	0.099 ± 0.018	0.0090 ± 0.002	5.510 ± 0.15
	F1	35%	5.30 ± 0.30*	0.1726 ± 0.030*	0.0132 ± 0.003	16.277 ± 0.14*
	F2	35%	4.95 ± 0.23*	0.1472 ± 0.015	0.0138 ± 0.002	11.774 ± 0.10*
	F3	30%	5.17 ± 0.23*	0.2383 ± 0.018*	0.0182 ± 0.002	8.876 ± 0.21*

Note: CK, experimental soil without fertilizer; F1, add fish waste fermented fertilizer in soil; F2, add fermented fish waste in soil without tobacco waste; and F3, add fermented fish waste + unfermented tobacco waste. Values are means ± s.e. * indicates differences between treatments at the 5% significant level.

Table 5. Soil nitrogen, phosphorus and potassium contents after different fertilization treatments.

Testing items	Fish waste fermented fertilizer	CK	F0	F1	F2	F3
Ammonium nitrogen (mg/kg)	16.037	5	298	355	350	310
Available phosphorus (mg/kg)	3.76	54	63	172	125	159
Available potassium (mg/kg)	7.78	8	117	46	101	58

Note: CK, experimental soil without fertilizer; F0, add fish waste fermented fertilizer in soil without planting crops; F1, add fish waste fermented fertilizer in soil; F2, add fermented fish waste in soil without tobacco waste; and F3, add fermented fish waste + unfermented tobacco waste.

phosphorus and available potassium in the soil. The content of ammonium nitrogen, available phosphorus and available potassium in F0, F1, F2 and F3 were higher than those of fish waste fermented fertilizer. Li (2019) found that the application of tobacco stem bio-organic fertilizer increased soil total nitrogen, available phosphorus, and available potassium contents compared with chemical fertilizer. These results suggest that fermented organic fertilizer can alter the activity of soil microorganisms and soil properties, provides essential nutrients that facilitate plant growth and development (Bagga et al. 2024).

Effect of different fertilization treatments on soil enzyme activities

Soil enzyme activity is an important indicator to evaluate the physicochemical properties and fertility status of the soil, and can indirectly reflect the conversion of certain nutrients in the soil (Ning et al. 2017; Zhu et al. 2024). In our study, the F1 treatment significantly increased the activities of soil urease, invertase and catalase by 14.90, 253.51, and 12.90% respectively, compared with CK (Fig. 4), which is consistent with the results of Li (2019). In contrast, F0, F2 and F3 treatments had a promoting effect on soil invertase activity and a significant inhibiting effect on urease and catalase activities. Ning et al. (2017) showed that soil enzyme activities vary under different fertilization regimes. This variation likely results from the different nutrient compositions in the fermented fertilizer, which can influence the structure of the soil microbial community and its metabolic level (Ma et al. 2014). The F0 group exhibited lower urease activity than the CK group due to a lack of enzymes secreted by plant roots (Bagga et al. 2024).

Effect of different fertilization treatments on soil microbial community

Soil microbial community composition is a driving force for organic cycling and transformation of soil nutrients, which influencing soil structure, fertility, and plant health (Banerjee and van der Heijden 2023; Hartmann and Six 2023). In the present study, eight of the top 10 genera in terms of abundance for the five treatments were identical: unclassified Enterobacteriaceae, *Acinetobacter*, *Pseudomonas*, *Sphingomonas*, *Massilia*, *Lysobacter*, unclassified Vicinamibacterales, and unclassified Vicinamibacteraceae (Fig. 5). Notably, *Stenotrophomonas* and *Brevundimonas* were not present in the CK group. After the applicatioon of fish waste with fermented fertilizer, *Acinetobacter*, *Pseudomonas*, *Stenotrophomonas* and *Massilia* emerged as the dominant genera, with their relative abundance increasing to 2.34–22.68%, 3.73–10.20%, 2.22–6.08% and 3.41–9.22%, respectively. Furthermore, fish waste inhibited the growth of *Lysobacter*, unclassified Vicinamibacterales, and *Sphingomonas* in the soil environment. *Massilia* and *Pseudomonas* are known for their denitrification functions (Briones-Roblero et al. 2017; Li et al. 2020), while *Enterobacter* and *Acinetobacter* are associated with nitrogen fixation (Mayta-Apaza et al. 2021; Yu et al. 2021), indicates that the application of fish waste fermented fertilizer changed the microbial functional groups in the soil and improved the utilization of fish waste fermented fertilizer.

KEGG variability analysis

KEGG pathways of bacterial community functions were predicted, and the differences among the CK, F1 and F3 treatments were compared (Fig. 6). The results showed that

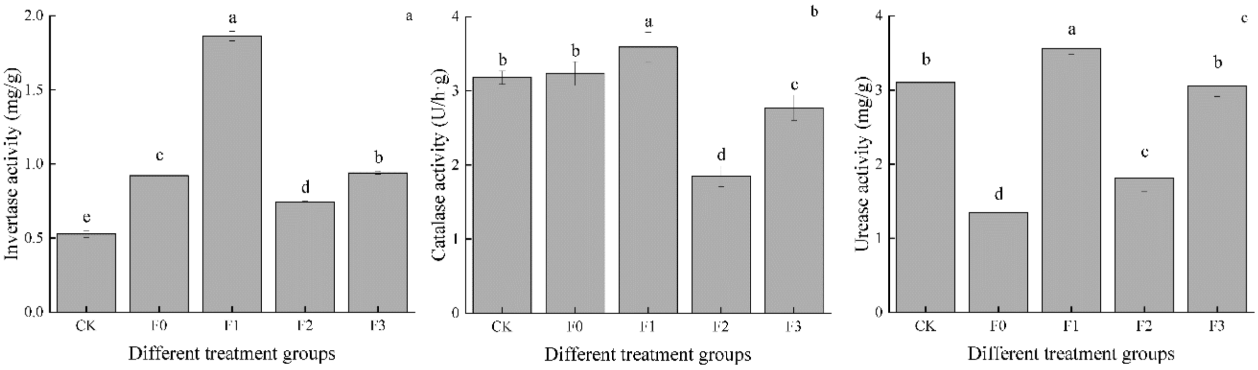

Figure 4. Effect of different fertilization treatments on soil enzyme activities. CK, experimental soil without fertilizer; F0, add fish waste fermented fertilizer in soil without planting crops; F1, add fish waste fermented fertilizer in soil; F2, add fermented fish waste in soil without tobacco waste; and F3, add fermented fish waste + unfermented tobacco waste. Vertical bars represent the means ± standard error. Significant differences among the treatments are indicated by different lower case letters ($p < 0.05$, Duncan's test).

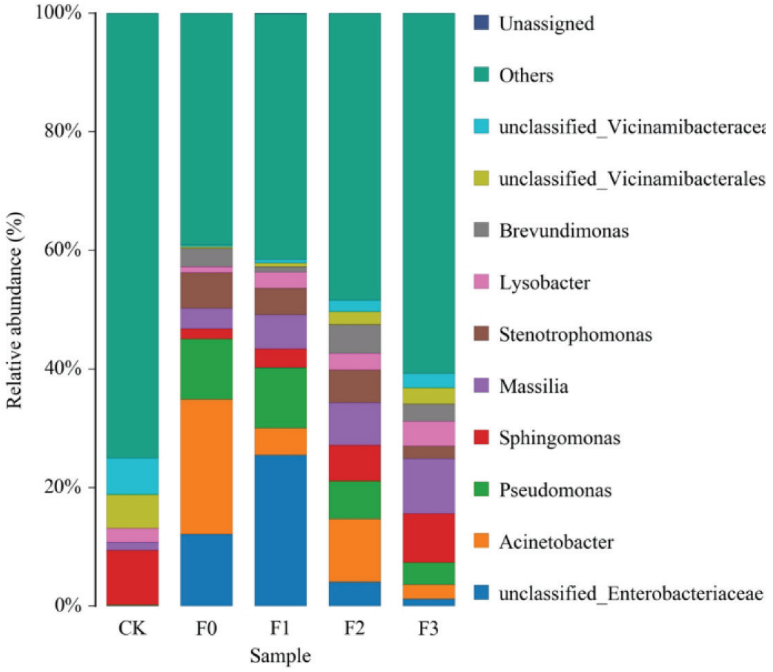

Figure 5. Relative abundance of bacteria of different treatments at the genus level. CK, experimental soil without fertilizer; F0, add fish waste fermented fertilizer in soil without planting crops; F1, add fish waste fermented fertilizer in soil; F2, add fermented fish waste in soil without tobacco waste; and F3, add fermented fish waste + unfermented tobacco waste.

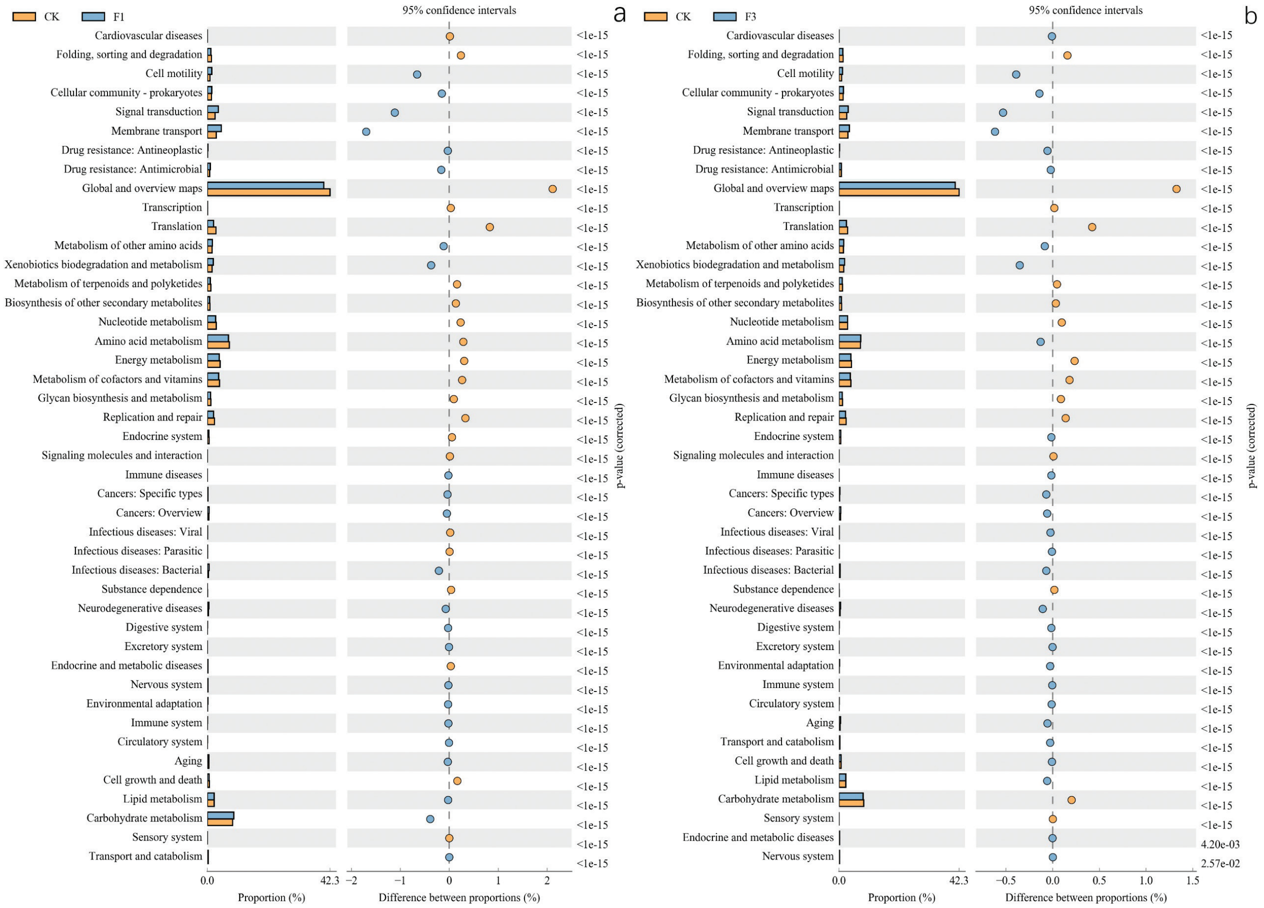

Figure 6. Predicted Kyoto Encyclopedia of Genes and Genomes functions of bacterial communities. CK, experimental soil without fertilizer; F1, add fish waste fermented fertilizer in soil; and F3, add fermented fish waste + unfermented tobacco waste.

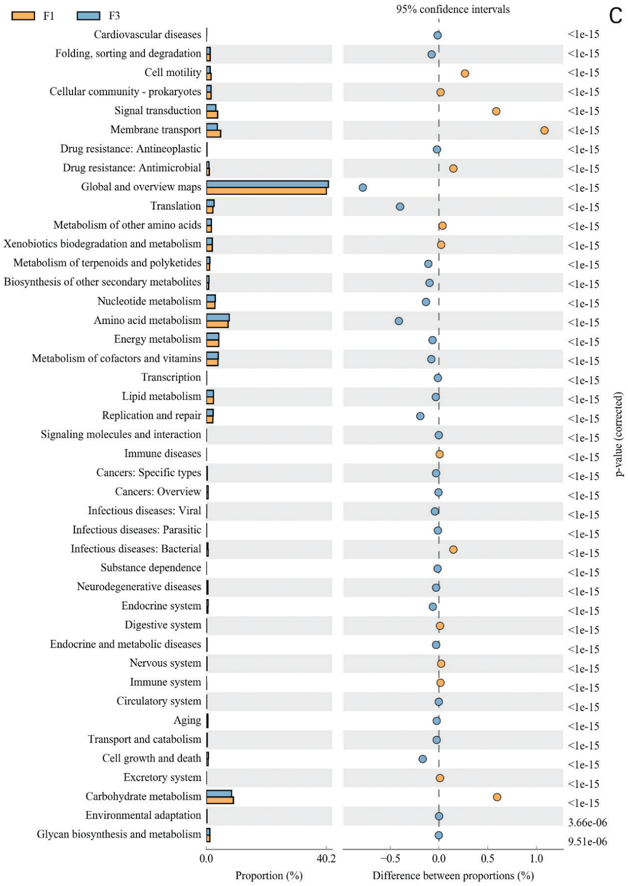

Figure 6. Continued.

the order of carbohydrate metabolism levels was F1 > CK > F3, while amino acid metabolism levels were F3 > CK > F1 groups, which indicates that fermented fish waste fertilizer had a significant effect on microbial carbohydrate and amino acid metabolisms. The changes in carbohydrate and amino acid metabolisms reflect the microbial community's ability to utilize different carbon sources and the energy provided by hydrolysing amino acids, ultimately influencing community structure and abundance (Guo et al. 2014). Moreover, the contribution of metabolism to fish waste fermentation fertilizer treatment was greater than that of no application of fertilizer treatment, which indicates that fermentation fertilizer might closely related to the changes of potential ecosystem functions (Zhou et al. 2022).

Conclusions

In summary, microbial fermentation technology was applied to fish and tobacco wastes, and the fermentation conditions were optimized by single-factor and orthogonal tests. The optimal fermentation conditions were: 0.5% sodium chloride and 1% ammonium nitrate in the fish waste and 30% tobacco waste fermentation substrate, a ratio of *B. subtilis*: *Rho. rubrosaensis*: *Rhi. oryzae* at 2:1:3, an inoculation amount of 4×10^{10} CFU/g, and a fermentation temperature of 34 °C. The physicochemical indexes of the fish waste fermented fertilizer prepared by solid state fermentation method conformed to the requirements of "Organic Fertilizer" (NY/T525–2021). Pot culture revealed that fish waste fermented fertilizer was decomposed after being applied to the soil, which had a positive effect on plant growth by improving soil enzyme activities and soil nutrients, altering microbial functional groups in the soil, and enhancing microbial carbohydrate metabolic activities. Further research is necessary to elucidate the mechanisms by which this fertilizer promotes plant growth and crop yields, with an aim to apply these findings in agricultural practices.

Author contributions

Conceptualization: XZ, AL. Data curation: HF. Funding acquisition: AL. Investigation: JY, KZ. Methodology: HF, CL. Writing – original draft: JY. Writing – review and editing: XZ.

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References

Ahuja I, Dauksas E, Remme JF, Richardsen R, Les A (2020) Fish and fish waste-based fertilizers in organic farming – With status in Norway: A review. Waste Management 115: 95–112. https://doi.org/10.1016/j.wasman.2020.07.025

Aranganathan L, Rajasree SRR (2016) Bioconversion of marine trash fish (MTF) to organic liquid fertilizer for effective solid waste management and its efficacy on tomato growth. Management of Environmental Quality 27: 93–103. https://doi.org/10.1108/MEQ-05-2015-0074

Bagga D, Chauhan S, Bhavanam A, Nikhil GN, Meena SS, Mohanty A (2024) Recent advancements in fermentation strategies for mass production and formulation of biofertilizers: towards waste valorization. Journal of Soil Science and Plant Nutrition 24: 5868–5897. https://doi.org/10.1007/s42729-024-01947-y

Banerjee S, van der Heijden MGA (2023) Soil microbiomes and one health. Nature Review Microbiology 21: 6–20. https://doi.org/10.1038/s41579-022-00779-w

Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu YX, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology 37: 852–857. https://doi.org/10.7287/peerj.preprints.27295v1

Briones-Roblero CI, Rodríguez-Díaz R, Santiago-Cruz JA, Zuiga G, Rivera-Ordua FN (2017) Degradation capacities of bacteria and yeasts isolated from the gut of *Dendroctonus rhizophagus* (Curculionidae: Scolytinae). Folia Microbiologica 62: 1–9. https://doi.org/10.1007/s12223-016-0469-4

Chen G, Liu X (2021) On the future fermentation. Microbial Biotechnology 14(1): 18–21. https://doi.org/10.1111/1751-7915.13674

Daccache MA, Koubaa M, Maroun RG, Salameh D, Vorobiev E (2020) Impact of the physicochemical composition and microbial diversity in apple juice fermentation process: A review. Molecules 25(16): 3698. https://doi.org/10.3390/molecules25163698

Dai Y, Xu Z, Wang Z, Li X, Dong J, Xia X (2023) Effects of fermentation temperature on bacterial community, physicochemical properties and volatile flavor in fermented soy whey and its coagulated tofu. LWT 173: 114355. https://doi.org/10.1016/j.lwt.2022.114355

Du J, Zhang Y, Qu M, Yin Y, Fan K, Hu B, Zhang H, Wei M, Ma C (2019) Effects of biochar on the microbial activity and community structure during sewage sludge composting. Bioresource Technology 272: 171–179. https://doi.org/10.1016/j.biortech.2018.10.020

Guo X, Liu S, Wang Z, Zhang X, Li M (2014) Metagenomic profiles and antibiotic resistance genes in gut microbiota of mice exposed to arsenic and iron. Chemosphere 112: 1–8. https://doi.org/10.1016/j.chemosphere.2014.03.068

Hartmann M, Six J (2023) Soil structure and microbiome functions in agroecosystems. Nature Review Earth Environment 4: 4–18. https://doi.org/10.1038/s43017-022-00366-w

Hassoun A, Cropotova J, Trollman H, Jagtap S, Garcia-Garcia G, Parra-Lopez C, Nirmal N, Ozogul F, Bhat Z, Ait-Kaddour A, Bono G (2023) Use of industry 4.0 technologies to reduce and valorize seafood waste and by-products: A narrative review on current knowledge. Current Research in Food Science 6: 100505. https://doi.org/10.1016/j.crfs.2023.100505

Hu J, Li S, Zhang Y, Du D, Zhu X (2024) Potential regulatory effects of arbuscular mycorrhizal fungi on lipid metabolism of maize in response to low-temperature stress. Journal of Agricultural and Food Chemistry 72(41): 22574–22587. https://doi.org/10.1021/acs.jafc.4c06908

Huang CH, Lee FL, Tien CJ, Hsieh PW (2011) *Rhodotorula taiwanensis* sp. nov., a novel yeast species from a plant in Taiwan. Antonie van Leeuwenhoek 99: 297–302. https://doi.org/10.1007/s10482-010-9489-2

Jung HY, Kim JK (2016) Eco-friendly waste management of mackerel wastewater and enhancement of its reutilization value. International Biodeterioration & Biodegradation 111: 1–13. https://doi.org/10.1016/j.ibiod.2016.04.002

Khiari Z, Kaluthota S, Savidov N (2019) Aerobic bioconversion of aquaculture solid waste into liquid fertilizer: effects of bioprocess parameters on kinetics of nitrogen mineralization. Aquaculture 500: 492–499. https://doi.org/10.1016/j.aquaculture.2018.10.059

Kim JK, Dao VT, Kong IS, Lee HH (2010) Identification and characterization of microorganisms from earthworm viscera for the conversion of fish wastes into liquid fertilizer. Bioresource Technology 101: 5131–5136. https://doi.org/10.1016/j.biortech.2010.02.001

Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Vega Thurber RL, Knight R, Beiko RG, Huttenhower C (2013) Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nature Biotechnology 31: 814–821. https://doi.org/10.1038/nbt.2676

Li W (2019) Development and soil improvement application of tobacco straw organic fertilizer. Hubei University.

Li Y, Zhao Y, Liu F, Li X, Zhao X (2020) Characterization of heterotrophic nitrification and aerobic denitrification of *Massilia neuiana*. Environmental Engineering 38(10): 103–107.

Li Z, Luo Y, Teng Y (2008) Soil and environmental microbiology research method. Science Press.

Liu S, Zhao L, Li M, Zhu Y, Liang D, Ma Y, Sun L, Zhao G, Tu Q (2024) Probiotic Bacillus as fermentation agents: Status, potential insights, and future perspectives. Food Chemistry X 22: 101465. https://doi.org/10.1016/j.fochx.2024.101465

Londoño-Hernández L, Ramírez-Toro C, Ruiz HA, Ascacio-Valdés JA, Aguilar-Gonzalez MA, Rodríguez-Herrera R, Aguilar CN (2017) *Rhizopus oryzae* – Ancient microbial resource with importance in modern food industry. International Journal of Food Microbiology 257: 110–127. https://doi.org/10.1016/j.ijfoodmicro.2017.06.012

Ma P, Li C, Lei M, Yang Y, Ma J (2014) Characteristics of soil microorganisms and enzyme activities in grassland, abandoned farmland

and cultivated land in riparian-slope of the Three Gorges Reservoir Area, China. Acta Ecologica Sinica 34(4): 1010–1020.

Mandi N, Lalevi B, Raievi V, Radojii V (2022) Impact of composting conditions on the nicotine degradation rate using nicotinophilic bacteria from tobacco waste. International Journal of Environmental Science and Technology 20(7): 7787–7798. https://doi.org/10.1007/s13762-022-04405-3

Mayta-Apaza AC, García-Cano I, Dabrowski K, Jiménez-Flores R (2021) Bacterial diversity analysis and evaluation proteins hydrolysis during the acid whey and fish waste fermentation. Microorganisms 9(1): 100. https://doi.org/10.3390/microorganisms9010100

Muscolo A, Mauriello F, Marra F, Calabro PS, Russo M, Ciriminna R, Pagliaro M (2022) AnchoisFert: A new organic fertilizer from fish processing waste for sustainable agriculture. Global Challenges 6: 2100141. https://doi.org/10.1002/gch2.202100141

Ning C, Gao P, Wang B, Lin W, Jiang N, Cai K (2017) Impacts of chemical fertilizer reduction and organic amendments supplementation on soil nutrient, enzyme activity and heavy metal content. Journal of Integrative Agriculture 16(8): 1819–1831. https://doi.org/10.1016/S2095-3119(16)61476-4

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research 41: 590–596. https://doi.org/10.1093/nar/gks1219

Shen K, Xia L, Gao X, Li C, Sun P, Liu Y, Fan H, Li X, Han L, Lu C, Jiao K, Xia C, Wang Z, Deng B, Pan F, Sun T (2024) Tobacco as bioenergy and medical plant for biofuels and bioproduction. Heliyon 10(13): e33920. https://doi.org/10.1016/j.heliyon.2024.e33920

Sun S, Guo C, Wang J, Ren L, Qu J, Guan Q, Dou N, Zhang J, Chen Q, Wang Q, Wang J (2024) Effect of initial moisture content, resulting from different ratios of vegetable waste to maize straw, on compost was mediated by composting temperatures and microbial communities at low temperatures. Chemosphere 357: 141808. https://doi.org/10.1016/j.chemosphere.2024.141808

Thirukumaran R, Priya VKA, Krishnamoorthy S, Ramakrishnan P, Moses JA, Anandharamakrishnan C (2022) Resource recovery from fish waste: Prospects and the usage of intensified extraction technologies. Chemosphere 299: 134361. https://doi.org/10.1016/j.chemosphere.2022.134361

Vehapi M, İnan B, Kayacan-Cakmakoglu S, Sagdic O, Ozcimen D (2023) Optimization of growth conditions for the production of *Bacillus subtilis* using central composite design and its antagonism against pathogenic fungi. Probiotics and Antimicroial Proteins 15: 682–693. https://doi.org/10.1007/s12602-021-09904-2

Wang D, Li J, Yao X, Wu Q, Zhang J, Ye J, Xu H, Wu Z, Cai D (2022) Tobacco waste liquid-based organic fertilizer particle for controlled-release fulvic acid and immobilization of heavy metals in soil. Nanomaterials 12(12): 2056. https://doi.org/10.3390/nano12122056

Ye C, Liu D, Huang K, Li D, Ma X, Jin Y, Xiong H (2023) Isolation of starch and protein degrading strain *Bacillus subtilis* FYZ1-3 from tobacco waste and genomic analysis of its tolerance to nicotine and inhibition of fungal growth. Frontiers in Microbiology 14: 1260149. https://doi.org/10.3389/fmicb.2023.1260149

Yu H, Liang H, Wang Z, Yang X, Li W (2021) Isolation, Identification and growth promotion of culturable nitrogen-fixing and ammonifying bacteria from rhizosphere of *Mikania mikania*. Acta Microbiologica Sinica 62(5): 1851–1863.

Yu X, Jiang Z, Chen Y (2020) Study on the preparation of antisepsis functional seafood flavor seasoning by response surface method based on two-bacteria fermentation of tuna offal. Chinese Condiments 45(07): 101–107.

Zhang J, Akyol C, Meers E (2023) Nutrient recovery and recycling from fishery waste and by-products. Journal of Environmental Management 348: 119266. https://doi.org/10.1016/j.jenvman.2023.119266

Zhang X (2015) Biotransformation of fish offal by microbial fermentation. Fujian Normal University.

Zhang Y, Wang H, Hu M, Cai R, Miao Y, Zhu X (2024) Heavy metals potentially drive co-selection of antibiotic resistance genes by shifting soil bacterial communities in paddy soils along middle and lower Yangtze River. Pedosphere 34: 606–619. https://doi.org/10.1016/j.pedsph.2023.01.012

Zhao Y, Liu S, Han X, Zhou Z, Mao J (2022) Combined effects of fermentation temperature and Saccharomyces cerevisiae strains on free amino acids, flavor substances, and undesirable secondary metabolites in huangjiu fermentation. Food Microbiology 108: 104091. https://doi.org/10.1016/j.fm.2022.104091

Zhou Z, Zhang Y, Zhang F (2022) Abundant and rare bacteria possess different diversity and function in crop monoculture and rotation systems across regional farmland. Soil Biology & Biochemistry 171: 108742. https://doi.org/10.1016/j.soilbio.2022.108742

Zhu X, Yuan J, Qu H, Hou F, Mao C, Lei J, Cao X, Li L (2024) Effects of different proportions of fruit tree branches on nicotine content and microbial diversity during composting of tobacco waste. Journal of Environmental Management 365: 121568. https://doi.org/10.1016/j.jenvman.2024.121568

Zhuang S, Liu X, Li Y, Zhang L, Hong H, Liu J, Luo Y (2021) Biochemical changes and amino acid deamination & decarboxylation activities of spoilage microbiota in chill-stored grass carp (*Ctenopharyngodon idella*) fillets. Food Chemistry 336: 127683. https://doi.org/10.1016/j.foodchem.2020.127683

Zou F, Zhao J, Lei Y, Zu C, Cao H (2016) Isolation, identification and enzymatic properties of a tobacco stone-degrading strain. Soil 48(5): 939–945.