Effects of treatment process on larvae growth performance and nutrient yield during cyanobacteria bioconversion by black soldier fly

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Supplementary material

TEXT S1

Enzyme assay methods

To extract crude enzymes, the larvae were added to a phosphate buffer solution and homogenized in an ice bath at a ratio of 1:10. After centrifugation at 8,000 g for 10 min at 4 °C, the supernatant was used for subsequent experiments. In the SOD determination steps, the control and experimental groups were set up for the experiment and the test solution was added into each group according to the instructions, after it was mixed thoroughly, the solutions were left to stand for 30 min, then the wavelength of the instrument was set to 450 nm to determine the absorbance of the solution. In the CAT determination steps, the control and experimental groups were set up for the experiment, then the test solution was added into each group according to the instructions, after the operation was completed the wavelength of the instrument was set to 405 nm and the absorbance of the solution was measured immediately. In the GSH determination steps, the control and experimental groups were set up, and then the test solution was added to each group according to the instructions, mixed thoroughly, and the absorbance was measured at 412 nm. In the MDA determination steps, the control and experimental groups were set up, the prepared test solution was added to each group according to the instructions, after centrifuged for 10 min, the supernatant was taken and the solution absorbance was measured at 532 nm.

In the assay of digestive enzymes, the extraction steps for the crude enzyme solution are the same as those described above. In the proteinase determination steps,

the test solution was added to the groups according to the instructions and the solutions were water-bath at 30 °C for 20 min, after which the solution absorbance was measured at 680 nm. In the amylase determination steps, the test solution was added to each group according to the instructions, after mixing, the solution was bathed in water at 95 °C for 5 min, finally the absorbance of the solution was measured at 540 nm. In the lipase determination steps, the test solution was added to each group according to the operating instructions, then the solution was shaken at 37 °C for 5 min, followed by standing for 5 min, finally the supernatant was taken to determine the solution absorbance at 710 nm.

The larvae survival rate (SR) was calculated using the equation (Gold *et al.*, 2020):

$$SR(\%) = \left(\frac{N_{end}}{N_{start}}\right) \times 100\% \tag{1.1}$$

where N_{end} is the number of surviving larvae at the end of the experiment, and N_{start} is the initial number of larvae added.

The material (dry matter) reduction rate (MR) was calculated by (Diener *et al.*, 2009):

$$MR(\%) = \left(1 - \frac{W_{residue}}{W_{initial}}\right) \times 100\% \tag{1.2}$$

where $W_{residue}$ is the total weight of the remaining substrate, and $W_{initial}$ is the total weight of feed added at the beginning of the experiment.

The bioconversion rate (BCR) and feed conversion rate (FCR) were estimated when 50% of the larvae were found to be in the prepupa stage. The BCR and FCR of the BSFL were calculated using the equations (Rehman et al., 2017):

$$BCR(\%) = \left(\frac{W_{prepupa}}{W_{initial}}\right) \times 100\% \qquad (1.3)$$

$$FCR(\%) = \left(\frac{W_{initial}\text{-}W_{residue}}{W_{gain}}\right) \times 100\% \qquad (1.4)$$

where $W_{prepupae}$ is the total weight of prepupa harvested at the end of the experiment, $W_{initial}$ is the total weight of feed added at the beginning of the experiment, $W_{residue}$ is the total weight of the remaining substrate, and W_{gain} is the total weight gain of the BSFL over the course of the experiment.

In this study, all experiments were repeated in triplicate. Data were statistically analyzed using SPSS version 26.0 (SPSS Inc., Chicago, IL, USA). Differences in experimental results among the groups were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's post-hoc analysis. P values of <0.05 were considered statistically significant.
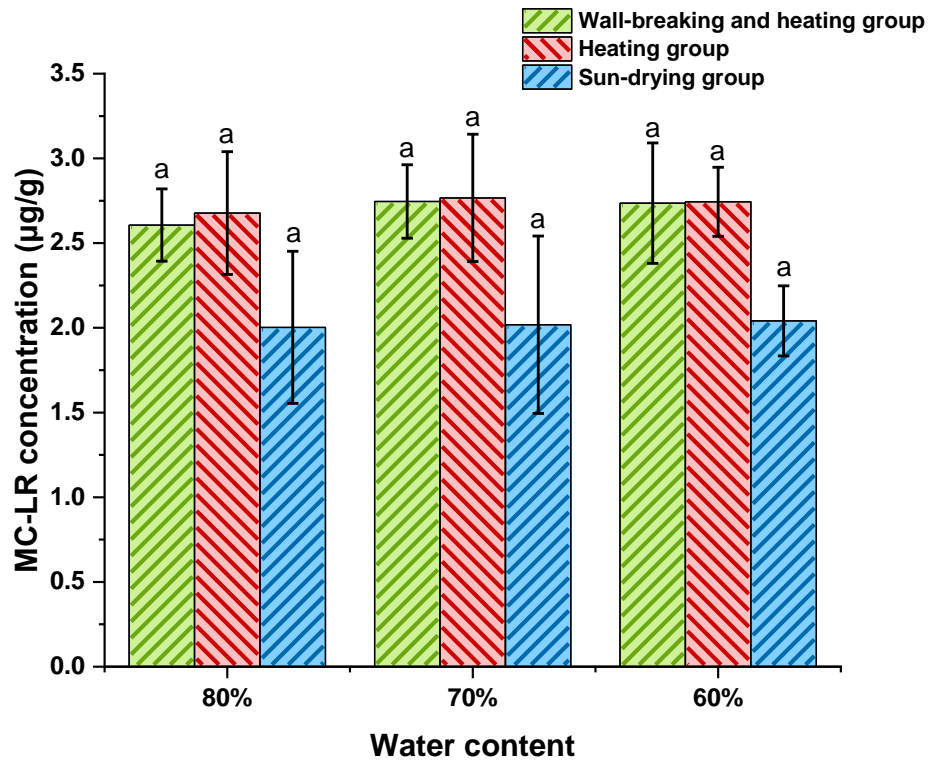
FIGURE S1 MC-LR content of BSF larvae in different groups. The values are reported as mean ± standard deviation of three experiments. Error bars represent standard errors.

TABLE S1   Growth performance and nutrient composition of BSFL using wheat bran as feed substrate.

	Feed substrate	Total larvae Weight (g)	Survival rate (%)	Bioconversion rate (%)	Dry mass reduction (%)	Feed conversion ratio
Growth performance	Wheat bran	22.73 ± 0.95	98.67 ± 0.85	4.91 ± 0.28	49.80 ± 1.50	10.36 ± 0.52
		Water content (%)	Crude protein (dry matter basis) (%)	Crude fat (dry matter basis) (%)	Crude ash (dry matter basis) (%)	
Nutrient composition	Wheat bran	77.90 ± 0.90	41.89 ± 1.37	24.37 ± 0.96	15.63 ± 0.73	

Note: Substrate moisture water was 70%.

TABLE S2 Economic evaluation of the different treatment processes.

	Groups	WT80	HT80	ST80	WT70	HT70	ST70	WT60	HT60	ST60	Note
	Cyanobacteria	/	/	/	/	/	/	/	/	/	Free of charge
	Wheat bran (CNY/T)	800	800	800	800	800	800	800	800	800	
	Transport (CNY/T)	220	220	220	220	220	220	220	220	220	About 20km
Raw materials procurement and handling	Instruments depreciation Charge (CNY)	165	120	/	165	120	/	165	120	/	Based on 3% of equipment cost
	Labor cost (CNY/T)	100	50	150	100	50	200	100	50	250	
	Heating duration (h)	4	4	/	6	6	/	7.5	7.5	/	
	Electricity costs (CNY)	328	328	/	492	492	/	615	615	/	Electricity price: 1.025 CNY/kW·h
Breeding cost	Site cost (CNY)	300	300	500	300	300	500	300	300	500	

	BSFL egg (CNY)	20	20	20	20	20	20	20	20	20	
	Labor cost (CNY)	600	600	600	600	600	600	600	600	600	
	Environmental charge (CNY)	200	200	200	200	200	200	200	200	200	
	Brief	2733	2638	2490	2897	2802	2540	3020	2925	2590	
	Larvae weight produced (KG/T)	213.03	217.90	281.87	156.23	171.10	290.50	162.17	172.80	182.37	
Output benefits	Larvae price (CNY)	2130.33	2179	2818.67	1562.33	1711	2905	1621.67	1728	1823.67	Larvae price: 10 CNY/KG
	Frass weight produced (KG/T)	524.54	513.74	493.54	573.10	568.99	489.53	567.48	557.73	549.69	
	Frass price (CNY)	188.84	184.95	177.68	206.32	204.84	176.23	204.29	200.78	197.89	frass price: 0.36 CNY/KG

	Brief	2319.17	2363.95	2996.34	1768.65	1915.84	3081.23	1825.96	1928.78	2021.56
Summary		-413.83	-274.05	506.34	-1128.35	-886.16	541.23	-1194.04	-996.22	-568.44

Note: Cost based on quotation with the manufacturer and may vary with quantity, larvae frass can be used as an organic fertilizer at a price referring to the median price of organic fertilizers.

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