Supporting information

Deeper insights into the effect of humic acid on kitchen waste anaerobic digestion: enzyme activities, microbial community dynamics, and key metabolic pathways

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Text S1

2. Materials and methods

2.3. Physical, chemical and enzymatic analysis

For ROS production, we adopt 2'7'-dichlorodihydrofluorescein diacetate (H_2DCFDA; Molecular Probes, Biosharp, CN) as probes for quantification according to Wei et al. (2019). The CDF can be oxidized to fluorescent dichlorofluorescein (DCF) by ROS. 3 ml of digestates were diluted to 15 ml by PBS buffer (0.1 mol/L, pH=7.4) and divided into three equal volume parts. For the first part, the dilution was decomposed by 300 W ultrasound for 5 minutes and centrifuged at 10000 rpm for 10 minutes to obtain the supernatant, so as to obtain all ROS in the digestates. The second part of the dilution was centrifuged at 10000 rpm for 10 minutes to obtain the ROS in the supernatant of the fermentation broth. As for the dilution in the third part, the pellets in dilution were washed by PBS buffer for three times and suspended again. After decomposing by ultrasonic, the suspension was centrifuged at 10000 rpm for 10min, and the supernatant was obtained. In this step, the intracellular ROS in the digestates was gained. These supernatants were then mixed with H2DCFDA (50 μmol/L) and incubated in the dark for 30 minutes at 37±1℃. After incubation, the fluorescence intensity of CDF was measured by a multifunctional enzyme marker (TECAN, Austria) with an excitation wavelength of 485 nm and an emission wavelength of 520 nm.

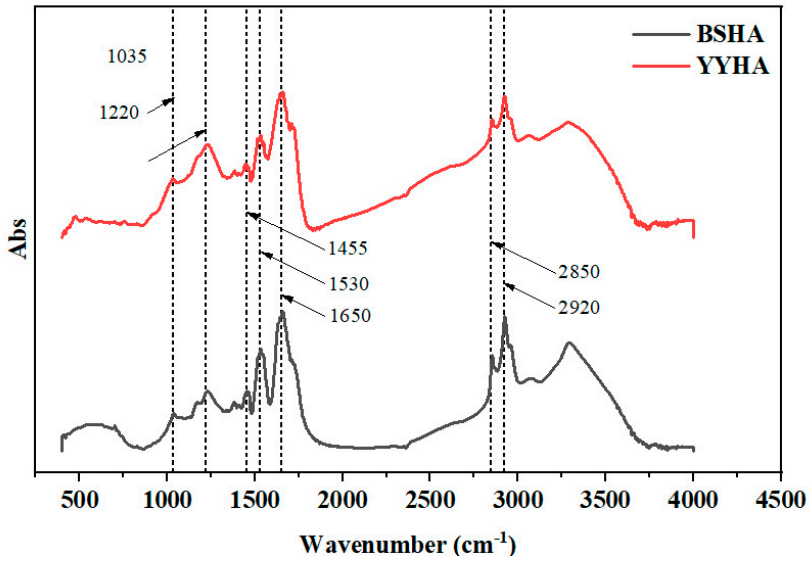

Figure. S1 FTIR spectra of BAHS and YYHA

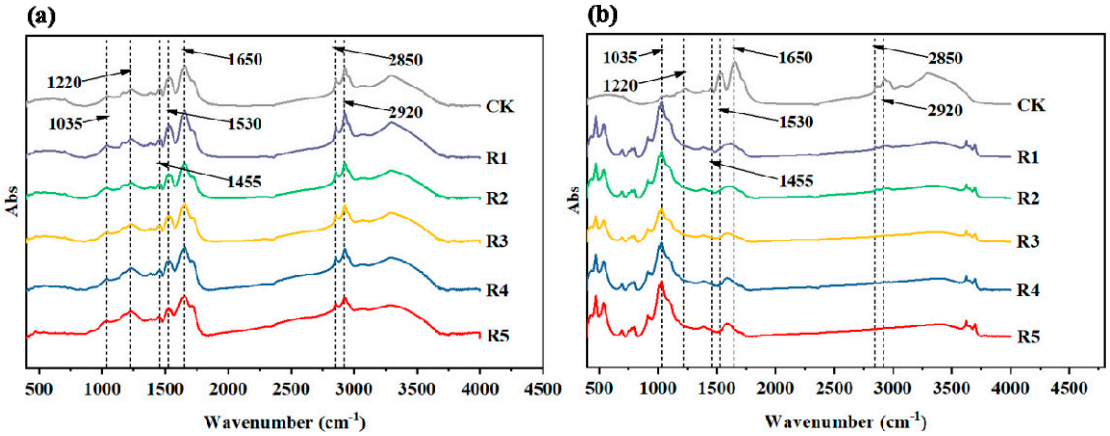

Figure. S2. Comparison of the FTIR spectra among all experiment groups (a)before and (b)after AD.

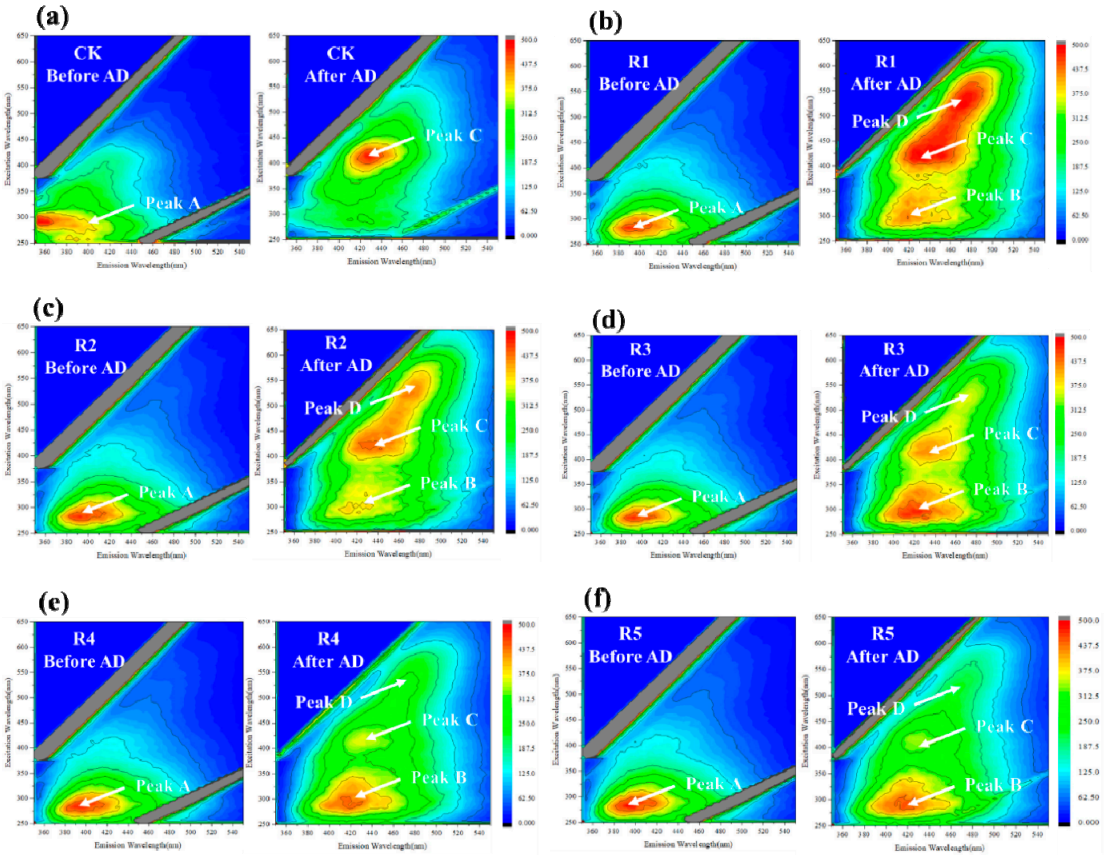

Figure. S3 3D-EEM Characteristics of HA in (a)CK, (b)R1, (c)R2, (d)R3, (e)R4 and (f)R5 before and after AD process.

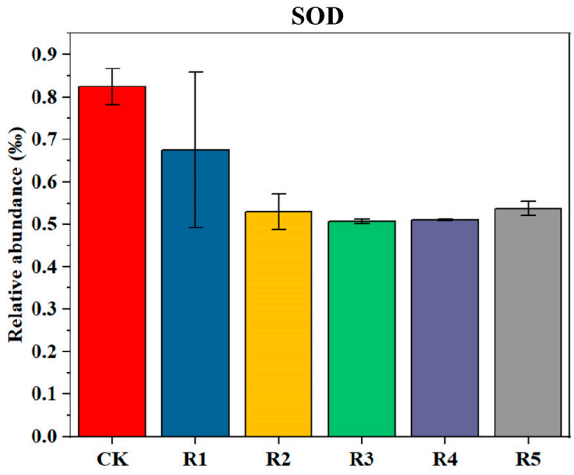

Figure. S4 the key enzymes-encoding gene abundance of SOD.