Optimization for the degradation of food waste by a novel anti-acidification and salt-tolerant microbial consortium

Qingbo Meng 1,2,3, Yude Gao 1,2,3,*, Shuangke Li 1,2,3,*, Bini Jiang 1,2,3, Mingfei He 1,2,3, Hao Bu 1,2,3, Hongling Wang 1,2,3, Di Wu 1,2,3

1Institute of Resources Comprehensive Utilization, Guangdong Academy of Sciences, Guangzhou, China;
2State Key Laboratory of Separation and Comprehensive Utilization of Rare Metals, Guangzhou, China
3Guangdong Provincial Key Laboratory of Development and Comprehensive Utilization of Mineral Resources, Guangzhou, China
*Correspondence: 13202001016@163.com (Q.M.); shuangke0215@sina.com (S.L.)

Abstract: Improving the microbial degradation capacity of food waste (FW) is a great challenge due to its acidity and high salinity. In this paper, an anti-acidification and salt-tolerant microbial consortium (AASTMC) obtained from acid and salinity mutagenesis of Bacillus amyloliquefaciens, Bacillus cereus, Yarrowia lipolytica, and Trichoderma reesei was used to degrade residential food waste. The process parameters that influenced the degradation rate of organic matter were identified by response surface methodology (RSM) with a central composite design (CCD). The effect of inoculation amount, temperature, and bulk density on the degradation rate of organic matter decreased in turn. The degradation rate of organic matter reached 94.87% under the optimum parameters of inoculation amount: 5%, bulk density: 0.04 g/cm³, and temperature: 32.33°C.

1. Introduction
Food waste (FW) gives rise to a significant environmental, economic, and social issue. The reduction and management of hundreds of millions of tons of FW has become a worldwide problem [1]. The leading factors that restrict the comprehensive treatment of FW from residential areas are unclassified domestic waste and the poor state of the FW collection and transportation system. At present, the compulsory classification of domestic waste in China creates a prerequisite for the centralized treatment of FW. Although the characteristics of FW are closely related to its source and the eating habits of consumers, FW has commonalities of high organic content, loose physical structure, high salinity and oil content, high protein (high nitrogen content), high moisture content (MC), and a weakly acidic pH [2, 3]. FW contains a large amount of easily degradable organic substances (such as sugars, starches, lipids, and proteins) and is suitable for disposal by biological degradation [4, 5]. Aerobic composting and anaerobic digestion are currently the most widely used bioengineering techniques for the management of FW [6, 7]. However, they still have some defects, such as a long processing time, many influencing factors, a complex and changeable intermediate process, and inconsistent results. In contrast, the biodegradation of food waste with composite microbial inoculants has the significant advantages of high efficiency, stability, no resulting pollution, and wide applicability [8, 9].
The major components of food waste are polysaccharides (starch and cellulose), protein, and lipids. *Bacillus amyloliquefaciens* (BA), *Bacillus cereus* (BC), *Yarrowia lipolytica* (YL), and *Trichoderma reesei* (TR) could effectively break down starch, cellulose, grease, and protein, respectively [10-13]. This suggests that the biodegradation of FW would be enhanced by the addition of a mixed culture of BA, BC, LY, and TR. In this study, the following innovative work was carried out: (1) the domestication and culture of microorganisms that can selectively degrade protein, fat, cellulose, and starch by adding acid mixture and NaCl; (2) the degradation of FW in a residential area by adding original strains and an anti-acidification and salt-tolerant microbial consortium (AASTMC); (3) the optimization of the degradation conditions of FW by AASTMC using response surface methodology (RSM); and (4) the clarification of the effects of inoculation with AASTMC on organic matter metabolic networks.

2. Materials and methods

2.1. Materials

FW was collected from eight sampling points in a residential neighborhood named Zhuguang Yujingjunting (113°24′5″ east longitude and 23°6′46″ north latitude) in Guangzhou, China. The sampling method CJ/T313–2009 was adopted. A total of 50 kg of obtained FW was shredded into pieces with diameters of less than 5 mm. The sample was then well mixed before the experiments.

*Bacillus amyloliquefaciens* (BA, GIM 1.129), *Bacillus cereus* (BC, GIM 1.199), *Yarrowia lipolytica* (YL, GIM 2.197), and *Trichoderma reesei* (TR, GIM 3.553) strains were purchased from the Guangdong Microbial Culture Center (Guangzhou, Guangdong, China). BA and BC were cultured in pre-sterilized nutrient agar medium. YL and TR were cultured in malt extract agar medium and synthetic potato medium, respectively. The chemical composition of the synthetic potato medium was as follows: 1 L 20% potato extract, 20 g glucose, 3 g KH₂PO₄, 1.5 g MgSO₄.7H₂O, 8 mg Vitamin B₁, 15 g agar, pH=6.

2.2. Optimization of degradation conditions

RSM, which was designed by Design-Expert 8.0.5 software based on central composite design (CCD) with three variables, was used for the optimization of the degradation efficiency of organic matter. The amount of the inoculants, bulk density, and temperature were chosen as the three independent variables in the experiment. The actual values and corresponding coded values of the three independent variables are listed in Table 1. In addition, 20 group experiments were designed in this study (see Table 2).

According to Table 2, 250 mL Erlenmeyer flasks containing a certain amount of FW were sterilized in 121°C high pressure steam for 15 minutes. Then, AASTMC seed solution at the stable period was inoculated. A five-day degradation test of FW was performed in a 150 rpm TS-200B-wide temperature thermostat shaker culture (Shanghai Tensuc Lab Instruments Manufacturing Co., Ltd., Shanghai, China). All of the experiments were done in triplicate and the average of loss of organic matter obtained was taken as the response (Y). The second order polynomial coefficients were calculated and analyzed using the Design Expert software (Version 8.0.5, Stat-Ease Inc., Minneapolis, USA) statistical package.

| Independent variables | Range and levels |
|-----------------------|-----------------|
| A-Amount of inoculants (%) | −1 0 1 |
| B-Bulk density (g/cm³) | 0.04 0.12 0.2 |
| C-Temperature (°C) | 28 35 40 |
Table 2. The CCD with three independent variables.

| Run order | Amount of inoculants (%) | Bulk density (g/cm³) | Temperature (°C) |
|-----------|--------------------------|----------------------|------------------|
| A1        | 1                        | 0.04                 | 28               |
| A2        | 2                        | 0.04                 | 35               |
| A3        | 2                        | 0.12                 | 35               |
| A4        | 5                        | 0.12                 | 35               |
| A5        | 2                        | 0.12                 | 35               |
| A6        | 2                        | 0.12                 | 35               |
| A7        | 2                        | 0.2                  | 35               |
| A8        | 5                        | 0.2                  | 28               |
| A9        | 1                        | 0.12                 | 35               |
| A10       | 2                        | 0.12                 | 40               |
| A11       | 2                        | 0.12                 | 35               |
| A12       | 1                        | 0.2                  | 28               |
| A13       | 1                        | 0.2                  | 40               |
| A14       | 1                        | 0.04                 | 40               |
| A15       | 2                        | 0.12                 | 35               |
| A16       | 5                        | 0.04                 | 28               |
| A17       | 2                        | 0.12                 | 28               |
| A18       | 2                        | 0.12                 | 35               |
| A19       | 5                        | 0.04                 | 40               |
| A20       | 5                        | 0.2                  | 40               |

2.3. Analytical methods

The contents of total carbon (TC), total nitrogen (TN), and H and S elements were measured using a Vario EL Cube type element analyzer (Lementar, Germany) in CHNS mode with 20 mg dried and ground FW sample, and C/N and C/H ratios were obtained [14]. The contents of K₂O, CaO, MgO, and P₂O₅ were determined by atomic absorption spectrophotometry after dry ashing [15]. One gram of dried sample was burned in a 600°C muffle furnace for 4 h, then digested in aqua regia and analyzed by atomic absorption spectrometer (Atomic Absorption Spectrometer, AAS, WFX-110B, Beijing Beifen-Rulli Analytical Instrument Co., Ltd., China). The fresh samples were dried in a hot air oven at 105°C temperature for 24 h to measure moisture content (MC) and total solid (TS) content [16]. The calculation of the organic matter and ash contents of FW was based on the reduction and remaining ratio of the dry basis, respectively, after being burned in the muffle furnace at 600°C for 4 h [17]. A 1:20 aqueous extract (w/v, dry weight basis) of FW with deionized water was used for the analysis of pH. The pH was measured using a pH meter (PHS-3C, China) [14].

2.4. Statistical analysis

Except for statistical analysis of RSM, all the physico-chemical properties of FW were analyzed using SPSS 25.0 (SPSS for Windows, Version 13.0, USA). One-way analysis of variance (ANOVA) with SPSS was performed to determine the significant differences, with a P-value < 0.05 suggesting a statistically significant difference. Statistical analysis of RSM was performed to evaluate ANOVA with Design-Expert software, including model significance, its associated probability p (F), a lack-of-fit test, and determination coefficient R² [18, 19]. The three-dimensional response surface and contour curves, according to quadratic polynomial models, were generated using Design-Expert software to determine the interaction between two examined variables.
3. Results and discussion

3.1. Characterization of food waste

The physicochemical properties of FW in this study are shown in Table 3. The TS, TC, and TN contents of FW were comparable to those obtained from the canteen of the Beijing University of Chemical Technology [20]. However, FW in this study had more protein and fat contents. Moreover, the contents of K₂O, MgO, and P₂O₅ were higher than the average of FW reported in other studies [15].

It can be seen from Table 3 that the ratio of starch to protein to cellulose to fat content was 1:9:1:13. The protein and fat content of the sample was significantly higher than that of starch and cellulose. The protein, fat, starch, and cellulose content of the FW was linked to the living standards and eating habits of the residential district.

3.2. Anti-acidification and salt-tolerance acclimatization and degradation test

In order to obtain the anti-acidification and salt-tolerant microbial consortium (AASTMC), BA, BC, YL, and TR were domesticated with both acid and salt resistance. Small molecular organic acids, including mainly acetic, propionic, butyric, and lactic acids, were produced in the degradation process of the proteins, starch, and fat in FW. The pH of the culture medium was thus adjusted by the above four acids with a mass ratio of 3:3:2.5:12.5 [21, 22]. In the experiment, the tolerances of microbial strains to the pH and/or salinity of the medium were enhanced by increasing the amount of acid mixture and NaCl until they could grow in pH=4.5 and 4% salinity medium normally.

The original microbial consortiums (OMC) were composed of BA, BC, YL, and TR strains in a ratio of 1:1:9:13, as consistent with the ratio of waste (starch content: protein content: cellulose content: fat content) in the FW. The anti-acidification and salt-tolerant microbial consortiums (AASTMC) were made up of domesticated BA, BC, YL, and TR strains in a ratio of 1:1:9:13. A total of 50 g FW was added to a 250 mL Erlenmeyer flask. The inoculation amount of OMC or AASTMC was 2%, and the bulk density was 0.12 g/mL. The oscillatory degradation test was carried out under the conditions of 150 rpm and 28°C for five days. The results were showed in Figure 1.

| Properties       | Food waste |
|------------------|------------|
| MC (%)           | 76.20±3.77 |
| TS (%)           | 23.80±3.77 |
| Ash (%)          | 5.30±1.06  |
| OM (%)           | 94.70±1.06 |
| pH               | 4.90±0.03  |
| Salinity (%)     | 3.81±0.29  |
| TC (%)           | 47.43      |
| TN (%)           | 3.011      |
| C/N ratio        | 15.75      |
| C/H ratio        | 8.77       |
| S (%)            | 0.14       |
| K₂O (%)          | 0.36       |
| CaO (%)          | 1.21       |
| MgO (%)          | 0.09       |
| P₂O₅ (%)         | 2.31       |
| Starch content (%)| 2.6        |
| Protein content (%)| 18.8      |
| Cellulose content (%)| 2.20     |
| Fat content (%)  | 27.5       |

All results except moisture content are on a dry matter basis. The results are based on three samples taken at times during the experiment.
As shown in Figure 1, after five days of degradation with the OMC, the loss of organic matter of FW was 73.72%, while the loss of organic matter of FW with the AASTMC was 80.57%, an increase of 6.85%. The degradation ability of the AASTMC was significantly improved compared to that of the OMC. The reason may be that the stronger environmental adaptation of AASTMC to the acid and high salinity of FW significantly shortened the degradation start time and promoted the growth and reproduction ability of the strains.

3.3. Optimization of degradation of FW by CCD-RSM

3.3.1. Model fitting analysis. Experiments were performed according to the 20 statistical designs in Table 2 to obtain the series of the decomposition of organic matter. Data were analyzed by the means of the most widely used second-order polynomial response surface function. In this study, the regression model with the amount of the inoculants (A), bulk density (B), and temperature (C) as independent variables and decomposition of organic matter (Y) as a dependent variable was obtained as follows:

\[ Y = 63.36 + 20.21A - 6.22B - 14.18C + 5.45AB + 3.76AC + 1.65BC + 8.77A^2 + 1.58B^2 - 21.77C^2. \]

Table 4. ANOVA analysis of CCD based RSM for degradation.

| Source                  | Sum of squares | Degree freedom (DF) | Mean square | F value | Prob > F | Remarks |
|-------------------------|----------------|--------------------|-------------|---------|----------|---------|
| Model                   | 8358.89        | 9                  | 928.77      | 349.35  | < 0.0001 | Significant |
| A-Amount inoculants     | of 4077.67     | 1                  | 4077.67     | 1533.79 | < 0.0001 |
| B-Bulk density          | 381.84         | 1                  | 381.84      | 143.63  | < 0.0001 |
| C-Temperature           | 1987.08        | 1                  | 1987.08     | 747.43  | < 0.0001 |
| AB                      | 249.66         | 1                  | 249.66      | 93.91   | < 0.0001 |
| AC                      | 119.69         | 1                  | 119.69      | 45.02   | < 0.0001 |
| BC                      | 21.96          | 1                  | 21.96       | 8.26    | 0.0165   |
| A^2                     | 111.39         | 1                  | 111.39      | 41.9    | < 0.0001 |
| B^2                     | 6.84           | 1                  | 6.84        | 2.57    | 0.1397   |
Analysis of variance (ANOVA) was used to evaluate the statistical significance of the aforementioned quadratic polynomial model. The results are displayed in Table 4. The results showed that $F = 349.35$, $p < 0.001$, which suggested that the regression equation could well reflect the actual relationship between the loss of organic matter and the three independent variables, and the model was statistically significant. The significance test results of independent variables showed that all were significant model variables ($p < 0.05$) except $B^2$. A, B, C, AB, AC, $A^2$, and $C^2$ ($p < 0.0001$) had a great effect on the decomposition of organic matter. In addition, the coefficient of determination ($R^2$), adjusted $R^2$ (adj. $R^2$), and CV (coefficient of variation) of the model were used to evaluate the fit of the quadratic polynomial model and its overall predictive ability. The model $R^2 = 0.9968$, indicating that the model could explain the change of the response value to the extent of 99.68%. The fitting degree of the model was high, and the experimental error was small. The adj. $R^2 = 0.9940$ meant that 99.40% of the data points in the predicted regression line can be explained simply by the independent variable that actually affected the dependent variable, which indicated that the fitting of the model had strong correlation. Pred. $R^2$ was 0.9798, indicating that the actual values of loss of organic matter were very close to the predicted values (see Table 5). The regression equation had goodness of fit, and the model was valid and significant. CV reflected the reproducibility of the experimental model. The CV of this experiment was 3.13% ($< 10%$), indicating that the experimental model was reproducible. The lack of fit expressed the effect of the difference between the actual value and the predicted value on the pure error. The lack of fit model was 20.24 ($p > 0.05$), which indicated that it was insignificant and the regression model was reliable.

### Table 5. Experimental values of loss of organic matter versus predicted values.

| Run order | Experimental | Predicted |
|-----------|--------------|-----------|
| 1         | 80.09        | 80.14     |
| 2         | 2.29         | 3.77      |
| 3         | 23.34        | 23.81     |
| 4         | 20.26        | 17.62     |
| 5         | 85.7         | 84.98     |
| 6         | 50.68        | 52.17     |
| 7         | 45.4         | 45.08     |
| 8         | 37.62        | 36.35     |
| 9         | 51.63        | 52.17     |
| 10        | 47.02        | 49.74     |
| 11        | 52.36        | 52.17     |
| 12        | 63.8         | 63        |
The effect of the amount of the inoculants, bulk density, and temperature on the decomposition of organic matter can be judged by the mean square value in Table 4. The greater the mean square value was, higher the influence degree. The mean square values of inoculation amount, bulk density, and temperature were 4077.67, 381.84, and 1987.08, respectively. Therefore, the order of influences on the decomposition of organic matter from strong to weak was the amount of inoculants, the temperature, and the bulk density.

3.3.2. Effect of process variables. Based on the optimization analysis, the degradation rate of AASTMC was predicted to be 96.36% under the conditions of 5% of inoculation amount, 0.04 g/cm³ bulk density, and 32.33°C. The response surface slope of the interactive effects between two factors of inoculation amount, bulk density, and temperature were determined according to the quadratic polynomial regression equation (Figures 2-4). The sensitivity of the response values (loss of organic matter) to the interactions between the factors (inoculation amount, bulk density, and temperature) depended on the steepness of the response surface slope. More precisely, the steeper the slope, the more sensitive the interaction. The effect of the interaction between factors on the loss of organic matter could be obtained according to contour shapes. An oval shape meant that the interaction between the two factors was significant, while a circular shape meant that the interaction was not significant.

According to Figures 2-4, all of the interactive effects among inoculation amount, bulk density, and temperature were significant. As can be seen from Figure 2, the slope of the response surface was steep, and the interaction between bulk density and temperature was strong. At 5% inoculation, the degradation of organic matter decreased as the bulk density increased from 0 to 0.125 g/cm³, while the degradation increased first and then decreased as the temperature was constantly raised from 28°C to 35°C.

![Figure 2. Response surface plots presenting the interaction between bulk density and temperature (amount of the inoculants, 5%)](image_url)
There was a similar pattern seen in the slope of the response surface and the interaction between inoculation amount and temperature, which can be observed in Figure 3. At the bulk density of 0.04 g/cm³, the organic matter degradation rate had a significant positive correlation as the inoculation amount was increased from 3% to 5% and a trend of increasing first and then decreasing while the temperature was raised from 28°C to 35°C. As shown in Figures 2 and 3, the response surface slopes were symmetrically distributed under the temperature of 32.33°C, which indicated the significant effect of temperature on the degradation of organic matter. This result was consistent with the conclusion of Liu et al. [23]. As suggested by Figure 4, the fact that the response surface slope was steep meant that the interaction between bulk density and inoculation amount was strong. Under the temperature of 32.33°C, the degradation rate of organic matter increased as the inoculation quantity increased, and the rate decreased with decreasing accumulation density before reaching the optimal level.
3.4. Degradation of FW with AASTMC under optimal conditions
Response surface methodology based on central composite design suggested the optimal degradation conditions: inoculation amount of AASTMC 5%, accumulation density of 0.04 g/cm³, and temperature of 32.33°C. The degradation of FW with AASTMC under optimal conditions was performed and the results were presented in Figure 5.

In the first two days of degradation, the degradation rate of organic matter increased slowly, because bacteria needed an adaptation process. On the third day, the degradation rate increased rapidly and the loss of organic matter increased by 57.35% compared to that on the second day. After five days of degradation, the loss of organic matter reached 94.87%. It was very close to the predicted value.

![Figure 5. Loss of organic matter with the progress of degradation.](image)

4. Conclusions
FW in this study had the characteristics of strong acidity, high protein content, and rich nutrients, such as K₂O, MgO, and P₂O₅. A novel anti-acidification and salt-tolerant microbial consortium (AASTMC) with a stable and efficacious ability to degrade FW was developed. The AASTMC exhibited tolerance and adaptability to high salinity and strong acidity. The degradation rate of FW was 6.85% higher than that of the OMC. The optimal degradation conditions suggested by the response surface methodology based on central composite design were as follows: inoculation amount of AASTMC 5%, accumulation density 0.04 g/cm³, and temperature 32.33°C. The actual degradation rate within five days was 94.87%.

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