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KITCHEN WASTE SOLID STATE FERMENTATION BY *Aspergillus niger* TO PRODUCE ACID PROTEASE

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Key words: kitchen waste, *Aspergillus niger*, solid state fermentation, acid protease

ABSTRACT

Acid protease production was carried out by solid state fermentation (SSF) with *Aspergillus niger* using kitchen waste as the principal raw material. The SSF conditions were studied and the optimal one was identified. This study explored effects of different water contents in SSF medium, different inoculum sizes and different fermentation time on acid protease activities by following the one-factor-at-a-time method. Based on the results obtained, the optimal fermentation conditions for the production of c acid protease, were are as follows: 30g of kitchen waste content, 20mL of initial water content, 4mL of inoculum, and 70h ofas fermentation time. Under these fermentation conditions, the activity of the acid protease was 223.518 U.

Palabras clave: desperdicios de cocina, Aspergillus niger, fermentación en estado sólido, proteasa ácida

RESUMEN

Se llevó a cabo la producción de proteasa ácida por fermentación en estado sólido (SSF, por sus siglas en inglés) con Aspergillus niger, utilizando desperdicios de cocina como principal materia prima. Se estudiaron las condiciones de la SSF y se identificó la óptima. La investigación exploró el contenido de agua en el medio y diferentes cantidades de inóculo y tiempos de fermentación en la actividad de la proteasa ácida con el método de un factor a la vez. Con base en los resultados obtenidos, las conndiciones óptimas de fermentación para la producción de proteasa ácida fueron: 30 g de desperdicos de cocina, 20 mL de agua iniciales, 4 mL de inóculo y 70 h de tiempo de fermentación. Bajo estas condiciones la actividad de la proteasa ácida fue de 223.518 U.

INTRODUCTION

Kitchen waste refers to the household wastes left over from household consumption, including the wastes and food residues left over from the food processing in families, restaurants, canteens and various food and beverage industries (Yang et al. 2017, Fu and Liu 2017, Lamas et al. 2016, Ali and Zayan 2017, Wahab and Adzmi 2017). Its main features are as follows: they are perishable, smelly, easy to breed and spread bacteria and viruses, and rich in organic nutrients; their sources are relatively concentrated and obvious, and they have high water content and are easy to be collected (Quan 2011, Sharifuzzaman et al. 2017). Due to the high water content and grease content in the kitchen waste, it is not conducive to incinerate household waste, and the landfill can easily pollute groundwater and generate much biogas, causing secondary pollution to the environment (Borogayary et al. 2018, Albrecht and Shaffer 2016, Khan et al. 2017, Khan et al. 2018). China is a country with large amounts of kitchen waste. Every day, a large amount of kitchen waste is thrown away, which is a waste of resources and polluting the environment. Many processing technologies are currently available, such as crushing disposal, earthworm treatment, organic fertilizer by aerobic fermentation, protein feed by thermophilic fermentation, extraction of biodegradable plastic, biogas production by anaerobic digestion, hydrogen production by biological fermentation (Wang et al. 2004, Yu et al. 1999, Ye et al. 2015, Fu and Liu 2017, Afzal et al. 2018). However, due to various reasons related to funding, siting, technologies and so on, they have yet to be widely used in the country. At present, the main method of treating kitchen waste in China is to throw it away or collect it and then send it to farms as feed. Although this simple feed treatment shows the value of recycling kitchen waste, it is not safe to use it to directly feed livestock due to its homologous pollution (Xu et al. 2011, Ashraf et al. 2017, Anees et al. 2017). Although kitchen waste contains toxic materials, its main components are vegetables, rice noodles, meat, and the like, and its main chemical composition is organic matters such as starch, cellulose, protein and lipid. Therefore, kitchen waste can be reused and recycled. Proper handling of them can deliver benefits to the society, the environment and the economy.

Acid protease is an enzyme that hydrolyzes proteins and polypeptides in acidic environments. Because of its good acid resistance, it is widely used as an important industrial enzyme in feed processing, brewing alcohol and beer, leather technology, and in light industry and food processing industry. People are closely followingwatching the potential of acid protease (Liu et al. 2004, Zhang et al. 2009, Yu et al. 2006, Wang et al. 1993, Mi et al. 2016, Mi et al. 2017, Ali and Iftikhar 2017, Shamsudin et al. 2017). Acid protease is mainly obtained through microbial fermentation currently, which can be generated by *Aspergillus niger*, *Aspergillus oryzae*, *Bacillus*, etc (Shen et al. 1999, Nongqwenga and Modi 2017). *Aspergillus niger* is the most commonly used fungus for producing acid protease due to it is less toxic and generates more metabolites. Microbial SSF requires fewer investments, and has the advantages of low energy consumption, easy operation, easy promotion,generating less pollution, and having simple post-processing and fermentation process. Microbial SSF also uses simple equipment and can be easily promoted. *Aspergillus niger* fit falls in the category of SSF filamentous fungi. To produce a large amount of acid protease at low cost so as to meet the market demand has become a vision for many researchers.

Kitchen waste is rich in protein, fat, carbohydrates and many kinds of inorganic salts, and can promote the growth and reproduction of a variety of microbes. Therefore, the fermentation conditions of acid protease, which is produced by *Aspergillus niger* SSF kitchen waste, were studied, and the optimal conditions were finally worked outidentified. It is expected to provide an example for the integrated use of kitchen waste and the production and preparation of acid protease.

MATERIALS AND METHODS

Materials and reagents

Kitchen waste, collected on June 18, 2017 at the restaurant on the first floor of the fourth canteen of Zhaoqing College.

Strain: *Aspergillus niger* (purchased from Guangdong Culture Collection Center, strain preservation number: CICC2377), Food Microbiology Laboratory of Zhaoqing College.

The main reagents: potato dextrose agar (PDA) medium, Guangdong Huankai Microbial Sci. & Tech. Co.,Ltd.; foline-phenol reagent, trichloroacetic acid, casein, lactic acid, L-tyrosine, anhydrous sodium carbonate and sodium lactate are of domestic analytical grade.

SSF medium: remove plastic, paper towels and other sundries in the kitchen waste, and then take gauze filter residue. Dry and crush the residue to obtain SSF medium. Take several of 250-ml flasks

of 250ml, each loaded with 30g of medium, and then add due amounts of water according to the design of the experiment. All media were sterilized at 121 ºC for 20 min.

Instruments and equipment

LRH-150 biochemical incubator; SW-CJ-2FD clean bench; Shimadzu UV-1240 UV-visible spectrophotometer; TDZ5-WS centrifuge; HH-6 digital thermostat water bath; PHS-25 pH meter; DHG-9070B digital electric oven; BF-08 small grinder.

Experimental methods

Measurement of the main components of the medium

(1) Measurement of crude protein content is in accordance with the national standard (GB 5009.5-2010 Kjeldahl method) (GB/T 6432-1994).
(2) Measurement of crude fat content is in accordance with the national standard GB/T 6433-2006 (Soxhlet extraction method) (GB/T 6433-2006).
(3) Measurement of starch content is in accordance with the national standard GB/T 5009.9-2008 (acid hydrolysis method) (GB/T 5009.9-2008).
(4) Measurement of water content is in accordance with the national standard GB/T 6435-2014 (direct drying method) (GB/T 6435-2014).
(5) Measurement of crude ash content is in accordance with the national standard GB/T 6438-2007 (high temperature burning method) (GB/T 6438-2007).
(6) Measurement of cellulose content is in accordance with the national standard GB/T 6434-2006 (filtration method) (GB/T 6434-2006).

Preparation of spore suspension

Aspergillus niger routinely separated and purified from the plate medium was inoculated on a beveled test tube and incubated at a constant temperature of 30℃ for 3-5 days until the beveled test tube was covered with spores. Add 10mL of sterile water, use glass beads to scatter the spores, and make a spore suspension. Uuse a hemocytometer to count the number of spores and save the spore suspension for future use (Zhang and Zhou 2011, Lin et al. 2014).

SSF enzyme production

TakeP 30g of smashed kitchen waste in a 250-mL Erlenmeyer flask, sterilize the wasteflask at 121 ºC for 30 min, and cool it to room temperature and add the spore suspension to it. Add a certain amount of sterile water, shake up the water and waste in the flaskk , keep them fermented at 30 ºC in a constant temperature incubator, shake them up several times during fermentation to ensure that *Aspergillus niger* spread evenly in the kitchen waste.

Preparation of crude enzyme solution

Smash and shake up enzymatic ferments obtained after a certain time of kitchen waste SSF by *Aspergillus niger*, take 5g of such ferments in a triangular flask, add 50 mL of 1% NaCl solution, leach them in water bath at 40 ºC for 30 min, and filter them with gauze and centrifuge to obtain crude enzyme solution (Zhang et al. 2009, Xie et al. 2005).

Measurement of acid protease enzyme activity

Measurement of acid protease is in accordance with the GB/T 28715-2012 (GB/T 28715-2012)

Drawing of the standard tyrosine curve

Respectively prepare 0.0-μg/mL, 10.0-μg/mL, 20.0-μg/mL, 30.0-μg/mL, 40.0-μg/mL, 50.0-μg/mL and 60.0-μg/mL L-tyrosine standard working solutions. Take 1.0 mL of each of these solutions, add into these solutions a certain amount of sodium carbonate solution and foline-phenol reagent, and keep these solutions fermented in water bath at 40℃ for 20 min. Then move these solutions out quickly, cool them to room temperature in cold water, and measure the absorbance at 680 nm with 10 mm cuvette. Take L-tyrosine content as the abscissa and the absorbance as the vertical axis. Then a standard curve can be drawn and an equation of linear regression can be obtained.

Definition of the protease activity: the amount of enzyme required to hydrolyze casein into 1 μg of tyrosine per minute at a pH of 3.0 under (40 ± 2) ºC is one unit of measurement of enzyme activity. The measurement is carried out according to the foline-phenol method, and a unit of measurement of enzyme activity is expressed in U.

Acid protease enzyme activity formula:

$$X_i = c \times V \times 4 \times N / (1.0 \times m \times 10)$$

Where: X_i is the protease activity (U); c is the tyrosine concentration (μg/mL) obtained from the standard curve;
V is the total volume of the extract (mL); 4 is the total volume of enzyme reaction (mL);
N is the second dilution times of the sample extract; 1.0 is the amount of enzyme (mL); involved in the reaction;
m is the sample's mass (g); 10 is the reaction time (min)

TABLE I FERMENTATION MEDIUM (KITCHEN WASTE) COMPOSITION

Item	Protein	Starch	Fat	Cellulose	Moisture	Ash content
Content/%	15.26	36.95	14.67	6.72	11.30	8.41

Acid protease separation (salting-out method)

Filter and centrifuge the crude enzyme solution, add into the solution solid ammonium sulfate until reaching the saturation point. After one whole night, remove the supernatant and dry the solid precipitated by the filter press at 40 ºC for 24 h. After drying and pulverizing, the crude enzyme product is obtained (Zheng 2006).

RESULTS AND ANALYSIS

Composition of the fermentation medium (kitchen waste)

Table I shows that the main components of kitchen waste are protein, starch and fat-based organic matters, which can promote the growth and reproduction of a variety of microbes and can be used to breed *Aspergillus niger*.

Drawing of standard tyrosine curve

The standard curve drawn according to the method of 1.4.7 is shown in **Fig. 1**, and the regression equation is obtained: y=0.0106x + 0.0021, R^2=0.9999.

Single-factor experiment of fermentation conditions

The experiment identified the optimal inoculum size, fermentation time and water holding capacity for using kitchen waste SSF by *Aspergillus niger* to produce acid protease.

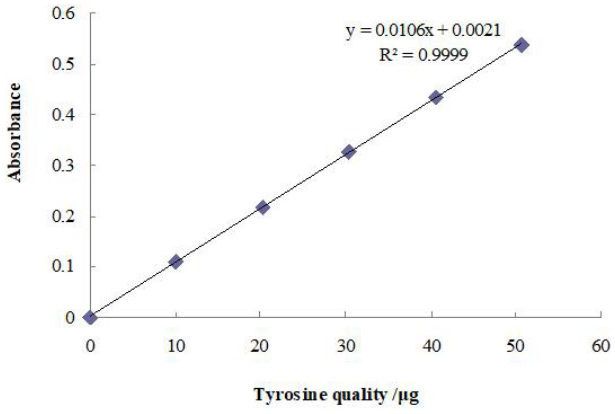

Fig. 1. Standard tyrosine curve

Effects of different inoculum sizes on the enzyme production

Respectively add into the kitchen waste solid medium 1, 2, 3, 4, 5, 6, 7 and 8 mL of spore suspension whose spore concentration is 2.5×10^6. Keep these solutions fermented in a 30 ºC-biochemical incubator for 70 h. Effects of different inoculum sizeson enzyme production were studied. The results are shown in **Fig. 2**. When the inoculum size was under 3 mL, increase of inoculum could promote the production of enzyme, and when above 3mL, increase of inoculum does not make much difference to enzyme production.

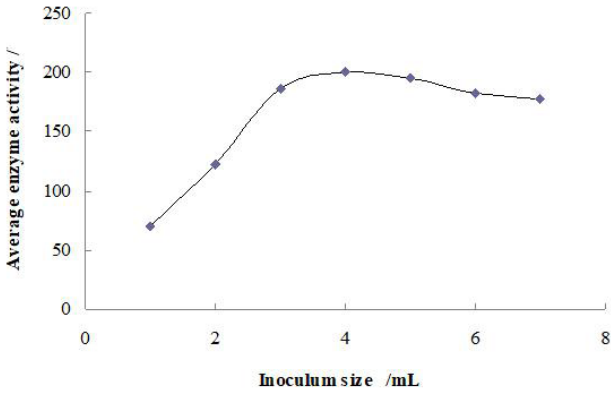

Fig. 2. Effects of different inoculum sizes on enzyme production.

Effects of different water contents on the enzyme production

Water content in the medium determines the growth and reproduction of *Aspergillus niger*. Too little water does harm to the growth of *Aspergillus niger* cells. Too high will inhibit its normal life activity. Respectively add into 4mL of inoculum and 30g of kitchen waste solid medium 5, 10, 15, 20, 25 and 30mL of sterile water . Then study the effects of different water contents on the production of enzymes. The results are shown in **Fig. 3**. The maximum amount of enzyme produced was 20mL. When the amount of water added was higher or lower than 20 mL, the amount of enzyme produced decreased remarkably. Water content had a significant effect on the production of enzyme.

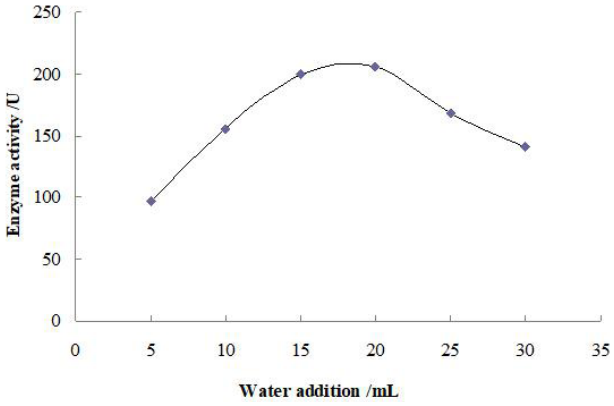

Fig. 3. Effects of different water contents on the production of enzymes

Effects of different fermentation time spans on the production of enzymes

The fermentation time span is one of the key factors in the production of acid protease. In the pre-fermentation stage, the activity of producing acid protease by *Aspergillus niger* increases with the growth and reproduction of bacterial cells, and the protease may be consumed in the late fermentation stage. 4mL of spore suspension, 30g of kitchen waste solid medium, and 20mL of sterile water were mixed up and fermented for respectively 40, 50, 60, 70, 80 and 90 h. The protease activity was measured, and the curve was obtained, as shown in **Fig. 4**. The figure shows that the enzyme activity increased rapidly within 70h of culture, and a slight decrease in enzyme activity is observed after 70 h.

Identify the optimal fermentation conditions by using orthogonal experiments

The best fermentation conditions of the culture medium are not simply the optimal superposition of each single factor. Interaction exists between the various conditions. When these conditions interact properly, *Aspergillus niger* can better facilitate growth and metabolism of enzymes. The three-factor and three-level orthogonal experiment on different inoculum sizes, water contents, culture time spans is shown in **Table II**, aiming to further find out the optimal culture conditions and to identify the optimal conditions for enzyme production.

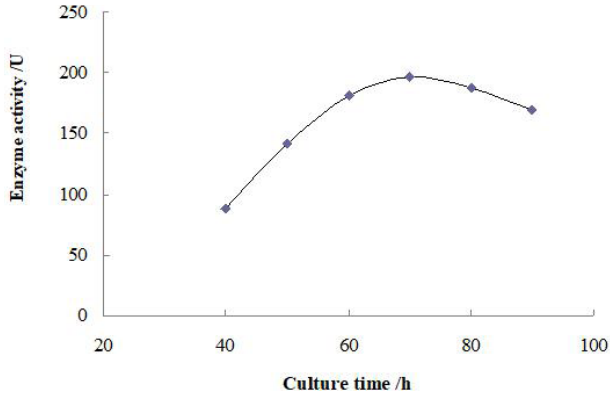

Fig. 4. Effects of different fermentation time spans on the production of enzymes.

TABLE II. ORTHOGONAL TEST DESIGN TABLE OF CULTURE CONDITIONS.

Level	Factor		
	A(Culture time span/h)	B (Water content/mL)	C (Inoculum size/mL)
1	65	18	3
2	70	20	4
3	75	22	5

As can be seen from **Table III**, the optimal formula for producing enzymes is $A_2B_2C_2$: 70h of culture time, 20mL of water, and 4mL of inoculum.

Extraction and separation of acid protease

According to the optimal formula, mix up 20mL of water, 4mL of spore suspension and 30g of kitchen

TABLE III. L_9 (3^4) ORTHOGONAL EXPERIMENTAL RESULTS AND ANALYSIS

S/N	Factor				Enzyme activity (U)
	A	B	C	Nullable column	
1	1	1	1	1	167.132
2	1	2	2	2	186.755
3	1	3	3	3	179.585
4	2	1	2	3	201.849
5	2	2	3	1	204.113
6	2	3	1	2	198.830
7	3	1	3	2	180.717
8	3	2	1	3	185.623
9	3	3	2	1	178.453
$\mathbf{K_1}$	1.420	1.463	1.468	1.463	
$\mathbf{K_2}$	1.609	1.534	1.509	1.507	
$\mathbf{K_3}$	1.450	1.482	1.502	1.509	
k_1	0.473	0.488	0.489	0.488	
k_2	0.536	0.511	0.503	0.502	
k_3	0.483	0.494	0.501	0.503	
R	0.063	0.024	0.014	0.015	

waste solid medium. After 70 h of culture at 30 ºC, carry out the experiments of the acid protease extraction and separation. Measure the acid protease activity of the crude enzyme solution obtained according to the optimal formula and the dried crude enzyme product. The acid protease activity of the crude enzyme solution and the crude enzyme product were respectively 223.518 U and 2954.74 U. Crude enzyme products obtained through the salting-out method contain a large amount of impurities, and to obtain more active acid protease products, further separation and purification are needed.

CONCLUSION

Kitchen waste is rich in various nutrients that can promote the growth and reproduction of a variety of microbes and is a good raw material for microbial fermentation. Using kitchen waste to produce acid protease not only can generate more active enzymes, but also can better utilize kitchen waste and deliver benefits to the society, environment and economy. Single-factor experiment and orthogonal experiment have identified the optimal conditions for producing the enzyme: mix up 20mL of sterile water, 30g of kitchen waste SSF medium, and 4mL of suspension of *Aspergillus niger* whose spore concentration is 2.5×10^6, and keep them fermented for 70h under constant temperature of 30 ºC. Under such culture conditions, the crude enzyme activity of acid protease reaches up to 223.518 U, indicating that using kitchen waste SSF by *Aspergillus niger* can produce more active acid protease.

This is just a preliminary experiment on the kitchen waste SSF technique. However, to use this technique in our daily life, it is necessary to experiment with the *Aspergillus niger* strain, medium supplement, culture temperature and the like. The obtained fermentation products and crude enzyme products also need further analysis.

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REFERENCES

Afzal M.N.I., Lawrey R., Anaholy M.S. and Gope J. (2018). A Comparative Analysis of The Efficiency and Productivity of Selected Food Processing Industries in Malaysia. Malaysian Journal of Sustainable Agriculture. 2(1), 19-28.

Albrecht F., and Shaffer G. (2016). Regional Sea-Level Change along the Chilean Coast in the 21st Century. Journal of Coastal Research. 32 (6), 1322-1332.

Ali S., Ali R. and Iftikhar A. (2017). Physico-chemical and microbiological assessment of some freshwater aquifers and associated diseases in district ghizer, gilgit-baltistan, Pakistan. Acta Scientifica Malaysia. 1(1), 08-12.

Ali S.M.M. and Zayan J.M. (2017). Drag Reduction for A Fast Back Passenger Car (Logan). Science Heritage Journal. 1(2), 17-20.

Anees M.M., Qasim M. and Bashir A. (2017). Physiological and Physical Impact of Noise Pollution on Environment. Earth Science Pakistan. 1(1), 08-11.

Ashraf M.A., Hussin N.H. and Gharibreza M. (2017). Studies on the Removal of Heavy Metals from Aqueous Solution Using Immobilized Typha angustata L. Earth Science Pakistan. 1(1), 12-16.

Borogayary B., Das A.K. and Nath A.J. (2018). Tree species composition and population structure of a secondary tropical evergreen forest in Cachar district, Assam. Journal of Environmental Biology. 39 (1), 67-71.

Fu H., Liu X. (2017). Research on the Phenomenon of Chinese Residents' Spiritual Contagion for the Reuse of Recycled Water Based on SC-IAT. Water 9 (84611). doi: 10.3390/w9110846.

Fu H.L. and Liu X.J. (2017). Research on the Phenomenon of Chinese Residents' Spiritual Contagion for the Reuse of Recycled Water Based on SC-IAT. Water, 9 (11), 846.

GB/T 28715-2012. (2012). Feed additives - Determination of Acid and Neutral Protease Activity in Feed Additives - Spectrophotometric Method. Beijing: Standards Press of China.

GB/T 5009.9-2008. (2008). Determination of Starch in Foods. Beijing: Standards Press of China.

GB/T 6432-1994. (2009). Determination of Crude Protein in Feeds. Beijing: Standards Press of China.

GB/T 6433-2006. (2006). Determination of Crude Fat in Feeds. Beijing: Standards Press of China.

GB/T 6434-(2006). Determination of Crude fiber in Feeds - Filtration Method. Beijing: Standards Press of China.

GB/T 6435-2014. (2014). Determination of Moisture in Feeds. Beijing: Standards Press of China.

GB/T 6438-2007. (2007). Determination of Crude Ash in Feeds. Beijing: Standards Press of China.

Khan A.M., Yusoff I., Abu Bakar N.K., Abu Bakar A.F., Alias Y. and Mispan M.S. (2017). Accumulation, uptake and bioavailability of rare earth elements (rees) in soil grown plants from ex-mining area in perak, malaysia. Applied Ecology and Environmental Research, 15 (3), 117-133.

Khan F., Khan M.I., Khan S., Zaman M.A.U., Rasheed H. and Khan A.R. (2018). Evaluation of Agronomic Traits for Yield and Yield Components in Wheat Genotypes with Respect To Planting Dates. Malaysian Journal of Sustainable Agriculture. 2(1), 7-11.

Lamas M.I., Rodriguez C.G., Rodriguez J.D. and Telmo J. (2016). Numerical Model of So2 Scrubbing with Seawater Applied to Marine Engines. Polish Maritime Research, 23 (2), 42-47.

Lin J.H., Xiao H. and Ma S.H. (2014). Verification of Shelf Life of Aspergillus niger Spore Suspension. Drug Standards of China, 15 (2), 117-119.

Liu X., Li J. and Liu K.W. (2004). Catalytic Effect of Aspergillus niger Acid Protease in Vinegar Brewing. Chemical Research and Application, 16 (4), 482-484.

Mi C., Shen Y., Mi W.J. and Huang Y.F. (2015). Ship Identification Algorithm Based on 3D Point Cloud for Automated Ship Loaders. Journal of Coastal Research, (73), 28-34.

Mi C., Zhang Z.W. and Huang Y.F. (2016). A Fast-Automated Vision System for Container Corner Casting Recognition. Journal of Marine Science and Technology, 24 (1), 54-60.

Nongqwenga N., and Modi A.T. (2017). Phosphorus and Potassium Quantity/Intensity Properties of Selected South African Soils (Kwazulu-Natal) and Their Correlation with Selected Soil Parameters. Applied Ecology and Environmental Research, 15 (3), 1-14.

Quan J.X. (2011). Resourceized Disposal and Comprehensive Utilization of Urban Kitchen Waste. Technology Innovation and Productivity, 214, 69-73.

Shamsudin S.B., Marzuki A., Jeffree M.S. and Lukman K.A. (2017). Blood lead concentration and working memory ability on malay primary school children in urban and rural area, Malacca. Acta Scientifica Malaysia. 1(1), 04-07.

Sharifuzzaman M.D., Lemckert C.D. and Etemad-Shahidi A. (2017). Dynamics of lock-release crystalline gravity currents. Geology, Ecology, and Landscapes, 1(4), 213-218.

Shen P., Fan X.R. and Li G.W. (1999). Microbiology Experiment. Beijing: Higher Education Press, 214-215.

Wahab W.A. and Adzmi A.N. (2017). The Investigation of Cytotoxic Effect of Cinnamomum Zeylanicum Extracts on Human Breast Cancer Cell Line (Mcf-7). Science Heritage Journal. 1(2), 21-26.

Wang Q.H., Ma H.Z. and Wang X.M. (2004). Recycling Technology of Kitchen Waste. Modern Chemical Industry, 24 (7), 56-59.

Wang X.G., Zhang A.Q. and Yu L.P. (1993). Study on the Application of Acid Protease in Soy Sauce Brewing. Biotechnology, 3 (4), 26-29+21.

Xie B.F., Cao Z.Y. and Zheng T. (2005). Purification and Characterization of Acid Protease from Aspergillus niger SL2-111. Chinese Journal of Applied and Environmental Biology, 11 (5), 618-622.

Xu C.Y., Song W. and Zhao S.Q. (2011). Study on Homologous Pollution of Kitchen Waste Feed Processing Technology. Environmental Sanitation Engineering, 19 (1), 9-10.

Yang A., Han Y., Pan Y., Xing H. and Li J. (2017). Optimum surface roughness prediction for titanium alloy by adopting response surface methodology. Results in Physics 7, 1046-1050. doi: 10.1016/j.rinp.2017.02.027.

Ye Q.X., Wei X.L. and Lin L.Y. (2015). Process Plan for Resourceized Utilization of Kitchen Waste. China Environmental Protection Industry, (10), 42-45.

Yu C.Z., Dong X.K. and Wang Q.J. (2006). Study on the Effect of Acid Protease 537 on Leather Extensibility of Sheep Wet Blue. China Leather, 35 (9), 16-20+25.

Yu P.H., Chua H. and Huang A.L. (1999). Transformation of industrial food wastes into polyhydroxyalkanoates. Water Science and Technology, 40 (1), 365-370.

Zhang A.M. and Zhou H.T. (2011). Guide Book for Food Science and Engineering Experiment Internship. Beijing: Beijing Normal University Press, 19-21.

Zhang X.F., Liu D.Y. and Xia H. (2009). Application of Acid Protease in Sweet Rice Wine Production. China Brewing, (2), 22-124.

Zheng B.D. (2006). Food Enzymology. Nanjing: Southeast University Press, 18-19.