JOURNAL OF PURE AND APPLIED MICROBIOLOGY, May 2014. Vol. **8**(Spl. Edn. 1), p. 97-103

Experimental Study on the Characters of Wastewater Treated by Microorganisms and its Agricultural Recycling

Maomao Hou[1], Xiaohou Shao[1], Shaojie Pu[2], Jing Chen[1] and Chunfang Wang[1]

[1]College of Water Conservancy and Hydropower, Hohai University, Nanjing 210098, China.
[2]Division of Urban and Rural Science and Technology Development,
Nanjing Municipal Science and Technology Commission, Nanjing 210019)
Postal address: Hohai University, Xi Kang Road No. 1, Gulou District, Nanjing210098, China.

(Received: 13 January 2014; accepted: 28 March 2014)

Effective microorganism (EM) technique is a preferable means on wastes treatment, and could promote to stimulate plant growth and soil fertility in agriculture. In this study, EM stoste was enriched by adding molasses and distilled water, and the wastewaters were treated with different EM stoste treatments and EM enrichment treatments, then the EM-treated wastewaters were used for the irrigation of tomatoes. The indexes including vitamin C, soluble protein, soluble sugar and nitrate were used to evaluate the tomato quality, and projection pursuit model was introduced to select the optimal scheme for wastewater treatments. Results showed that: (1) T3 was supposed to be the recommended enrichment scheme, proportion of which was 6% EM stoste, 6% molasses and 88% distilled water, (2) EM enrichment solution had more significant effects in decreasing the COD_{Cr} value of wastewaters since which enhanced harmony and adaptation of microorganisms, and S1 (600ml wastewater, 200ml activated sludge, 10ml EM enrichment solution, and under aeration condition) was preferable for the degradation of COD_{Cr} in wastewaters, (3) Dynamics of tomato plant height with different EM-treated wastewater treatments could be well presented by the exponential models, and R^2 reached a considerably high value of 0.9707- 0.9909, (4) According to the analysis of Vitamin C, soluble protein, soluble sugar and nitrate in tomato fruits with different schemes, S1 and S2 scheme appeared to obtain the tomatoes with better quality, (5) S1 was recommended as the optimal scheme based on the analysis of comprehensive indexes, indicating a preferable result in not only the treatment of wastewaters but also the cultivation of tomatoes. The study conclusions could provide practical basis for biological processing techniques of wastewater and the reutilization of wastewater in agricultural production.

Keywords: EM, wastewater, tomato, cultivation, enrichment.

EM (effective microorganism) is a combination of specially selected microorganism capable of producing multiple benefits, including predominant populations of lactic acid bacteria, yeasts, and smaller numbers of PSB, actinomycetes and other types of organisms [1-2], and they can quickly decompose organic matter, metabolize antioxidant substances and inhibit the proliferation of harmful microorganisms [3]. Japan was the country of origin in producing EM, the explorations and studies in the application of EM in wastewater treatments were also firstly carried out in Japan, for treating sewage water, industrial wastewater and eutrophication water [4-5]. The advantages of EM including: improving the bad smell of H_2S and biogas; obtaining great ability in water treatments; stabilizing the temperature of waters; decreasing the sludge quantity by 1/2 to 1/3; and reducing the aeration time. There were also studies about the application of EM on agricultural production [6-9]. EM was supposed to have positive effects on reforming soil nematode community structure,

* To whom all correspondence should be addressed.
E-mail: wuduguer0407@126.com

increasing crop yield and improving soil properties. However, rare studies were reported about using EM-treated wastewater to cultivate vegetables. In this study, the wastewaters were treated by different EM treatments, and the EM-treated wastewaters were applied for tomato cultivation, in order to discover the regularity of the response of wastewaters to EM treatments, and to select a scheme with best comprehensive benefits.

MATERIALS AND METHODS

Experimental Site

The experiments were initiated from May to October in 2012 at Vegetables and Flowers Institute of Jiangning (latitude 31°43' N, longitude 118°46'E), Nanjing, China. The average annual rainfall is about 1106.5mm, with the rainy season from the end of June to the middle of July, and the average yearly temperature is approximately 15.7! and average humidity is about 81%.

Materials

The EM stoste and the molasses were bought from A.M.L limited company [10], Nanjing branch; the wastewaters were derived from Liuhe Wastewater Treatment Plant, Nanjing, China; the tomato variety "*21st Century Crown*" was chosen as plant materials, on June 16th the six week old tomato seedlings were transplanted to the experiment fields, after that, conventional field managements were carried out fairly among the treatments.

Experimental Design

The experiments were divided into two components: lab experiment and field experiment. Irrigation waters used in field experiment were the EM-treated wastewaters from the lab experiment.

Lab experiment

EM stoste was mixed with molasses, distilled water for enrichment cultivation, and the different schemes were shown in Table. 1. After mixing homogeneously, these sealed EM-Calcium mixtures would be transferred to a orbital shaker with 37! constant temperature fermenting 3-6 days. Then the best EM enrichment scheme would be applied for the wastewater degradation based on the cost and eventual PH of the solutions. There were two operation conditions during the degradation: aeration and non-aeration, and the wastewaters were added with EM, EM enrichment solution and activated sludge, the detailed schemes for wastewater degradation were shown as Table. 2.

Field experiment

Prior to the field treatment, soil characters were: organic matter 14.34 g kg^{-1}, available nitrogen 104.17 ppm, available phosphorus 26.48 ppm, available potassium 184.70 ppm. Tomato seedlings were arranged as Fig. 1, several soil ridges were made to provide a suitable growing condition for tomatoes, the ridge was 60cm wide and 100cm apart, and about 6cm above the bare soil, two line tomato seedlings were transplanted in one ridge with a 40cm distance between them. Every 12 tomato seedlings were planted as a treatment in a 220cm×60cm block. Irrigation and fertilizer scheme of the treatments was accorded with the local farming practices during the tomato growth period. The only difference among field experimental treatments was the irrigation water variety, which was applied based on Table. 2, so there were 8 treatments for the tomatoes and each treatment had 3 replications.

Main tested index and methods

At tomato maturity, 2 marketable tomato fruits were harvested from one plant, and about 10 g tomato flesh per fruit was taken along the longitudinal axis then homogenized for the following measurements: Vitamin C content was measured by the 2, 6- dichloroindophenol titrimetric method [11]; soluble sugar was measured by the anthrone method [12]; soluble protein was measured by the Coomassie brilliant blue method [13]; nitrate content was measured by the ultraviolet spectrophotometry method [14-15].

RESULTS AND DISCUSSION

EM enrichment cultivation

The enrichment was supposed to be successful when the PH of solution decreased to 3.5 or below 3.5, according to this rule, 2% and 4% EM stoste was failure to enrich the microorganisms; this suggested that the enrichment of EM stoste needed adequate nutrients. On the other hand, PH of T3, T4 and T5 decreased to a satisfactory value, and the more EM stoste and molasses supplied, the faster the PH declined. However, the cost of T5 scheme was considerably high and hard to put into practice, with the comprehensive consideration, T3 was chosen as the optimal enrichment scheme and

Table 1. Experimental scheme on EM enrichment cultivation

Treatment	EM stoste (%)	Molasses (%)	Distilled water (%)
T1	2	2	96
T2	4	4	92
T3	6	6	88
T4	8	8	84
T5	10	10	80

Table 2. Experimental scheme on wastewater degradation

Treatment	Wastewater(ml)	Activated sludge (ml)	EM(ml)	Operation condition
CK1	800	0	10 (EM*)	Aeration
CK2	800	0	10 (EM*)	Non-aeration
S1	600	200	10 (EM*)	Aeration
S2	600	200	10 (EM*)	Non-aeration
S3	600	200	10 (EM)	Aeration
S4	600	200	10 (EM)	Non-aeration
S5	600	200	0	Aeration
S6	600	200	0	Non-aeration

Note: EM* represented the EM enrichment solution; EM represented the EM stoste.

Table 3. COD_{Cr} values of EM-treated wastewaters (mg/l)

Treatment	Original wastewater	Days after degradation treatment (d)			
		1	2	3	4
CK1	1742.8	2078.4	704.3	547.2	312.9
CK2	1742.8	2078.4	1867.5	1596.2	1477.6
S1	1312.6	1678.5	588.9	212.8	101.2
S2	1312.6	1678.5	1204.7	1026.7	939.9
S3	1312.6	1622.3	654.6	294.8	204.2
S4	1312.6	1622.3	1311.2	1127.4	997.6
S5	1312.6	1312.6	611.4	339.5	273.1
S6	1312.6	1312.6	1256.7	1099.3	1015.2

Table 4. Simulation models of tomato plant height

Treatment	Simulation models	R^2
CK1	$y = exp\ (-0.0003x^2 + 0.0649x + 2.0067)$	0.9725
CK2	$y = exp\ (-0.0876x^2 + 1.0138x + 1.9941)$	0.9802
S1	$y = exp\ (-0.0005x^2 + 0.0755x + 1.9081)$	0.9901
S2	$y = exp\ (-0.0004x^2 + 0.0666x + 1.9905)$	0.9756
S3	$y = exp\ (-0.0004x^2 + 0.0709x + 1.9228)$	0.9707
S4	$y = exp\ (-0.0005x^2 + 0.0788x + 1.8727)$	0.9909
S5	$y = exp\ (-0.0004x^2 + 0.0739x + 1.9114)$	0.9822
S6	$y = exp\ (-0.0005x^2 + 0.0770x + 1.8748)$	0.9829

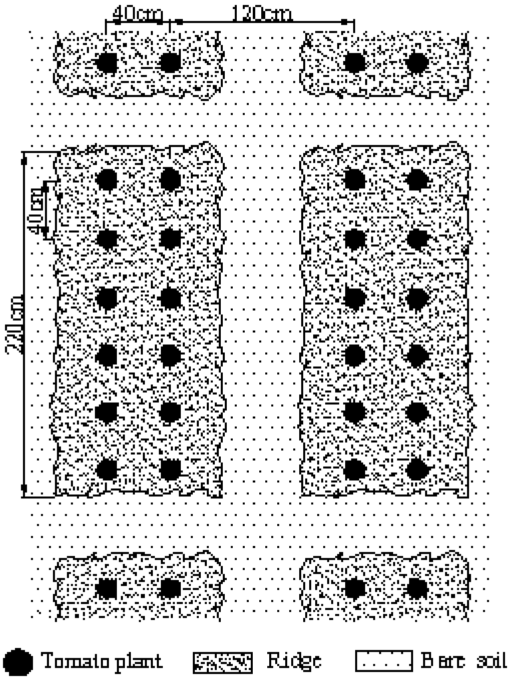

Fig. 1. The arrangement of tomato seedlings

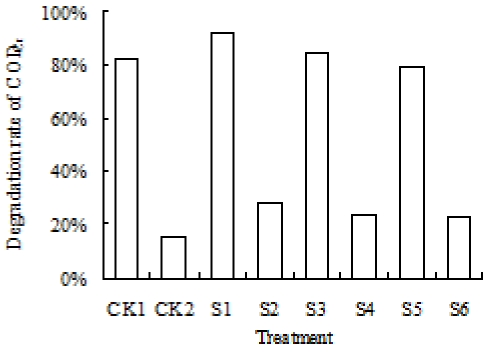

Fig. 2. Degradation rate of COD_{Cr} in wastewaters

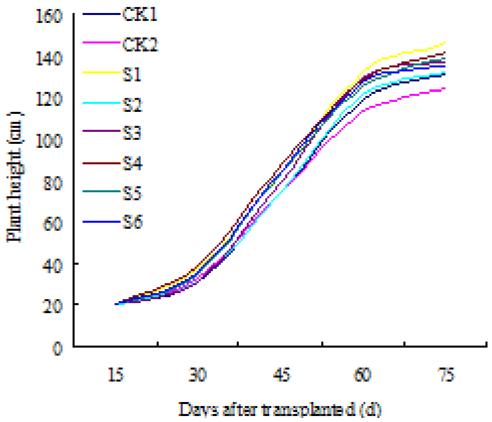

Fig. 3. Tomato plant height varying with days after transplanted

applied to the wastewater degradation although it needed longer time for cultivation.

COD_{Cr} values of EM-treated wastewaters

Table. 3 gave the dynamics of COD_{Cr} values of EM-treated wastewaters varying with days after treatments. According to the results, all treatments detected a decline in COD_{Cr} value, and the COD_{Cr} value of aeration treatments obtained much better effects. Compared with EM stoste treatments (S3, S4), the EM enrichment treatments (S1, S2) appeared to achieve preferable results, this implied that after the enrichment, original microorganisms were more adaptable and harmonious. Besides, degradation effects of treatments added with activated sludge were better than that of CK1 and CK2. To be more intuitive, the degradation rate of COD_{Cr} value in EM-treated wastewaters was shown in Fig. 2, great differences of COD_{Cr} degradation rate were found between aeration treatments and non-aeration treatments.

Dynamics and simulations of tomato plant height with EM-treated wastewater irrigation

Fig. 3 showed the tomato plant height varying with days after transplanted, on the whole, the changes could be divided into 3 stages: 15-30d was the slower growth stage; 30-60d was the faster growth stage; 60-70d was the slower growth stage again. The increasing tendency of plant height was similar among the treatments, and S1 got the highest tomato plant height of 146cm, indicating a better utilization result in agricultural production.

Simulation models of plant height dynamics were shown in Table. 4. The tomato plant height (y) presented exponential relationship with days after transplanted (x), and R^2 reached a considerably high value of 0.9707-0.9909.

Tomato quality with EM-treated wastewater irrigation

Human body can not compose vitamin C itself, vegetables are the main resources of absorbing Vitamin C for human bodies, and meanwhile, vitamin C is an important index to evaluate the vegetable quality [16-17]. Fig. 4 showed the main quality indexes and their values, and Fig. a showed the vitamin C in tomato fruits with different EM-treated wastewater treatments. Vitamin C content of S1 was higher than that of other treatments, which of S6 was the lowest, and significant difference was found among S1, S5 and S6.

Soluble protein has important physiological function; many studies showed that soluble protein had important relationship with the entire taste and flavor[18]. Fig. b gave the soluble protein content with different treatments, which of S2 and S6 was slightly higher than that of the other treatments, and CK1 detected the lowest value of soluble protein.

Soluble sugar combined with soluble sugar affects the taste significantly, and the content of soluble sugar is an important factor for the whole index system of tomato quality [15, 19]. According to Fig. c, soluble sugar content of S2 was significantly higher than that of CK1, CK2, S5 and S6, while no significant difference was found among S1, S2, S3, and S4.

NO^{3-}-N could be converted to NO^{2-}-N under the anaerobic conditions, and the NO^{2-}-N would make the Ferrum lose oxidation capacity, resulting in the anaerobic poisoning, meanwhile, NO^{2-}-N could react with amine derivatives, producing nitrosoamines—-a strong carcinogenic substance, thus the nitrate study had been a hot topic which caused a lot of attentions for many scholars [20]. In comparison according to Fig. d, S1, S2 and S3 appeared to obtain better values of nitrate.

Selection of optimal scheme on wastewater treatment

In order to select a scheme of wastewater treatment with the optimal comprehensive benefits, 6 main evaluation indexes were chosen, they were "COD_{Cr} degradation rate", "tomato plant height", "vitamin C", "soluble sugar", "soluble protein" and "nitrate", the value of these indexes was the higher the better except the index "nitrate". Projection pursuit classification model (PP) was built by Matlab 7.1, and RAGA was used to optimize the PP method. Before the course of optimization, the main parameters were set as: the original population

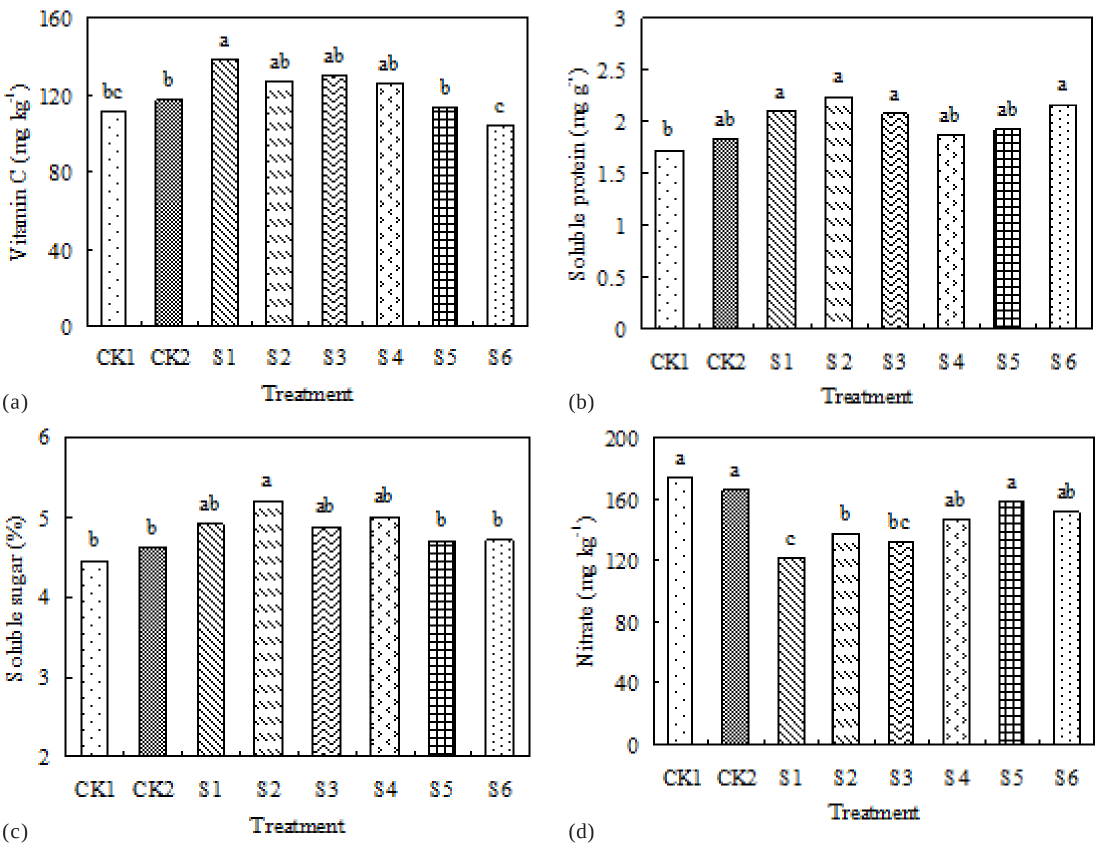

Fig. 4. Tomato quality indexes with different wastewater treatments. Columns with the same letter represent values that are not significantly different at the 0.05 level of probability according to the Duncan's multiple range test

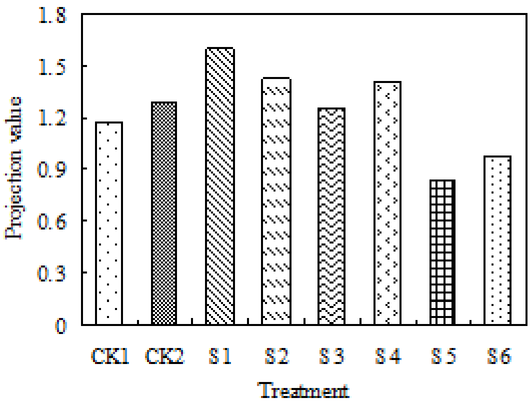

Fig. 5. Projection value of different wastewater treatments calculated by the projection pursuit model

size n=400; the probabilities of crossover Pc=0.8; the probabilities of mutation Pm=0.8; number of excellent individuals was 20; ±=0.05; accelerating 20 times [21-22]. According to the model's calculations, the projection value of CK1, CK2, S1, S2, S3, S4, S5, S6 was ordered to be $z_{(i)}*$= (1.172, 1.288, 1.599, 1.428, 1.254, 1.398, 0.834, 0.977), as was shown in Fig. 5. By the rules of projection value "the larger the better", S1 scheme was selected as the best scheme for wastewater treatment in this experiment.

CONCLUSIONS

Through above analysis, the conclusions could be obtained that:

1. The enrichment of EM stoste needed adequate nutrients. In comprehensive consideration of the cost and the PH of enrichment solution, T3 was supposed to be the recommended enrichment scheme, proportion of which was 6% EM stoste, 6% molasses and 88% distilled water.
2. Compared to EM stoste, EM enrichment solution had more significant effects in decreasing the COD_{Cr} value of wastewaters since which enhanced harmony and adaptation of microorganisms. Aeration treatments achieved obviously better results in wastewater treatments in comparison with non-aeration treatments. Besides, adding activated sludge was effective for COD_{Cr} degradation. On the whole, S1 (600ml wastewater, 200ml activated sludge, 10ml EM enrichment solution, and under aeration condition) was preferable for the degradation of COD_{Cr} in wastewaters.
3. Dynamics of tomato plant height with different EM-treated wastewater treatments could be well presented by the exponential models, and R^2 reached a considerably high value of 0.9707- 0.9909.
4. Different wastewater treatments had different effects on tomato quality indexes. According to the analysis of Vitamin C, soluble protein, soluble sugar and nitrate in tomato fruits with different schemes, S1 and S2 appeared to obtain the tomatoes with better quality.
5. According to the calculations of projection pursuit model, S1 was recommended as the optimal scheme based on the analysis of comprehensive indexes, indicating a preferable result in not only the treatment of wastewaters but also the cultivation of tomatoes.

ACKNOWLEDGEMENTS

This work was financed by the fund of the Ministry of Water Resources Public Welfare Project (201301017), Natural Science Foundation of China (51179054), Creative Funds of Jiangsu province (CXZZ13_0266) and state-supporting technology project of China (2012BAB03B03).

REFERENCES

1. Zhou Q, Li K, Jun X, Bo L., Role and functions of beneficial microorganisms in sustainable aquaculture. *Bioresource Technology*, 2009; **100**: 3780-3786.
2. Qiu Z, Liu Y, Yang L, Wu M, Wang Y., Treatment of landfill leachate by effective microorganisms. *International Journal of Environment and Pollution*, 2009; **38**: 39-47.
3. Higa T., Great revolution to save the earth in Chinese edition. China Agriculture Publisher University, 1997.
4. Zakaria Z, Gairola S, Shariff N M. Effective microorganisms (EM) technology for water quality restoration and potential for sustainable water resources and management; proceedings of the 5th Biennial Conference of the International Environmental Modelling and Software Society: Modelling for Environment's Sake, iEMSs 2010,

July 5, 2010 - July 8, 2010, Ottawa, ON, Canada, F, 2010 [C]. iEMSs Secretariat c/- IDSIA.
5. Zhou S, Wei C, Liao C, Wu H., Damage to DNA of effective microorganisms by heavy metals: Impact on wastewater treatment. *Journal of Environmental Sciences*, 2008; **20**: 1514-1518.
6. Daly M J, Stewart D P C, Influence of "Effective Microorganisms" (EM) on Vegetable Production and Carbon Mineralization–A Preliminary Investigation. *Journal of Sustainable Agriculture*, 1999; **14**: 15-25.
7. Daming R., Studies on the action mechanism of effective microorganisms in crop production. *Journal of maize science in China*, 1999; **7**: 62-64.
8. Hu C, Qi Y., Long-term effective microorganisms application promote growth and increase yields and nutrition of wheat in China. European Journal of Agronomy, 2013; **46**: 63-67.
9. Hu C, Qi Y., Effective microorganisms and compost favor nematodes in wheat crops. Agron Sustain Dev: 2013; 1-7.
10. Zhou Y X, Zhen S C, Zhai Y M, The application of EM-treated dairy effluent on the flue-cured tobacco irrigation. *J Food Agric Environ*, 2013; **11**: 960-964.
11. Lima-Silva V, Rosado A, Amorim-Silva V, Muñoz-Mérida A, Pons C, Bombarely A., Genetic and genome-wide transcriptomic analyses identify co-regulation of oxidative response and hormone transcript abundance with vitamin C content in tomato fruit. BMC Genomics, 2012; **13**: 1-15.
12. Yang L, Qu H, Zhang Y, Li F., Effects of partial root-zone irrigation on physiology, fruit yield and quality and water use efficiency of tomato under different calcium levels. *Agricultural Water Management,* 2012; **104**: 89-94.
13. Li J, Wang P, Li J., Effect of irrigation amount on physiology, biochemistry and fruit quality of greenhouse tomato under sub-low temperatures. Nongye Gongcheng Xuebao/ Transactions of the Chinese Society of Agricultural Engineering,2010; **26**: 129-134.
14. Murillo-Amador B, Jones H G, Kaya C, Aguilar R L, García-Hernández J L, Troyo-Diéguez E., Effects of foliar application of calcium nitrate on growth and physiological attributes of cowpea (Vigna unguiculata L. Walp.) grown under salt stress. Environmental and Experimental Botany, 2006; **58**: 188-196.
15. Wang F, Du T, Qiu R. 2011. Deficit irrigation scheduling of greenhouse tomato based on quality principle component analysis. Nongye Gongcheng Xuebao/Transactions of the Chinese Society of Agricultural Engineering, 27: 75-80.
16. Eze J I, Ojike O., Studies on the effect of different solar dryers on the vitamin content of tomato (*Solanum lycopersicon*). *Journal of Renewable and Sustainable Energy,* 2012; 4.
17. Khan I, Azam A, Mahmood A., The impact of enhanced atmospheric carbon dioxide on yield, proximate composition, elemental concentration, fatty acid and vitamin C contents of tomato (Lycopersicon esculentum). *Environmental Monitoring and Assessment*, 2013; **185**: 205-214.
18. Yinli L, Inosako K, Yamada S, Yanmei B, Xingjun L, Zexiu W. The correlation between microenvironment and tomato fruit quality in greenhouse; proceedings of the International Conference on Consumer Electronics, Communications and Networks, CECNet 2011, April 16, 2011 - April 18, 2011, XianNing, China, F, 2011 [C]. IEEE Computer Society, 2011 .
19. Balibrea M E, Martinez-Andujar C, Cuartero J, Bolarin M C, Perez-Alfocea F., The high fruit soluble sugar content in wild Lycopersicon species and their hybrids with cultivars depends on sucrose import during ripening rather than on sucrose metabolism. *Functional Plant Biology*, 2006; **33**: 279-288.
20. Flores P, Botella M A, Cerda A, Martinez V., Influence of nitrate level on nitrate assimilation in tomato (Lycopersicon esculentum) plants under saline stress. Canadian Journal of Botany, 2004; **82**: 207-213.
21. Hou M M, Shao X H, Chen L H, Chang T T, Wang W N, Wang Y F., Study on fertilizer N leaching, accumulation, and balance in tobacco fields with N-15 tracing technique. *J Food Agric Environ*, 2012; **10**: 1284-1289.
22. Shao X H, Hou M M, Chen L H, Chang T T, Wang W N., Evaluation of Subsurface Drainage Design Based on Projection Pursuit. Energy Procedia, 2012; **16**: Part B: 747-752.