

# Effect of a residue after evaporation from industrial vitamin C fermentation on chemical and microbial properties of alkali-saline soil

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**Abstract:** Residue after evaporation (RAE) from industrial vitamin C fermentation is emitted as a waste product at an amount of 60,000 tons per year in China. The disposal of RAE is difficult because of its high chemical oxygen demand ( $1.17 \times 10^6$  mg/l) and low pH (0.27). We hypothesized that RAE could be used as an ameliorant for alkali-saline soils, and tried to verify it by carrying out a pot experiment of pakchoi cultivation and to explore its effect on soil chemical and microbial properties. The results showed that pakchoi yield was increased by 28.13% and pakchoi quality was also enhanced under RAE treatment. The improved chemical and microbial properties of treated soil were also observed: soil pH was decreased from 9.19 to 9.03; total organic carbon, available phosphorus and available potassium were increased by 49.15%, 34.91% and 42.02%, respectively; number of culturable bacteria, actinomycetes and fungi, microbial biomass carbon and enzyme activity number were improved by 52.97%, 104.05%, 79.09%, 57.82% and 31.16%, respectively. These results suggested the residue application led to an improved soil quality and subsequently a higher yield and quality of pakchoi. This study provided a strong evidence for the feasibility of RAE as an ameliorant for alkali-saline soil.

**Keywords:** Residue after evaporation, vitamin C fermentation, alkali-saline soils, chemical properties, microbial functional diversity.

## INTRODUCTION

Residue after evaporation (RAE) of fermentation liquid from vitamin C industry is remnant liquid at kettle bottom in 2-keto-L-gulonic acid (2-KGA) fermentation process after steps of centrifugation, microfiltration, ion exchange, concentration under vapouring and crystallization. As the biggest vitamin C producing country, China produces annually about 100,000 tons vitamin C and about 60,000 tons RAE are emitted concomitantly as a waste-product. The disposal of RAE is very difficult because of its high COD value ( $1.17 \times 10^6$  mg/l) and low pH value (0.27). The current treatment method of RAE, in which RAE is employed as a raw material of oxalic acid production, will be abandoned soon due to few effective components in RAE, high cost of production process and secondary pollution. Another option is burying or incineration, which leads to a considerable waste disposal cost (about \$ 120/ton) and a problem of consequential environmental pollution. Therefore, how to deal sustainably with RAE becomes a big challenge to vitamin C industry.

It was estimated that there were 950 million hectares of alkali-saline lands globally (Sumner and Naidu, 1998). In China, the area of alkali-saline lands is about 36 million hectares, occupying 4.88% of available lands (Yang, 2008). Due to its poor fertility and physical properties, the alkali-saline soils normally show a low or even no crop

yield. Under the pressures of the increased population and the limited agricultural land resources, amelioration and utilization of alkali-saline lands are in urgent need in China.

Because of abundant organic acids in RAE (such as 2-KGA, formic acid, oxalic acid, and butylcarboxylic acid, etc. table 1), which should achieve both of alkali neutralization and organic matter improvement of alkali-saline soils, we speculated that RAE could be used as a potential ameliorant for alkali-saline soils. However, to our best knowledge, there is no report on this till now. Therefore, in this study, we try to verify this hypothesis by culturing pakchoi under RAE treatment in a pot experiment and to explore its effect on soil chemical and microbial properties. If RAE could be successfully used as an efficient ameliorant, it would lead to win-win benefits of both environmental sustainability and agricultural development.

## MATERIAL AND METHODS

### *RAE and soil tested*

The RAE was collected from Northeast Pharmaceutical Group Co., Ltd., China, and its basic chemical properties of RAE are shown in table 1. The RAE is a water-soluble thick liquid with a high COD value ( $1.17 \times 10^6$  mg/l) and a low pH value (0.27). The tested soil was sampled from 0-20 cm depth of an alkali-saline land in a coastal area of Dawa county, Panjin city, China (40°55'N, 121°59' E). It

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is a clay loam, classified as Halaquepts according to US Soil Taxonomy (Soil Survey Staff, 1998). The pH (soil: H<sub>2</sub>O=1:2.5) was 9.21, electrical conductivity (soil: H<sub>2</sub>O=1:5) was 1.67 dS/m and other basic properties of this coastal alkali-saline soil were shown in table 2. Before pot cultivation, the soil sample was air dried, crushed to pass a 2-mm sieve after removing visible roots and crop residues.

#### Pot cultivation of pakchoi

Experiment of pot pakchoi cultivation was carried out from May 5, 2012 to July 20, 2012. One and a half kilograms of soil (dry weight) was placed in cylindrical plastic pots. There were two treatments (five replicates for each): 1) RAE treatment of pot cultivation applied with RAE solution; 2) control treatment applied with tap water. During the period of pre-incubation for 2.5-months before seeds of pakchoi (*Brassica rapa L. ssp. chinensis*) were sowed, 75 ml RAE solution (diluted with tap water in a ratio of 1:60) was added to the soil in each pot of the RAE treatment twice a week. After sowing of pakchoi, 75 ml RAE solution (diluted with water in a ratio of 1:250) was irrigated to each pot twice a week in the RAE treatment. Tap water was substitute for RAE solution in the control treatment, as well as in the pre-incubation. The seedlings were thinned to 10 strains per pot when their height was about 2 cm.

#### Sampling and measurements

After 75 days of cultivation, whole plant of pakchoi was harvested, washed and weighed. Indices of pakchoi quality, i.e. vitamin C, soluble sugar, protein and chlorophyll contents in leaf were determined according to the reported procedures (Zhang, 2003). At the same time, the soil in each pot was collected, homogenized and divided into 2 portions. One portion was air-dried and screened through a 2 mm sieve for analyzing chemical characteristics, another portion was stored at 4° for determining microbial characteristics.

Soil pH (soil: H<sub>2</sub>O = 1:2.5) and electrical conductivity (EC) (soil: H<sub>2</sub>O = 1:5) were measured with an electrical pH meter and a conductivity meter, respectively. Cation exchange capacity, exchangeable sodium percentage (ESP), total organic carbon, total nitrogen, total phosphorus, available phosphorus, total potassium and available potassium were determined according to the reported methods (Okalebo *et al.*, 1993). Enumeration of culturable bacteria, fungi, and actinomycetes were performed using the soil dilution plate method (Nair and Rao, 1977). The media used were nutrient agar (for bacteria) (Allen, 1959), Martin's Rose Bengal agar (for fungi) (Martin, 1950), and Gauze 1 meidium (for actinomycetes) (Gauze *et al.*, 1983). Microbial biomass carbon was determined using chloroform fumigation extraction method (Vance *et al.*, 1987). Activities of soil enzymes, i.e. dehydrogenase (DH), alkaline phosphatase (AP), urease (UR), catalase (CA), protease (PR), amylase

(AM) and invertase (IN), were determined according to the reported procedures (Cole, 1977; Frankenberger and Johanson, 1983; Tabatabai, 1982). Enzyme activity number (EAN) index was calculated as  $EAN = 0.2 \times (0.15 \times DH + CA + 1.25 \times 10^{-5} \times AP + 4 \times 10^{-2} \times PR + 6 \times 10^{-4} \times AM)$  (Riffaldi *et al.*, 2002). Community level physiological profiles (CLPP) was determined using a MicroResp™ method (Campbell *et al.*, 2003). Microbial functional diversity was evaluated by Shannon-Weaver index (H'):  $H' = -\sum P_i (\ln P_i)$ , where P<sub>i</sub> is the ratio of the activity of a particular substrate to the sum of the activities of all tested substrates.

## RESULTS

#### Yield and quality of pakchoi

Significant enhancements were observed in the height, leaf width and root length of pakchoi (increased by 7.22%, 5.44% and 17.38% respectively in the RAE treatment). Thereafter, pakchoi yield was significantly increased by 28.13%, from 9.74 g pot<sup>-1</sup> in the control to 12.48 g pot<sup>-1</sup> in the RAE treated soil. Despite the contents of chlorophyll and protein of pakchoi were not increased, the contents of vitamin C and soluble sugar in pakchoi were significantly increased by 55.64% and 21.85%, respectively (table 3), which indicated a higher quality of pakchoi. Therefore, both yield and quality of pakchoi were improved by applying RAE to alkaline-saline soil.

**Table 1:** Chemical properties of RAE

Indices	Value
pH	0.27
COD (mg/l)	1.17×10 <sup>6</sup>
Total organic C (g/l)	175.5
Total N (g/l)	4.77
Available N (mg/l)	114.2
Total P (g/l)	0.19
Available P (mg/l)	4.40
Total K (g/l)	2.02
Available K (mg/l)	149.6
2-KGA (g/l)	202.6
Oxalic acid (g/l)	27.57
Formic acid (g/l)	3.34
Butylcarboxylic acid (g/l)	0.40

COD : Chemical O<sub>2</sub> demand

#### Chemical properties of soil

Chemical properties of the soils under RAE and control treatments were shown in table 4. The addition of RAE decreased the soil pH from 9.19 to 9.03, but no alteration of electrical conductivity, cation exchange capacity and exchangeable sodium percentage was found. Total organic C, total N, available P and available K content in soil, which are important soil indices to plant growth,

were increased by 49.15%, 52.21%, 11.86%, 34.91% and 42.02%, respectively, when compared to those of control treatment. However, no significant difference of available N, total P and total K of soil between two treatments was observed.

**Table 2:** Properties of tested soil

Indices	Value
Sand (2-0.05 mm) (%)	24.75
Silt (0.05-0.002 mm) (%)	37.17
Clay (<0.002 mm) (%)	38.08
pH (soil/water = 1:2.5)	9.21
EC (dS/m)	1.67
CEC (cmol/kg)	38.56
Soil bulk density (g/cm <sup>3</sup> )	1.67
Total organic C (g/kg)	7.28
Total N (g/kg)	0.60
Total P (g/kg)	0.44
Total K (g/kg)	13.06

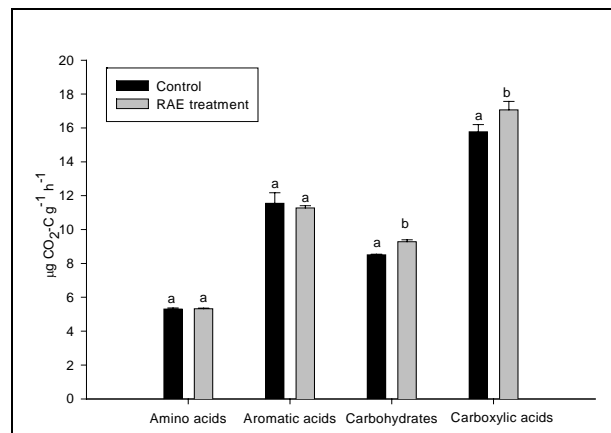
EC : Electrical conductivity

Enzyme activities of the amended soil were also stimulated by RAE addition. Table 5 showed that dehydrogenase, catalase, alkaline phosphatase, amylase, invertase and protease activities of the soil were enhanced significantly by 23.68%, 14.08%, 44.74%, 40.14%, 24.97% and 32.41%, respectively. However, the urease activity did not alter significantly. Enzyme activity number (EAN), an integrated index of soil enzyme activities were increased significantly by 31.16%.

#### Community level physiological profiles (CLPP) of the soil

Substrate utilization patterns of soil microbial communities were described by community level physiological profiles (CLPP) in this study. Mean C substrate utilization rate for the 15 tested substrates of soil in the RAE treatment was 9.73  $\mu\text{g CO}_2\text{-C/g/h}$ , which was increased by 5.99% significantly, compared to that of control (9.18  $\mu\text{g CO}_2\text{-C/g/h}$ ). However, Shannon diversity did not change significantly (table 6). Among these 15 C sources, L-malic acid, oxalic acid, D-fructose and  $\alpha$ -ketoglutaric acid were the carbon sources consumed preferentially by soil microbe in both treatments. Each of them accounted for more than 10% of the total utilization rate. Each of the microbial  $\text{CO}_2$  evolution induced by 3,4-dihydroxybenzoic acid, citric acid and trehalose, was about 5%-8% of the total induced respiration. While, the sum of the remained eight carbon sources made up less than 30% of the total induced respiration (table 6). In addition, among the four carbon resource groups, carboxylic acid was the highest consumed group, followed by aromatic acid, and then carbohydrate and amino acid. Consequently, the utilization rates of carboxylic acid and carbohydrate of soil with RAE application were enhanced significantly by 9.06% and

8.22%, while the acid did not differ significantly (fig. 1) utilization rate of aromatic acid and amino.



**Fig. 1:** The utilization rates of four carbon source groups in alkali-saline soil.

## DISCUSSION

In the pot experiment of this study, the significant improvements of both the pakchoi yield and the soil characteristics of the ameliorated soil by RAE application were observed. Here, we try to explore its effect on chemical and microbial characteristics of alkali-saline soil.

**Table 3:** Yield and quality indices of pakchoi under the treatments

Indices of pakchoi	Control	RAE treatment
Growth		
Height (cm)	8.86±0.54a	9.50±0.29b
RL (cm)	2.82±0.39a	3.31±0.17b
LW (cm)	3.31±0.10a	3.49±0.10b
Yield (g/pot)	9.74±1.06a	12.48±2.05b
Quality		
VC(mg/kg)	85.58±7.18a	133.2±8.63b
SS(mg/g)	11.99±1.43a	14.61±1.34b
Ch(mg/g)	0.44±0.08a	0.48±0.06a
SP(mg/g)	1.68±0.26a	1.91±0.21a

RL: Root length, LW: Leaf width.

VC: vitamin C, SS: soluble sugar.

Ch: Chlorophyll, SP: soluble protein

Values are the means of 5 replicated samples  $\pm$  SD. Values in the same row with different letters were significantly different ( $p < 0.05$ ).

#### Effects of RAE application on soil chemical properties

Despite there were increased crop yields in some cases, it is general that no decline in soil pH was found when organic amendments were applied to alkali-saline soil, such as vermicompost, farmyard manure and wheat straw (Goyal et al., 1999; Wu et al., 2013). On the contrary, application of chemical amendments, such as sulphuric acid (Sadiq et al., 2007), ferrous sulphate (Mahdy, 2011),

could decrease pH of alkali-saline soils, but without a supplement of organic matter to the soil. The results of this study showed us a slight, but statistically significant soil pH decline (from 9.19 to 9.03) after RAE application. Therefore, there existed two benefits from RAE application: soil pH decline and organic matter supply.

**Table 4:** Chemical characteristics of soil under the treatments

Indices	Control	RAE treatment
pH	9.19±0.06a	9.03±0.07b
EC	1.65±0.11a	1.70±0.15a
CEC	38.92±0.55a	39.25±0.20a
ESP	28.53±0.23a	28.63±0.29a
TOC	7.06±0.49a	10.53±0.19b
TN	0.59±0.04a	0.66±0.02b
AN	29.97±3.88a	29.59±5.93a
TP	0.44±0.07a	0.45±0.04a
AP	11.63±1.33a	15.69±3.39b
AK	573.12±53.36a	813.96±5.54b

EC: Electrical conductivity (dS/m)  
 CEC: Cation exchange capacity (cmol/kg)  
 ESP: Exchangeable sodium percentage (%)  
 TOC: Total organic C(g/kg), TN: Total N (g/kg)  
 AN: Available N(mg/kg), TP: Total P (g/kg)  
 AP: Available P(mg/kg), AK: Available K (mg/kg)

**Table 5:** Number of microbes, microbial biomass and enzyme activities of soil under the treatments

Indices	Control	RAE treatment
Microbes		
Bacteria	1.85±0.34a	2.83±0.18b
Fungi	2.20±0.39a	3.94±0.71b
ACT	3.21±0.65a	6.55±1.48b
MBC	124.06±24.47a	195.79±55.55b
MQ	1.74±0.29a	1.85±0.50a
Enzyme		
DH	0.38±0.01a	0.47±0.03b
CA	0.71±0.08a	0.81±0.04b
AP	198.11±24.79a	286.74±12.34b
AM	171.42±24.73a	240.23±36.28b
IN	7.85±1.14a	9.81±0.67b
UR	63.64±9.92a	59.04±6.47a
PR	151.2±11.0a	200.2±34.4b
EAN	1.38±0.09a	1.81±0.27b

Bacteria: (10<sup>7</sup> cfu/g), Fungi: (10<sup>4</sup> cfu/g)  
 ACT: Actinomycetes(10<sup>5</sup> cfu/g)  
 MBC: Microbial biomass C (mg/kg)  
 MQ: microbial quotient  
 DH: Dehydrogenase (µg TPF/g/h)

CA: Catalase (%O<sub>2</sub>/g/3min)  
 AP: Alkaline phosphatase (µg nitrophenol/g/h)  
 AM: Amylase (µg glucose/g /16h)  
 IN: Invertase (mg glucose/g/24h)  
 UR: Urease (µg NH<sub>4</sub>-N /g/2h)  
 PR: Protease (µg tyrosine /g /2h)  
 EAN: Enzyme activity number

The improved chemical properties of the ameliorated alkali-saline soil, especially organic C and total N, were observed in this study. It was due to the high concentrations of both carbon materials (e.g. 2-keto-L-gulonic acid, oxalic acid and sorbose, etc) and nitrogen materials (e.g. protein, polypeptide and nucleoside) in RAE. Available phosphorus and available potassium contents in soil were enhanced by 34.91% and 42.02% by RAE application, respectively. Previous studies showed that the contents of available phosphorus and available potassium in soil were not increased by application of some organic amendments, such as cattle dung, microbial fertilizer (Li *et al.*, 2011; Wu *et al.*, 2013). Other organic amendments, such as wasted culturing material of *Auricularia auricular* (Xie *et al.*, 2008), sewage sludge (Criquet *et al.*, 2007), were reported to increase the level of available phosphorus and potassium greatly due to abundance of these nutrients in them. Although the contents of available phosphorus and potassium in RAE were very low (table 1), their contents in the treated soil were significantly raised by RAE application. This was because that a high concentration of organic acids in RAE could reduced adsorption of phosphorus to the soil particle, then dissolved and activated the soil phosphorus (Fox *et al.*, 1990). Due to release of soil potassium resulted from acidic hydrolysis induced by organic acid (Song and Huang, 1988), the content of available potassium of soil with RAE was also enhanced. The improved chemical properties of soil under RAE treatment in this study, except soil pH, is in agreement with those studies on wastewater sludge (López-Valdez *et al.*, 2010) and livestock manure (Wu *et al.*, 2013).

**Effects of RAE application on soil microbial properties**

The increased culturable microbes number and microbial biomass C, the sensitive and potential index for characterizing the change of soil quality, were observed in this study. This was due to the higher concentrations of low-molecular-weight organic acids in RAE, which was usually considered to be the most important substrates for soil microbes (Berg, 2009; Schnurer *et al.*, 1985). The microbial quotient of soil (the proportion of soil microbial biomass carbon to total organic carbon), an indicator for carbon availability, could be increased through addition of organic matter, and was generally within the range of 1–5% (Jenkinson and Ladd, 1981). In our work, the microbial quotient of the treated soil was also within this range but relatively low, which indicated a lower microbial biomass in this soil compared to that in fertile soil. No significant increase in microbial quotient of the treated soil resulted from the increased total organic

carbon due to RAE incorporation, despite of the remarkable increased biomass carbon in it (table 5).

The increases in amylase and invertase activities, which are closely involved in carbon-cycle, may be explained by an increase in carbon availability and the subsequently improved microbial activities as a consequence of RAE incorporation. The increased microbial biomass carbon and enzyme activities induced by RAE application in this study were consistent with those studies under the treatments with municipal solid waste (Jedidi *et al.*, 2004) and livestock manure (Liang *et al.*, 2003).

**Table 6:** The ratio of sole substrate utilization rate to total substrate utilization rate in soil

Carbon source	Control	RAE treatment
Amino acid group		
ALA	3.79±0.06a	3.55±0.07b
CYS	3.92±0.05a	3.68±0.08b
ABA	3.66±0.02a	3.45±0.08b
LYS	3.50±0.07a	3.36±0.08a
NAG	3.59±0.01a	3.41±0.10a
Aromatic acids group		
DA	8.04±0.31a	7.39±0.04b
Carbohydrates rroup		
ARA	3.91±0.05a	3.70±0.11b
FRU	11.92±0.6a	12.91±0.28b
GAL	4.03±0.06a	4.00±0.06a
GLU	3.59±0.03a	3.37±0.06b
TRE	6.17±0.12a	6.44±0.19a
Carboxylic acids group		
KA	13.95±0.28a	12.74±0.82a
CA	5.48±0.02a	5.35±0.11a
MA	12.28±0.22a	13.56±0.47b
OA	12.19±0.34a	13.09±0.39b

ALA: L-Alanine, CYS: L-Cysteine HCl  
 LYS: L-Lysine  
 NAG: N-acetyl-Glucosamine  
 ABA: Gamma amino butyric acid  
 DA: 3,4-Dihydroxybenzoic acid  
 ARA: L-Arabinose, FRU: D-Fructose  
 GAL: D-Galactose, GLU: D-Glucose  
 TRE: Trehalose, KA:  $\alpha$ -Ketoglutaric acid  
 CA: Citric acid, MA: L-Malic acid  
 OA: Oxalic acid

Because the soil enzymatic activities are substrate-specific and are biological catalysts of specific reactions, it is difficult to obtain an overall status of soil enzymes in a soil from activity value of one enzyme alone. Hence, researchers have used activities of several soil enzymes as a basis for empirical indexes of soil quality, which included enzyme activity number index (EAN), biological index of fertility (BIF) and metabolic potential (MP). Beck reported EAN values were ranged from 1 to 4 for a cultivated soil (Beck, 1984). In this study, EAN values of the tested soil were within this range, and EAN of the

ameliorated soil was increased significantly by 31.16%, confirming a greater metabolic activity in it.

The increases of the utilization rates of carboxylic acids and carbohydrates in RAE treated soil (fig.1) may be due to the additions of abundant organic acid (e.g., 2-KGA, oxalic acid) and carbohydrates (e.g., sorbose) from RAE to soil. It would lead to an increased enrichment of the microorganisms who consume organic acid and carbohydrates. While, the increase of mean C utilization rate of the soil indicated an increase of available organic carbon supply.

## CONCLUSIONS

Our study is the first to report that RAE was tested as a potential ameliorant for an alkali-saline soil in a pot experiment of pakchoi cultivation. Both yield and quality of pakchoi were markedly enhanced by RAE application. The improved chemical properties of the soil, such as soil pH, total organic carbon, available phosphorus, available potassium, were observed. Microbial properties, such as culturable bacteria, actinomycete, and fungi, microbial biomass carbon, enzyme activities, the mean substrate utilization rate reflected by CLPP were also raised by RAE application. Therefore, this study provided a strong evidence for feasibility of RAE as an ameliorant for alkali-saline soil.

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