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ORIGINAL ARTICLES

Utilization of Citrate Wastes by *Scenedesmus* sp. I- Enhancement of Vegetative Growth

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ABSTRACT

Concerning environmentally hazardous wastes, economical regards and aiming at minimizing the production costs, green alga *Scenedesmus* sp. was laboratory grown under different concentrations of citrate wastes generated from citric acid production which characterized by high CNPK. The superior concentration of citrate wastes (10ml.l^{-1}) was incubated under different depleted concentrations from nitrogen, phophorus and potassium ranged from 0 to 100% of the initial concentration. Growth measurements i.e., dry weight and total chlorophyll were estimated. Growth indicator as dry weigh or total chlorophyll was accelerated proportionally to the applied volume of wastes. Obtained results showed that 10 ml.l^{-1} of wastes enriched cultures surpass other applied concentrations (*i.e.*, 20, 30, 40 and 50 ml.l^{-1} of wastes). Dry weight failure as well as chlorophyll decomposition were progress in association to the rate of nutrients depletion mainly nitrogen with 10ml.l^{-1} of wastes, while citrate wastes contain more nitrogen phophorus ratio as compared with those of BG-II growth medium (control), nitrogen level was found to be the limiting element that accelerates the rate of chlorophyll decomposition. No inhibitory effects on growth dry weight was observed when citrate supported cultures were re-incubated under completely depleted concentrations of phosphorus and potassium, while total chlorophyll represented the opposite manner.

Key words:

Introduction

Microalgae have a great potential for the removal of nitrogen and phosphorus from the growing media. However, for nutrient removal, *Chlorella* (Lee and Lee, 2001; Gonzales *et al*., 1997), *Scenedesmus* (Martinez *et al*., 1999, 2000), and *Spirulina* (Olgu´ın *et al*., 2003) are the widely microalgae used.

The nutrition demand for algae mass production is mainly carbon dioxide dependent and mineral nutrients that represented the main yield costs (Zaborsky, 1985). Therefore, many authors tend to use certain species of microalgae for treating wastewater, in which the microalgae are able to consume many minerals and organic compounds found in wastewater as pollutants (Laliberte *et al*., 1994). Unlike higher plants, algae also have a good potential to utilize organic carbon including sugars and their derivatives under heterotrophic growth conditions. Recently, certain industrial liquid wastes could be used for growing algae that could be significantly downed the algae yield costs (El-Sayed, 2004 a). Using such material almost provided growth medium by extra buffering action against the rise of pH through organic acids generated by fermentation besides the individual content of elements mainly carbon, nitrogen, phophorus and potassium as well as a sufficient quantity of micronutrients. For more downing costs some commercial fertilizers compounds including urea, phosphoric acid and potassium sulfate could be used for algal growth and proliferation (El-Sayed *et al*. 2001 and El-Sayed, and Abdel-Maguid 2010). Residuals from citrate industries produced by fungal fermentation containing sufficient amounts of minerals were used under the current study to enhance vegetative growth, dry weight and total chlorophyll of the preisolated green alga *Scenedesmus* sp. under indoor conditions which in turn led to minimizing production costs.

Materials and methods

The Alga and growth conditions:

Polyethylene tubes containing 2000 ml of nutrient growth media were used for growing *Scenedesmus* sp. El-Sayed (2004 b). Illumination was provided by day light lamps (12x40w) from one side. Aeration was provided by compressed air from the upper hold throughout 3mm polyethylene tubes ended by sand distributer. Growth media (BG11 as a control made by sterilized tap water) contained 0.3, 0.1, 0.05 g.l^{-1} of urea, phosphoric acid and potassium sulphate; respectively dissolved in one liter of sterilized tap water (El-Sayed *et al*., 2001).

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Concerning citrate wastes treatment, early experiments were conducted by enriching the original growth media with different volumes of citrate wastes *i.e.*, 0.0, 10, 20, 30, 40, and 50 ml.l^{-1} in three replicates. Incubation was carried out at the ambient room temperature for 10 days. Chemical and physical-prosperities of wastes are listed in Table (1). Growing media treatments and their initial content of nitrogen phophorus and potassium were listed in Table (2). Wastes analysis showed the extra supplementation of nitrogen, phophorus and potassium as compared with the initial concentrations of the original growth medium (BG-II).

Table 1: Some chemical prosperities of citrate waste.

pH	E.C	O.M	O.C	T.N	P	K	C:N	Fe	Zn	Mn	Cu
	dS.m^{-1}	%		g.l^{-1}			ratio	ppm			
4.87	4.95	0.80	0.47	0.65	0.13	1.3	0.73:1	395	21	9	13

*O.M.= Organic matter , O.C.= Organic carbon.

The second series of experiments was performed by growing the alga with the superior citrate waste concentration (10ml.l^{-1}); where the alga was incubated by the aforementioned technique with different depleted concentrations of nitrogen, phophorus and potassium. For each element, depletion was ranged from 0 to 100% of full BG-II growth medium.

Table 2: Basal growing medium BG11 treated with citrate wastes and initial content of NPK (g. l^{-1}).

Treatment	N (g.l^{-1})	P (g.l^{-1})	K (g.l^{-1})
Control	0.138	0.0311	0.0208
Control+ 10ml wastes	0.1445	0.0324	0.0338
Control+ 20ml wastes	0.151	0.0337	0.0468
Control+ 30ml wastes	0.1575	0.0350	0.0598
Control+ 40ml wastes	0.164	0.0363	0.0728
Control+ 50ml wastes	0.1705	0.0376	0.0858

Growth measurements and chemical analysis:

Daily measurement of dry weight was routinely carried out by filtering a define volume of algal slurry (5-10 ml.l^{-1}) over pre-weighted dried membrane filter (0.45 µm). Filters were dried (105^{o}C/30min.); kept over anhydrous calcium chloride till room temperature and then re-weighted. The difference between weights expressed the algal dry weight.

Total chlorophyll was determined in 95% DMSO extract as described by Burnison (1980). A modification was done by precipitating the algal biomass over membrane filter after dry weight determination at 70^{o}C. Filters were soaked in 5-10ml of 95% DMSO, filtered and re-extracted if necessary. Chlorophyll absorbance was measured at 666nm and concentration was calculated according to Seely *et al*. (1972). Nitrogen was determined based on micro-kjeldahl method using steam distillation unit Bushii 320 (Ma and Zauzag, 1942). Phophorus was spectrophotometrically measured by Vanedate method (APHA, 1998). Potassium was photometrically determined by flame emission. Iron, manganese, zinc and copper were photometrically measured by atomic absorption.

Results and Discussion

Growth dry weight:

The preliminary results showed the proportional effect of citrate wastes on *Scenedesmus* sp. growth and dry weight accumulation in 10 days as well as the given concentrations of wastes. The applied volumes of citrate wastes at the rates of 10-50ml.l^{-1} enhanced the algal dry weight; therefore, the maximum peak of algal dry weight percent was observed with 10 ml of citrate wastes (Table 3). Accordingly, the variations in the algal growth between the initial and end waste concentrations showed the increases of algal dry weight due to waste treatments in the concentration 10 ml.l^{-1} compared with others (Fig.1a). Such finding could be confirmed when data were subjected to growth rate (µ); where the decline on growth rate was observed to be in the opposite association with the given concentration. Increasing citrate wastes led to decreasing growth rate (Fig.1b).

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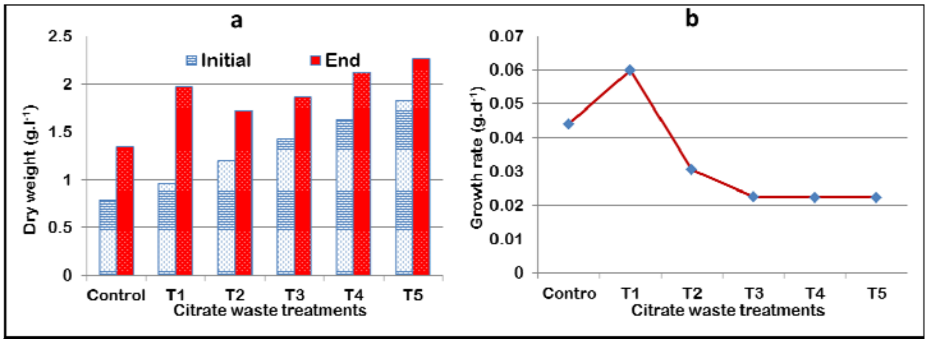

Fig. 1: a) Dry weight and b) Growth rate as dry weight g. day^{-1} (µ) of *Scenedesmus* sp. grown under different citrate waste concentrations.
[Control=(BG-II); T1= BG-II+10ml.l^{-1} of CW (Citrate Wastes); T2= BG-II+20ml.l^{-1} of CW; T3= BG-II+30ml.l^{-1} of CW; T4= BG-II+40ml.l^{-1} of CW and T5= BG-II+50ml.l^{-1} of CW.]

The decline on growth determined as dry weight with other The Decreasing of algal growth when treated with 20, 30, 40 and 50 ml.l^{-1} of wastes, could be ascribed to the deficiency of phophorus content as well as the hyper doses of potassium, however the examined alga able to utilize more concentration, but in balanced quantities. On the other hand, algae utilize phophorus only as orthophosphate, while the supported from wastes was organic form (El-Sayed *et al*., 2008). Otherwise, the increments observed on dry weight with higher concentrations of citrate wastes could be ascribed to the individual amounts of solids including carbon, nitrogen, phophorus and potassium. Here, citrate wastes treatment T1(10 ml.l^{-1}) support growth medium by extra amount of total solids which in turn interfaces with dry weight determination and making incorrectly comparison. Thus, a comparison was performed as what is the increase of dry weight was suggest as percentage increase. As shown in Table 3, percentage increases were recorded toward T1, cont., T2, T3, T4 and T5; however the rise of dry weight at zero time attributed to the initial solute of wastes.

Table 3: Dry weight increasing percent of *Scenedesmus* sp. as affected by citrate waste doses.

Treatments	Control	T1(10ml)	T2(20ml)	T3(30ml)	T4(40ml)	T5(50ml)
Increase (%)	169.6	2.05.2	144.2	131	130.7	123.5

Control=(BG-II); T1= BG-II+10ml.l^{-1} of CW (Citrate Wastes); T2= BG-II+20ml.l^{-1} of CW; T3= BG-II+30ml.l^{-1} of CW; T4= BG-II+40ml.l^{-1} of CW and T5= BG-II+50ml.l^{-1} of CW.

Nitrogen treatment:

Dry weight acceleration was found to be nitrogen supplementation independent and negative correlations with the rate of nitrogen reduction and the dry weight decline was observed with cultures which completely nitrogen starved, however it approximately equal control result(culture that received full BG-II growth media). The obtained results could be attributed to the relatively rate of nitrogen deficiency. Such algal cultures also received extra amount of other nutrients from the given citrate wastes including nitrogen which in turn play the same role. With other treatments, dry weight accumulation was found in a negative relationship with the rate of nitrogen reduction and the dry weight decline was observed with cultures which completely nitrogen starved. The obtained results could be attributed to the rate of nitrogen deficiency (Fig.2a).

Table 4: Nitrogen depletion treatments and dry weight & total chlorophyll increases of *Scenedesmus* sp. under treating of 10 ml of citrate wastes to one liter of basal growing medium BG11.

Element	Control	N series % + 10ml wastes				
Conc.%	Full BG-II	100	75	50	25	0.0
N (g.l^{-1})	0.138	0.1445	0.11	0.0755	0.041	0.0065
D.W. Inc.%	176.7	231.1	192.2	184.4	180	165.6
T. Ch. Inc.%	241.9	320.7	300.9	165.3	68.5	55.9

D.W. Inc.% = Dry weight increasing percent. .
T. Ch. Inc.% = Total chlorophyll increasing percent. .

Accordingly, 75, 50 and 25% of the recommended nitrogen quantities with 10 ml of wastes allow serving growth and completely utilized the nutrients supported by wastes. When cultures received only 25% or less of the recommended nutrients dose as well as 10 ml.l^{-1} of wastes alone, growth doesn't represent extending and dry weight failure as well as chlorophyll decomposition was observed.

When data expressed as total chlorophyll, the same pattern of dry weight was observed, whereas chlorophyll was more affected by nitrogen depletion rather than dry weight. Maximum chlorophyll accumulation was observed with cultures received 10ml of citrate wastes plus 100% of the recommended nitrogen dose followed by those received 75%. Other treatments resulted in chlorophyll decomposition as compared with control cultures; however dry weight was increased under such concentration. Such effect could be ascribed to the effect of individual solids of citrate wastes on dry weight and the consumption of organic carbon via carotenoids assimilation on the expense of total chlorophyll, where control cultures exhibited the same trend of dry weight accumulation (Fig. 2c). Percentage increase (Table 4) and specific growth rate (Fig. 2b&d) confirm such finding and indicating that even completely N-depleted cultures able to grow well in spite chlorophyll status. Results also showed that fewer than 50 and 75% of the initial nitrogen concentration of BG-II growth media plus 10 ml of citrate waste are adequate concentrations save growth and reliable economic.

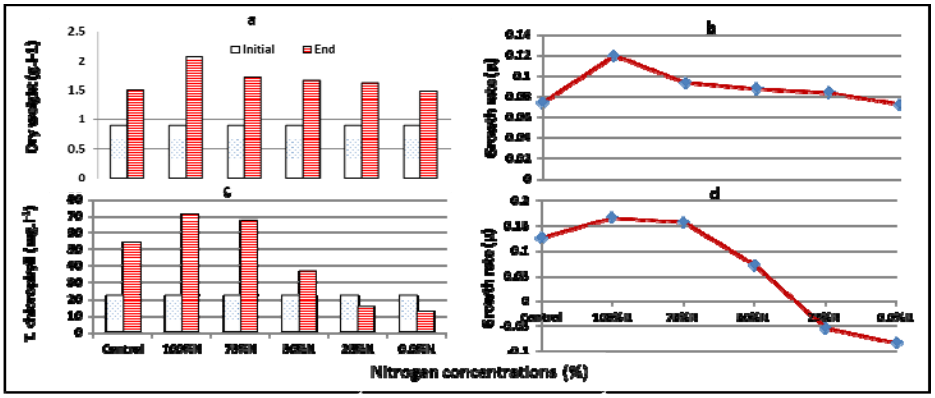

Fig. 2: a) Dry weight, b) Growth rate as dry weight g. day^{-1} , c) Total chlorophyll and d) Growth rate as total chlorophyll mg.l^{-1} of *Scenedesmus* sp. grown under different depleted nitrogen concentrations (%) of the original growth BG11 media with 10ml.l^{-1} of citrate wastes.

Similar finding showed that rinse water (RW) generated from olive-oil extraction industry used for *Scenedesmus* biomass production resulted in maximum specific growth rate, 0.044 g.d^{-1} with 5% RW due to nitrogen deficiency. However, biomass productivity proved greater in the culture with 100% RW. It was also found that the composition of the lipid fraction of the biomass depended on the percentage of wastewater used as the nutrient medium, reaching the highest percentages of monounsaturated, polyunsaturated, and essential fatty acids in the culture with 100% RW (Hodaifa *et al*., 2008). This might be goes back to nitrogen deficiency with high organic carbon content. Cell densities reached low, but of the same order of magnitude as those registered for *Chlorella vulgaris* and *Nannochloropsis oculata* grown on industrial wastewaters from a citric-acid production plant or a shrimp farm (Valderrama *et al*., 2002 and Yusoff *et al*., 2001). In addition, increasing of lipids is the first monitor of complicated causes media mainly nitrogen deficiency.

Phophorus treatment:

When cultures were incubated; by the same technique; with phophorus depleted media; dry weight was increased with all treated cultures as compared with control cultures (Table 3 &Fig. 3a).

No inhibitory effect due to phophorus depletion was observed. Here, the increment effect as compared with control cultures might be goes back to the extra nitrogen and nitrogen supplementation by citrate wastes in the relatively absent of phophorus . In addition, the examined alga might be not able to utilize such phosphate source. In addition, the most inhibitor effect was observed with 0.0% enriched cultures (Fig. 3b).

As for total chlorophyll (Table 5 &Fig. 3c); the opposite manner was observed, where, control and 100 % P cultures surpasses all the other treated cultures (75, 50, 25 and 0 % P cultures). The inhibitory effect might be attributed to the phophorus deficiency for those cultures received less fewer than full medium plus wastes and citrate might be contains a minor amount of inorganic phosphate.

In this case the decomposition of chlorophyll took place on the expense of carotenoids bioaccumulation. Data also showed that a slight differences among the different suboptimal concentrations than the optimum (Fig. 3d). Here, phophorus omitting seems to be more effective on chlorophyll biosynthesis than nitrogen.

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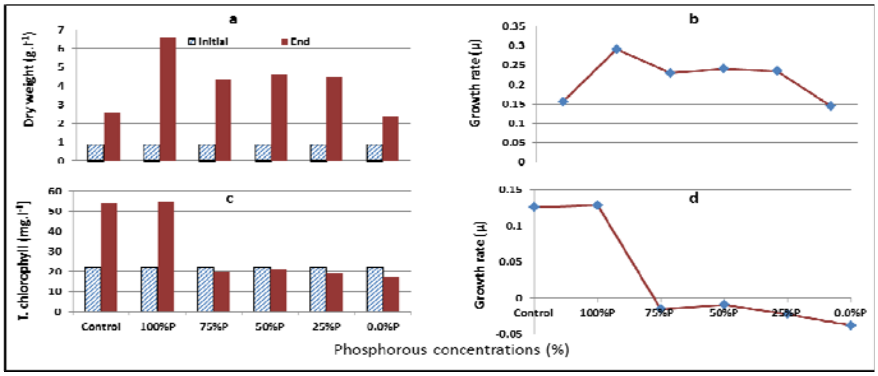

Fig. 3: a) Dry weight, b) Growth rate as dry weight g. day^{-1}, c) Total chlorophyll and d) Growth rate as total chlorophyll mg.l^{-1} of *Scenedesmus* sp. grown under different depleted phophorus concentrations (%) of the original growth BG11 media with 10ml.l^{-1} of citrate wastes.

Table 5: Effect of phophorus depletion treatments on dry weight and total chlorophyll of *Scenedesmus* sp. under treating of 10 ml of citrate wastes to one liter of basal growing medium BG11.

E	Control	P series % + 10ml wastes				
Conc.%	BG-II	100	75	50	25	0.0
P (g.l^{-1})	0.0311	0.0324	0.02465	0.0169	0.00915	0.0013
D.W. Inc.%	297.7	767.4	500	537.2	516.3	274.4
T. Ch. Inc.%	241.9	246	89.6	93.7	85.6	76.6

D.W. Inc.% = Dry weight percentage increase.
T. Ch. Inc.% = Total chlorophyll percentage increase.

However, Increasing of phophorus concentration leads to the increase in growth parameters especially at lower temperature, *i.e.*; 20^{o}C (Martinez *et al*., 1999). Hashimoto *et al*.(1989) reported that such effect could go back to the increasing of cellular nucleic acids and compounds of high energy content under high growth rate conditions. In addition, the internal P content declined with higher incident light intensity. This fact can be interpreted as a cellular response to avoid accumulating ATP when sufficient light energy was available.

Potassium treatment:

Potassium treatments represented a quite different manner (Table 6 &Fig. 4). Lower and moderate level of potassium deficiency resulted in dry weight increases (75, 50 and 25 % of full media plus 10 ml waste). Extreme potassium deficient cultures (0.0%) showed a slight dry weight decreases as compared with the superior cultures. In all cases no inhibitory effect was observed as potassium concentrations were depleted and control culture surpasses other treated cultures in concern dry weight accumulation. Data were found in quite similar trend for both dry weight and total chlorophyll. It may be concluded that however potassium concentrations were decreased, but the initial content of the deficient cultures was found in sufficient as compared with control cultures.

Concerning total chlorophyll, potassium deficient cultures showed a stimulatory effect on chlorophyll accumulation at lower and moderate deficient range (75 and 50% K). A slight inhibitory effect was observed with 25 %K, while 0.0% K represented a dramatic decrease. The most stimulatory effect was observed with cultures that received full BG-II plus 10 ml of citrate wastes (100% K).

Increasing of algal dry weights under all given concentrations (Fig.1) indicated the suitability of such wastes to algal growth and no inhibitory effect due to undesirable compounds was observed. The growth variation among the different treatments could be ascribed to the differences between the routinely used NPK ratio (6.5:0.13:1.3). It was found that, the examined alga succeeded to grow well (indoor and outdoor) under 0.65:0.1:0.01 (El-Sayed, 2001). In addition, such alga able to grow well under extra concentration of such elements, but in the same ratio (El-Sayed *et al*., 2008 and El-Sayed, and Abdel-Maguid 2010). Citrate wastes supported growth medium by a sufficient amount of nitrogen and potassium. Concerning phosphorus, citrate wastes supported growth media by organic phosphate even though at insufficient amount, where algae have no potential to utilize the organic phosphate.

Valderrama *et al*. (2002) reported that effluent water quality decreases with increasing nutrient concentrations and algae culture can remove nitrogen more effectively compared to phosphorus. The whole consumption rate in our case could be roughly calculated weather algae consumed the initial of 50ml^{-1} in fully omitted growth media. Chemical analysis at the end of exponential phase represented the high removal rate of nutrients

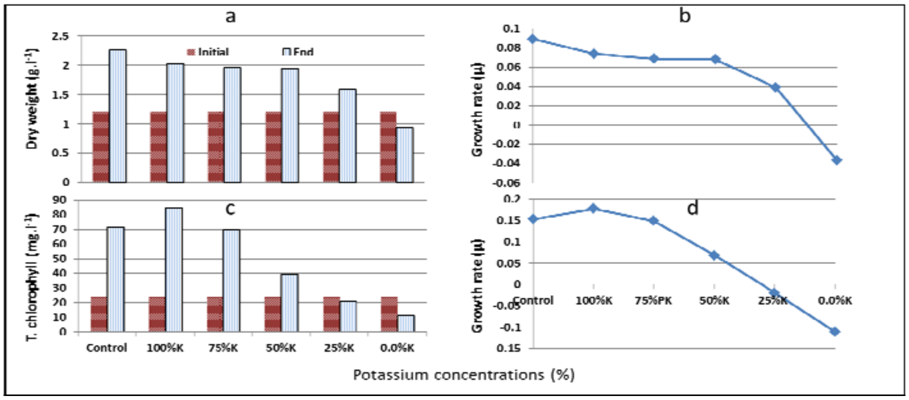

Fig. 4: a) Dry weight, b) Growth rate as dry weight g. day^{-1} , c) Total chlorophyll and d) Growth rate as total chlorophyll mg.l^{-1} of *Scenedesmus* sp. grown under different depleted potassium concentrations (%) of the original growth BG11 media with 10ml.l^{-1} of citrate wastes.

Table 6: Effect of potassium depletion treatments on dry weight and total chlorophyll of *Scenedesmus* sp. under treating of 10 ml of citrate wastes to one liter of basal growing medium BG11.

E	Control	K series % + 10ml wastes				
Conc.%	BG-II	100	75	50	25	0.0
K (g.l^{-1})	0.0208	0.0338	0.0286	0.0234	0.0182	0.013
D.W. Inc.%	186.8	167.8	161.9	161.2	131.4	77.7
T. Ch. Inc.%	291.4	346.5	283.2	161.6	86.9	46.1

D.W. Inc.% = Dry weight percentage increase.
T. Ch. Inc.% = Total chlorophyll percentage increase.

Removing of phophorus by *Scenedesmus obliquus* was increased with the dilution rate with no detectable direct influence of light intensity. Thus it may be suggested that increasing of phophorus removing parallel to increasing of dilution rate could be attributed to the buffering action of dilution against growth curve, where, dilution prolonged the exponential phase (Martinez *et al*., 2000). Growth enhancement in treatments receiving 10-20 ml.l^{-1} citrate wastes could be attributed to the increase of phophorus and potassium concentrations as well as the fast consumption of nitrogen by algae rather than phophorus and potassium (Table.4). Consumption rate per hour was highly increased. Acid reaction was raised due to algal growth however it's followed by alkaline elements consumption. The net gain of such treatment is to decrease the total solids expressed as electric conductivity. The remainder sodium of citric crystallization represented the maximum flow consumption as compared with other nutrients except nitrogen.

Summary:

Successive mass production of microalgae depending upon the minimizing ofthe running costs mainly mineral nutrition and carbon source which represent the maximum.Concerning environmental hazard, use of citrate wastes allow the save discarding of these wastes which contain a high content of organic nutrients mainly carbon, nitrogen, phophorus and potassium beside the adequate amount of micronutrients.

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