

Original Research Article

Vegetable wastes: An alternative resource for biogas and bio compost production through lab scale process

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ABSTRACT

Keywords

Vegetable wastes, biogas, biocompost, *Bacillus* sp., *Alcaligenes* sp., *Corynebacterium* sp. (SM23)

In the present study, the vegetable wastes namely, beat root, carrot and potato etc. were collected from Salem and Omalur vegetable markets, Tamil Nadu, India. Different ratio (1:1, 2:1 and 3:1) of vegetable wastes and cow dung was used for the production of biogas. Here, the ratio 2:1 consisting of vegetable wastes and cow dung showed maximum amount of biogas production in the experimental period. After the biogas production the effluent was used for plant growth study. Green gram was used in this study to find out the efficiency of anaerobically digested effluent. The shoot and root length of the plant was measured during the study period. In an another study, there are 30 different bacterial strains were isolated and identified from the vegetable wastes. From the 30 isolates, *Bacillus* sp. (SM4), *Alcaligenes* sp. (SM6) and *Corynebacterium* sp. (SM23) were selected as efficient starch, cellulose and lignin hydrolyser and those were acted efficiently in the compost process of market wastes with short duration. After the composting process the significant parameters pH, calcium, chloride, nitrogen, phosphorous, potassium, iron, zinc and copper were checked.

Introduction

Vegetable waste is a biodegradable material generated in large quantities from various sources like, domestic, industries, municipalities and markets. Now a day's these kinds of wastes are dumped on land to rot in the open, which not only emits a foul odor, but also creates a big nuisance by attracting birds, rats and pig's vectors of various diseases. These wastes can be treated for bio-energy production through

fermentation under controlled conditions or else used for composting.

Adopting anaerobic biological treatment of the organic fraction of municipal solid waste is a process which has received increased attention during the last few years (Raynal *et al.*, 1998; Convvverti *et al.*, 1999). Conversion of these wastes to methane provides some energy and can have a

beneficial effect on the environment. The production of methane during the anaerobic digestion of biologically degradable organic matter depends on the amount and kind of material added to the system (Convverti *et al.*, 1999). Preliminary work on anaerobic treatment of fruit and vegetable waste (FVW) in a batch digester (Bouallagui *et al.*, 2001) was inhibited by the volatile fatty acid (VFA) accumulation and irreversible decreasing pH problems. The present study focuses on the strategic application of bioconversion processes on Vegetable wastes used to develop environmentally friendly and low-cost operating systems for production of biogas. The production of biogas through anaerobic digestion offers significant advantages over other forms of bio energy production. It has been evaluated as one of the most energy-efficient and environmentally beneficial technology for bio energy production.

Composting is the controlled, microbial aerobic decomposition and stabilization of organic substrates it can protect plants from soil (Alvarez *et al.*, 2006; Coosemans, 1999) or seed borne pathogens (Schuler *et al.*, 1993). It is the most economical and sustainable option for organic waste management it a natural process of organic waste treatment which is currently practiced with various modifications to the technology. Temperature is a primary factor to bring microbial community succession during composting. Different microbial communities dominate during these various composting phases, and each adapted to a particular environment.

In the agricultural waste composting, lingo cellulose accounts for the major part of biomass and consequently, its degradation is essential for the operation of composting (Tuomela *et al.*, 2000; Dixon and Langer, 2006). Numerous studies have been

addressed the use of compost in nursery plant production and have analyzed its chemical, physical and biological properties (Sanderson *et al.*, 1980). More seriously, the dumping of municipal solid waste without proper treatment, proper supervision and control leads to fly breeding and other infectious diseases in the society. The Municipal Solid Waste rules estimated that 1.0 to 1.1 kg of solid waste generated per day per person in a well-developed city.

Compost improves the soil in a number of ways and reduces waste going to landfills. When added to soil, compost increases soil water and nutrient holding capacity. It improves plant productivity and cover. Increased plant cover reduces water runoff and the potential for soil erosion and nutrient pollution.

The main determinant for efficient agronomic use is nitrogen availability. It is important to develop integrated approaches to compost use in agriculture, which take into account agronomic benefits and environmental risks, while identifying the financial implications of compost application. Vegetable waste compost application to agriculture and towards the development of management systems among to maximize agronomic benefit and reduce the environmental risk. In the present study was aimed on bioremediation of vegetable solid waste through production of biogas and biocomposts.

Materials and Methods

Sample Collection and Processing

Market vegetable wastes (Beetroot, Carrot and Potato etc) were collected from Uzhavar Sandhai and Omalur vegetable market, Salem, Tamil Nadu, India. The samples were transport to the laboratory using sterile

plastic bags for further analysis. The spoiled vegetables were crushed by Mortar and pestle and from that the bacterial colonies were enumerated by serial dilution method. The colonies were counted and enumerated and morphologically different strains were selected and stored in agar slants at 4°C.

Anaerobic treatment of vegetable wastes through biogas production

The vegetable wastes namely fruit based wastes, Beetroot, Carrot and Potato were collected and chopped in to several pieces and air dried. The materials were crushed until to get stickiness and used as semisolid medium. Bioremediation of such wastes were performed through biomethanation process using a biodigester. The digester is 2.5 litre capacities and made up of dark glass. The top of the digester was closed with holed rubber cork connected with a silicon tube. Other end of tube was tightly closed with a stopper. To this digester, 1.5 litre semisolid medium was taken and enriched with cow dung as inoculums. The ratio of semisolid medium was prepared as shown the following protocols:

- a) Vegetable wastes alone (10%) = 1500ml (without amendments)
- b) Vegetable wastes (10%) + cow dung (10%) = 750 ml + 750 ml (1:1ratio)
- c) Vegetable wastes (10%) + cow dung (10%) = 1000 ml + 500 ml (2:1ratio)
- d) Vegetable wastes (10%) + cow dung (10%) = 1125 ml + 375 ml (3:1ratio)
- e) Cow dung alone (10%) = 1500 ml (without amendments)

Similar study was carried out in the wastes of tubers. The set up was kept under open space for the period of 15 days at room temperature. The substrate was mixed manually in each day to obtain biogas significantly. Every 3 days gas produced

was measured by downward displacement of water and expressed in ml (Saravanane *et al.*, 2001).

Aerobic treatment of vegetable wastes by composting

Isolation, identification and screening of amylase producing bacteria

Starch agar medium was prepared and sterilized at 121°C. The medium was poured into sterile plates and allowed for solidification. All the test organisms were inoculated by spot and they were incubated at 37°C for 24-72 hours. After incubation the medium was flooded with iodine solution. The iodine reacts with starch to form a dark blue-coloured complex. Clear area around the growth of the culture after the addition of the iodine indicates the breakdown of starch by the organism due to its production of amylase. The clear zone around the colonies were measured and noted. The starch hydrolysing bacterial strains were identified by biochemical analysis according to the Bergey's Manual of Determinative Bacteriology. The bacterial consortium was selected based on their antagonistic character and they were identified up to the generic level. Based on this results the three strains *Bacillus* sp. (SM4), *Alcaligenes* sp. (SM6) and *Corynebacterium* sp. (SM23) were selected and made consortium.

Compost process by aerobic bacterial consortium

A loopful of culture [(A: *Bacillus* sp. (SM4), B: *Alcaligenes* sp. (SM6) and *Corynebacterium* sp. (SM23)] was inoculated individually in presterilized 100 ml nutrient broth. The flask was kept in a shaker at 120 rpm for 16-18 h at 37°C. The culture broth was centrifuged at 10000 rpm for 20 min.

Cell suspension was prepared using sterile distilled water and adjusted to 1OD using UV Visible spectrophotometer (Model: Cyberlab UV100, USA). One percent (10^7 CFU/ml) of the above suspension was used as inoculum for further study.

Compost pit was prepared based on the method chart out by Subba Rao (1999). The pit was filled with vegetable wastes by layering and each layer is about 10 cm thickness. Between the layers the potential bacterial consortium presumptively selected by hydrolysis test was enriched under aseptic condition. The layering of solid wastes was repeated until the pit was filled. Finally, a layer of sterile mud paste was made on top of the pit. After a month, composted materials were thoroughly mixed and physicochemical characteristics were checked by standard methods.

Impact of anaerobically digested liquid waste on seed germination and plant growth

Tarson cups was filled with fertile soil and used for plant growth study. To it various concentration 10, 20, 30, 40 and 50% anaerobically digested effluent was added to maintain moisture content constantly. Four seeds of green gram (*Phaseolus aureous*) were shown in each cup and allowed to germinate. The control was supplied with tap water and the remaining cups were supplied with respective concentration of effluent regularly. The seed germination and growth was measured until 16th day. Finally the plants were uprooted and the height of the root and number of nodules were recorded (Ayyasamy *et al.*, 2008). The experiment was conducted in triplicates, unless otherwise stated. Data points in the tables and figures represent the means; with all the error bars shown (1 standard error of mean). Both the mean and standard

deviation were performed with appropriate usage of statistical package on Microsoft Excel Version 2010.

Results and Discussion

Anaerobic treatment of vegetable wastes by biogas production

The vegetable and tubers wastes were anaerobically treated and biogas was released from the set up and measured for the periods of 27 days with different combination and the quantity of gas was expressed in ml. The biogas production from vegetable and tuber wastes is represented in Figure 1. The raw vegetable waste act as control, it produced gas on 3, 6 and 9th day but on 12th and 27th day, there is decreased quantity of gas production. On 12th day it was 220 ml and on 27th day it was nil. Vegetable waste was treated with cow dung at 1:1 ratio and the result of the gas production shows maximum on 3rd day with the gas production of 540 ml and on 6th day it was 250 ml then the gas production decreases gradually and reaches 45 ml on 21th day. The vegetable waste and cow dung at 2:1 ratio shows on 3rd day the gas production was 580 ml which was higher and on 18th day it was 180 ml then the gas production gradually decreases as 50 ml on 21st day. In the ration of 3:1 produces maximum gas on 3rd day it was 330 ml and on 21st day the gas production was decreased (30 ml).

In the raw tuber wastes used as a control, produces gas on 3, 6, 9 and 12th day but on 15th and 18th day, there is decreased quantity of gas production, on 15th day it was 130 ml and on 18th day it was 110ml. In the 1:1 ratio of tubers and cow dung shows maximum gas production of 650 ml and on 6th day it was 310 ml then the gas production decreases gradually and reaches 60 ml on 24th day. In

the 2: 1 ratio, result showed maximum gas production on 3rd day which was 450 ml and on 12th day it was 200ml then the gas production gradually decreases at 21st day as 30 ml. In the 3:1 ratio the gas production was 520 ml which was maximum on 3rd day and on 24th day the gas production was 40 ml. The production of gas for every 3 days was measured from the anaerobically treated vegetable waste by the downward displacement of water.

Quantitatively tuber waste has a greater potential for biogas production since it gave the largest volume of biogas during the retention period compared to all the substrates under investigation. The volume of maximum gas production of tuber was 1850 ml in entire days. The other ratios of tuber and cow dung showed more or less equal consumption of gas production.

Compost process under aerobic condition

There are three bacterial strains (*Bacillus* sp. SM4, *Alcaligenes* sp. SM6 and *Corynebacterium* sp. SM23) were selected for degrade starch and other organic matter in vegetable waste. Similar to this study, In one of the report says that, among bacteria, *Bacillus* sp. is widely used for thermostable amylase production to meet industrial needs. *B. subtilis*, *B. stearothermophilus*, *B. licheniformis* and *B. amyloliquefaciens* are known to be good producers of amylase and these have been widely used for commercial production of the enzyme for various applications. Boyer and Ingle (1972) said that *Bacillus* species are considered to be the most important sources of amylase and have been used for enzyme production.

Compost layers were prepared by using dry and wet vegetable waste under anaerobic condition. During the composting, microbial load in compost was detected for each 15 days of time intervals; it reveals microbial

load increased during the compost. The color changes of the compost from soil color to dark brown. Microbial and physico-chemical parameters of the microbial compost were analyzed and the results are given in Table 1, and Table 2.

Initially, the colour of organic matters seems to be brown and turns into black at the end of composting process. The good quality of bio-compost was obtained on final day. The macro nutrients were obtained as nitrogen phosphorous and potassium. The chemical characteristics of bio-compost were discussed. pH value of raw vegetable waste compost is observed as 8.0. During the composting process, pH value of substrate was decreasing due to mixing of inoculants and this was slightly decreasing from 7.7. It shows that the alkalinity of the bio-compost is slowly reducing in the process.

The total nitrogen present in the vegetable market waste was 38.00. In all three layers, the total nitrogen is increasing about 92% the increase of total nitrogen shows the good quality of bio-compost. The concentration of total potassium in the vegetable waste compost was found 4 % and it is slightly increasing to a very minimum to about 15 to 25%. The concentration of total phosphorous of vegetable waste compost was determined as 21%. There was a considerable increase of total phosphorous in Bed-I it is from 32 to 46%.

The concentration of organic carbon of raw market waste was 26.42. The carbon contents of market waste were utilized as energy by the earthworms and hence organic carbon concentration is gradually reducing. The organic carbon concentration in Bed-I is 16.67, in Bed-II it is 20.67 and in Bed-III it is 13.62. The reduction of organic carbon shows that the earthworms rapidly multiplying and decomposing the organics.

Figure.1 Biogas production from vegetable and tubers wastes under laboratory process

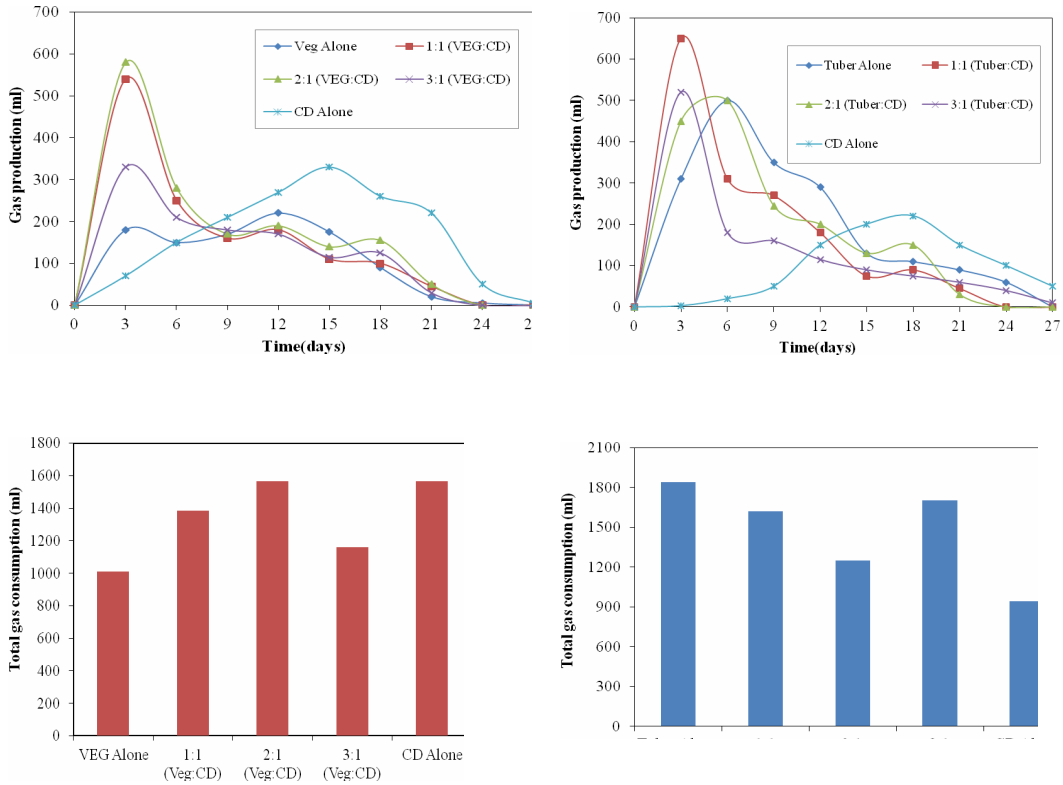


Figure.2 Effect of biodigested vegetable wastes on the growth of green gram
 A. Vegetables alone B. 1:1 ratio C. 2:1 ratio D. 3:1 ratio E. CD alone

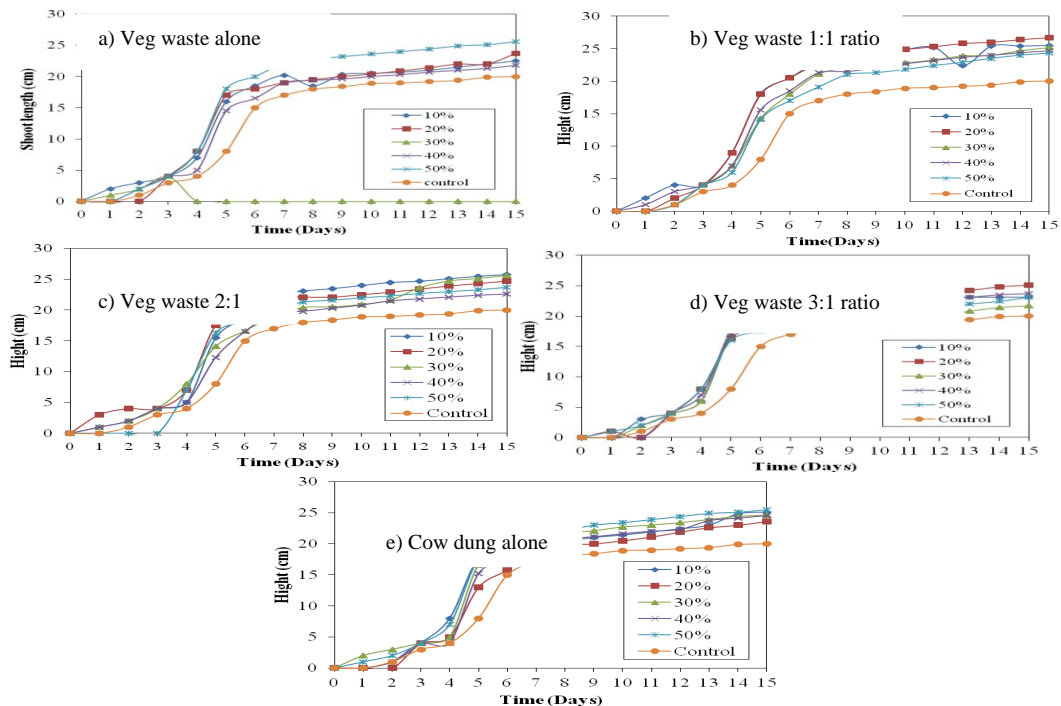


Figure.3 Effect of bio-digested tuber wastes on the growth of green gram

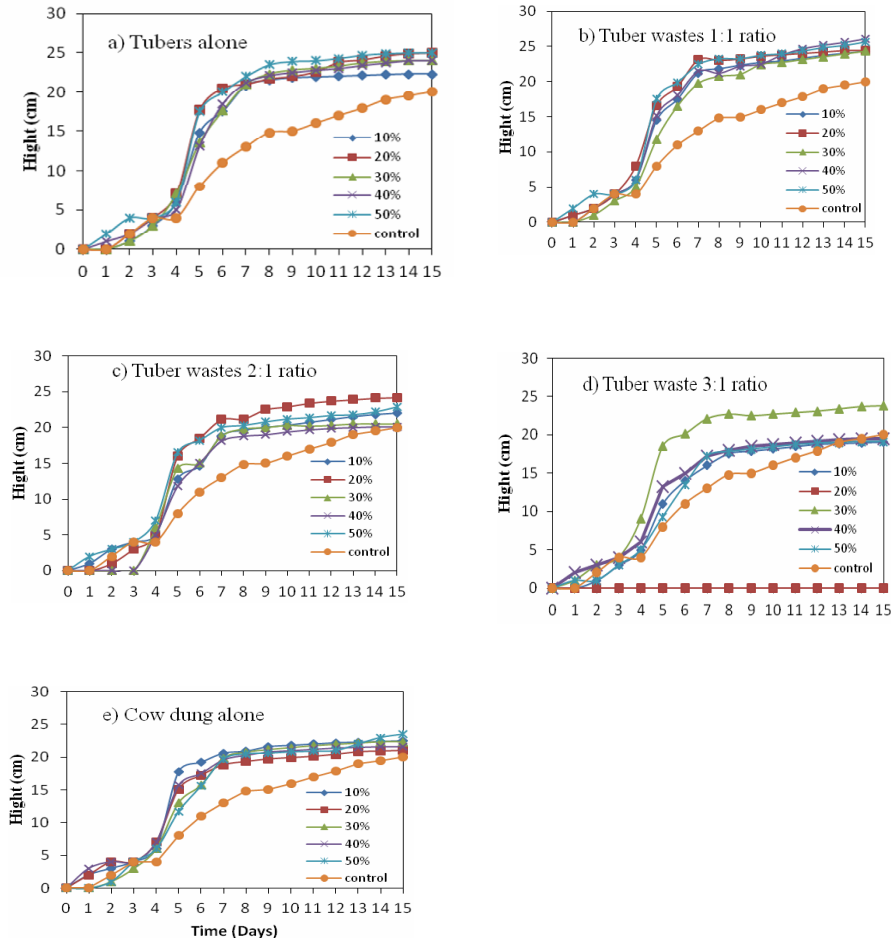


Table.1 Detection of microbial load in prepared compost

S.NO	Compost type	Dilution Factor	Colonies (CFU/g)					
			1 st Day	5 th Day	10 th Day	15 th Day	20 th Day	25 th Day
1	Dry weed compost	10 ⁻⁵	50	TNTC	200	TNTC	TNTC	TNTC
		10 ⁻⁶	150	100	150	TNTC	TNTC	TNTC
		10 ⁻⁷	50	50	80	TNTC	TNTC	TNTC
2	Wet weed compost	10 ⁻⁵	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
		10 ⁻⁶	30	200	170	260	TNTC	TNTC
		10 ⁻⁷	10	50	60	130	TNTC	TNTC
3	BCD	10 ⁻⁵	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
		10 ⁻⁶	200	250	200	TNTC	TNTC	TNTC
		10 ⁻⁷	50	150	230	TNTC	TNTC	TNTC
4	BDE	10 ⁻⁵	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
		10 ⁻⁶	150	270	200	TNTC	TNTC	TNTC
		10 ⁻⁷	75	100	75	200	TNTC	TNTC
5	Sterile soil without inoculums	10 ⁻⁵	TNTC	200	TNTC	TNTC	TNTC	TNTC
		10 ⁻⁶	120	220	200	TNTC	TNTC	TNTC
		10 ⁻⁷	100	154	100	TNTC	TNTC	TNTC

Table.2 Micro and macro nutrients in the microbial compost

Parameters	WET WEED	DRY WEED	BCD	BDE	SOIL+ VEG	CONTROL
Calcium carbonate	High	High	High	High	No	No
Salt level(dsm ⁻¹)	1.0	1.2	1.0	1.0	0.1	0.20
pH	7.8	7.7	7.7	7.7	7.9	8.0
N (mg/kg)	83	91	88	92	84	38.00
P (mg/kg)	20	25	25	20	15	4.00
K (mg/kg)	475	490	490	490	326	210.00
Fe (mg/kg)	11.69	7.11	10.86	10.16	23.0	9.92
Mn ppm	6.00	3.95	7.26	4.56	11.48	4.80
Zn ppm	1.12	1.30	0.9	1.16	1.80	0.62
Cu ppm	1.03	2.10	1.40	1.70	7.10	0.97

Plant growth irrigated with anaerobically digested vegetable wastes

A laboratory experiment was designed to know the effect of different concentration (10 and 50%) of anaerobically digested liquid wastes of vegetables and tubers on seed germination in Green gram (*Phaseolus aureus*) (Fig.2 and 3). At lower concentration of effluent the speed of germination, peak value and germination values are higher than the responses at higher concentrations. In one the literature showed lowering the concentration of effluent (25%) was shown to support 100% seed germination in kidney bean and millet, but osmotic pressure associated with higher concentration of sugar factory effluent were found to reduce the germination in kidney bean and millet (Ajmal *et al.*, 1983). It indicates that salt concentration that governs the seed germination and it varies from crop to crop because each crop has their own tolerance to the different salt concentrations.

The speed of germination, peak value and germination value of tested vegetation also varied with respect to different concentration of anaerobically digested liquid wastes. When compared to water control, the green gram plant growth was greater in all the ratios of anaerobically

digested liquid wastes of 10, 20, 30, 40 and 50% of concentration.

When compared to water control, all the ratios of anaerobically digested vegetable wastes of bio gas effluent show the better growth at the 10% of concentration. At the 50% of concentration, vegetables waste control and vegetables waste 2:1 shows the equal growth and also the water control shows good result on the plant growth. But the vegetables waste 3:1 has lesser impact on the plant growth. Vegetables waste 1:1 and vegetables waste 2:1 shows the better result on the plant growth at the 50% of concentration. The water control and vegetables waste 3:1 gives the equal growth and also vegetables waste control shows lesser impact on the plant growth at the 50% of concentration.

The shoot length of green gram was severally influenced by various concentration of effluent obtained from tubers treatment (Fig.3). Some essential micronutrients are needed in small quantities for plant growth. However, the excessive level could prove toxic to plant growth. Muthusamy and Jayabalan, (2001) clearly reported the toxicity of industry effluent on the growth, physiological and biochemical contents of *Gossypium*

hirsutum in soil medium. Accordingly, in our study, the plant growth was highly affected due to their pH and presence of higher dissolved solids in the anaerobically digested liquid wastes. There was slight variation in the shoot length when the pots irrigated with treated effluent at all the tested concentrations after the 15th day. Among the two different vegetables waste, anaerobically digested liquid wastes of bio gas effluent exhibits their greater influence on the seed germination and plant growth.

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