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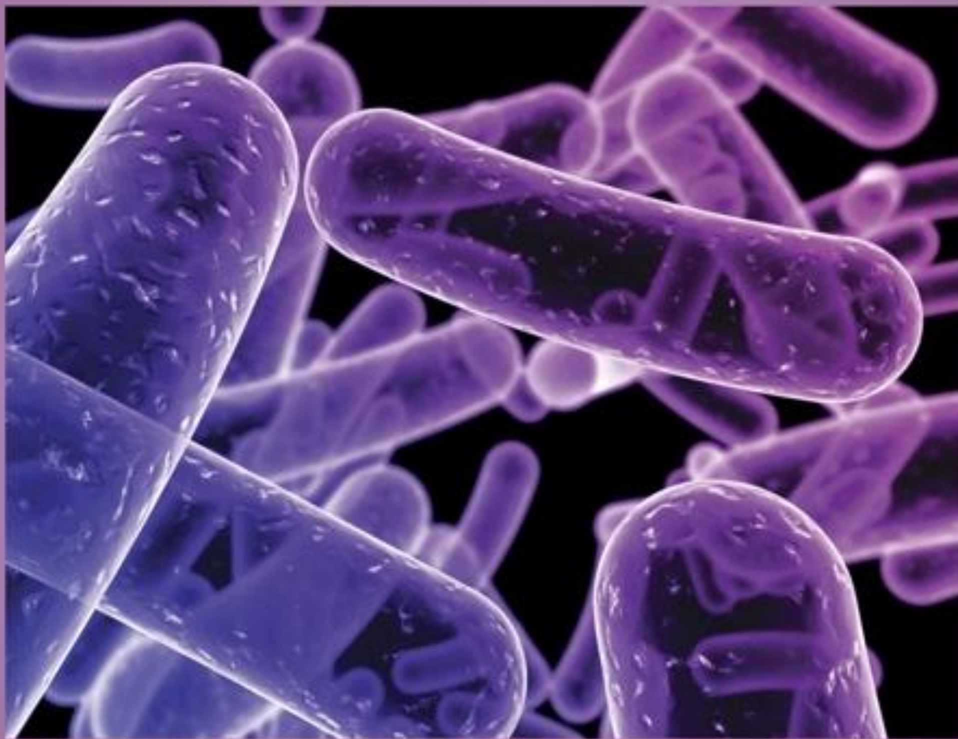


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# Production of Valuable Compost from Sugarcane Wastes

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**ABSTRACT**

In this study, development biofertilizer is produced from sugarcane residues was compared with commercial biological fertilizers. Development Fertilizers from sugarcane wastes have been analyzed chemically and microbiologically. Factors affecting the ripening of the compost and growth of microorganisms in the dander, (temperature, humidity and pH) were monitored. The final values for each were 45, 30 and 8., respectively.

The compost ripening was followed by the analysis of organic matter and organic carbon, their respective values were 20.7 and 63.54, respectively, and the C: N ratio was 1:16 upon completion of ripening. The ratio of major elements N, P, K, which was 2.4, 4.78 And 0.06 respectively at the end of the ripening period. Bacterial isolated from sugarcane wastes were belonged to *Bacillus genera,* while fungal isolates belonged to *Aspergillus genera*.

## INTRODUCTION

Sugarcane bagasse is a residue that the sugar industries produce in great quantities. Bagasse is the by-product of the milling that remains after sugar is removed from the plant. It is a fibrous residue, which mainly contains cellulose, hemicellulose and lignin (A. Akbar Babael, *et al.,* 2016). Approximately 1.6 billion tons of sugar cane are produced annually worldwide, generating about 279 million tons of biomass residues consisting of bagasse and leaves (A. K. Chandel, *et al.,* 2012). The sugarcane industry in Egypt goes back to the year 710 AD (Hassan, S.F. & Nasr, M.I., 2008). Cane plantations are concentrated in the area of Upper Egypt. The total amount of cane cultivated in Upper Egypt is about 16 million tons per year (Hamada, Y.M., 2011).

Most of these wastes, particularly the bagasse, are usually burned or converted into boiler fuel due to the lack of proper management techniques that resulted in atmospheric pollution due to toxic gas emissions and therefore posed a threat to human health (N. P. Pandit and S. K. Maheshwari 2012).

Bioconversion of the leftover bagasse into value-added products could therefore have both sustainable economic and strategic advantages. Because the sugarcane bagasse is abundantly available, this waste can be used to generate value-added commodities such as biofertilizer by co-composting it with other undesirable materials.

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Composting is one of the most recommended methods of organic waste recycling, as it closes the natural cycle and returns its nutrients back to the soil. It is deemed the "highest recycling form" (Epstein, E., 1997). Composting can improve soil conditions and plant growth, decrease the potential for erosion and runoff and, if properly produced, can add humus to the soil. Generation of composted fertilizer from lignocellulosic residues of sugar by-products preserves the health of plant and soil properties and protects the plant from diseases caused by soil (Sardar, S *et al.,* 2012).Bagasse may be used in composting when first shredded, enriched with other substances and/or inoculated with certain microorganisms that degrade cellulose (Tengerdy and Szakacs, 2003).Pretreatments work by disturbing the lignocellulosic network, reducing the amount of lignin and hemicelluloses and altering the crystalline form of the cellulose to make it more vulnerable to enzymatic attacks (Silverstein *et al.,* 2007).At the same time, antagonists develop during compost ripening. Composts therefore can reduce the incidence of different plant diseases (Fuchs, 2002).

Composts are known for suppressing plant diseases by incorporating physicochemical and biological features. Physical or chemical aspects of composts directly or indirectly reduce the severity of disease affecting pathogen or host growth (Jeanine *et al.,* 2002). Therefore, the main objective of this study is to get use of sugarcane wastes to develop a valuable fertilizer.

## MATERIALS AND METHODS

### Samples Collection and Preparation Procedure:

Two samples of fresh bagasse were collected carefully and about 10 g of each were carefully stored to isolate bagasse microorganisms. The bagasse is treated with steam at 2 bar for an hour to kill the nematodes that may be found in bagasse and also to digest the cellulose into simple organic material to facilitate microbial hydrolysis.

The test specimens shall consist of about 100-200 g of the sample obtained in such a way as to ensure that it is representative of a whole lot of material being tested. The moisture content was determined after 4 hof drying 105°C (NREL BAT Team laboratory analytical procedure 2004).

### Compost Piles Components:

Natural organic waste bagasse was sampled in May 2016 from Sugarcane factories and refining at Nag-Hammady city, Qena, Egypt.

Different piles were prepared as shown in Table (1). The compost piles were processed in a wooden model divided into two levels equipped with electric antennas for continuous ventilation with constant flipping during the composting process. One pile was placed on each level. The pile was equipped with a length of 1m, a width of 0.5m, a height of 0.8m.

**Table 1:** Compost combinations

| Pile Name | Piles components | | | | Moisture solutions | | |
|---|---|---|---|---|---|---|---|
| | Bagasse | Tree leaves | Sawdust | Poultry residue | water | Molas solen | West Honey |
| Blank | 80 % | 10 % | 5 % | 5 % | ✓ | × | × |
| Mature molass C1 | 80 % | 10 % | 5 % | 5 % | × | ✓ | × |
| A mature molass C2 | 80 % | 10 % | 5 % | 5 % | × | × | ✓ |
| Mix C3 | 80 % | 10 % | 5 % | 5 % | × | ✓ | ✓ |

The piles were moisturized daily for the first three days then moisturized every two days. Samples from each pile were collected every 4 days for analysis until the end of the experiment after 4 weeks.
The solutions were Prepared for moisturizing compost as follows:

Solution of molasses at a concentration of 10%, Solution of yeast powder with a concentration of 10%, Solution of EM material at a concentration of 10% and Solution of cooking sugar cane juice at a concentration of 10%. In blank were moisturized by tab water only and samples were moisturized by waste of sugarcane juice solution 10% (Olynciw, 2002). From each pile, two samples were taken at different positions. Samples were transported to the laboratory rapidly and all the analyses were made on the same day.

**Physical and Chemical Parameters:**

During the process, the pile temperature was monitored daily using a composting digital thermometer that was inserted in the piles at various heights. During 8 hours drying samples at 105oC determined the moisture content. And then calculate the difference. Compost samples were diluted 1:10 in distilled water placed in a shaker for 24 hours and finally filtered and calculated using filter paper the pH was determined with the application of AS-501 pH Analyzer (STEM Corporation, England).

For analysis of metals, filtrates of the last two samples collected after 4 weeks were digested in digestion system attached to scrubbing unit according to Page *et al.* (1982) and then analyzed for the content of N, K, and P. Analysis was carried out using Buck Scientific INC 210– Atomic Absorption Spectrophotometer (AAS) according to Lindsay &Novel (1978).
Organic carbon, C:N ratio, and organic matter (OM) was determined by using the Walkley-Black wet combustion method (Man, 1996).

Finally, compost total nitrogen content was determined using the Kjeldahl technique (Nelson &Sommers, 1973).

**Isolation of Microorganisms from Bagasse and Compost:**

1 g of bagasse was diluted with 49 ml of distilled sterilized water. Samples were vigorously shaken to form a uniform solution with concentrations of 10-1. The decimal serial dilutions (10-1 to 10-7) were prepared using the method of (Ejifor & Okafor 1985).
For the isolation of fungi, the plate count method (Raper & Fennell, 1965) was used as follows: a volume of 500 μL of the diluted sample, from compost serial dilutions, was used to inoculate Czapek's agar medium (Smith & Dawson, 1944). Chloramphenicol (0.05 mg/ml) was used as bacteriostatic agent (Al-Doory, 1980). The plates were incubated at 28 ºC for 5-7 days during which the developing fungi colonies were counted and identified (Domsch *et al.,* 1980).
For the isolation of bacteria, by using nutrient agar medium (NA). The plates were incubated at 37 ºC for 24 - 48 h (Seeley &Van Demark, 1981). For the isolation of yeast, by using Yeast Extract-Peptone-Dextrose (YPD) Agar that comprised ($gl^{-1}$): 10 g yeast extract, 20 g peptone, 20g dextrose and 20 g agar Final pH (at 25°C) 6.5±0.2 (Ausubel F. M. *et al.,* 1994).

**Identification of Microbial Isolates:**

Identification of bacteria was carried out following the morphological and biochemical characteristics of isolates (Bergey's Manual of Systematic Bacteriology, 1994).
Identification of fungal species and genera were carried out according to Raper&Fenell (1965), Ellis (1971, 1976) and Bissett (1991).

**Isolation of Cellulolytic Bacteria**

Cellulolytic bacterial strains were isolated from all samples using serial dilutions and spread plate technique One gram of each sample was suspended to suspend the cells in 9.0 mL of physiological saline (0.85 percent NaCl) and an aliquot of this mixture was transferred to two selective media. The first medium, called the agar medium of cellulose-containing gL-1(cellulose 2, gelatin 2, MgSO4 0.25, KH2PO4 0. 5, Agar 15) Adjusted pH at 6.8-7.2. Incubated at 37 °C for 96 hours. All the

different bacterial colonies which appeared after the incubation period on the cellulose agar medium plates were selected and subjected to the purification process. Repeated streaking on the same medium used for the isolation process was used to purify the bacterial colonies.

For further identification and screening for cellulase processing, the isolated colonies were preserved at 4oC. Carboxymethyl cellulose agar containing CMC as an asol carbon source is the second medium used for isolation. The CMC agar medium used for isolation of cellulolytic bacteria containing gL-1(peptone 10, carboxymethylcellulose (CMC) 10, K2HPO4 2, agar 15, MgSO4.7H2O 0.3, (NH4)2So4 2.5 and gelatin 2) Adjusted pH at 6.8-7.2. Incubated at 37 °C for 48 hours (Yin LJ, Huang PS *et al.,* 210). Bacterial colonies were purified by repeated streaking. The purified colonies were preserved at 4 °C for further identification and screening for cellulose enzyme production.

## RESULTS AND DISCUSSION

**Physical And Chemical Parameters:**

**Temperature (C);**

The temperature increased gradually in all compost types at the 12 days to reach to 50-55 °C and then it is obviously reduced to 45 °C at the 6$^{th}$ day. It was noticed that, the C2 and C3 composts exhibited higher temperature than control sample and C1 in a period from 0-12 days as shown in Figure (1). It may be attributed to the effect of the biological processes as a result of the microbial activity in the compost, as mentioned by (Yoohyun Lee 2016).

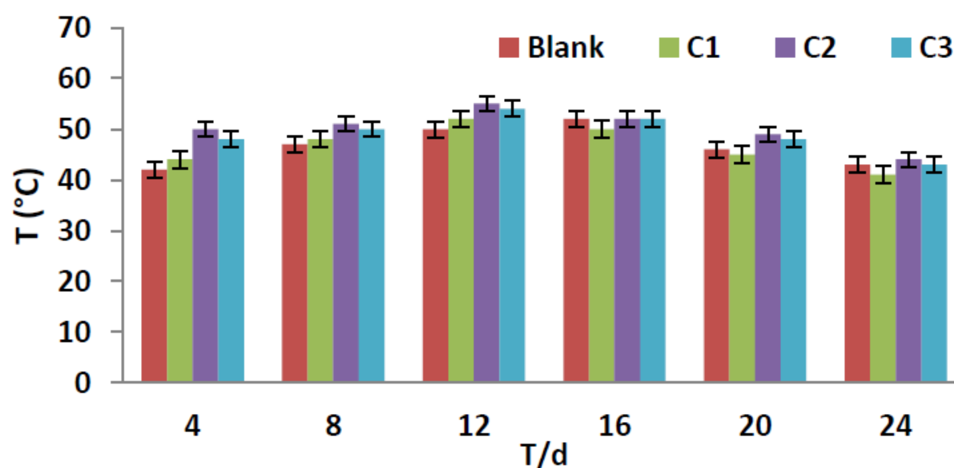


**Fig (1): Different temperature in compost types**

**Moisture (%):**

During the follow-up of Moisture throughout the curing stages of the compost, it was found that it ranges between 51 to 59 in both the control (blank) and the compost as shown in Figure (2), while the humidity ranged from 25 to 32 at (El-Sayed G. Khater 2015). This difference may be due to the high content of moisture in the bagasse than other materials, as it contains a high percentage of fiber.

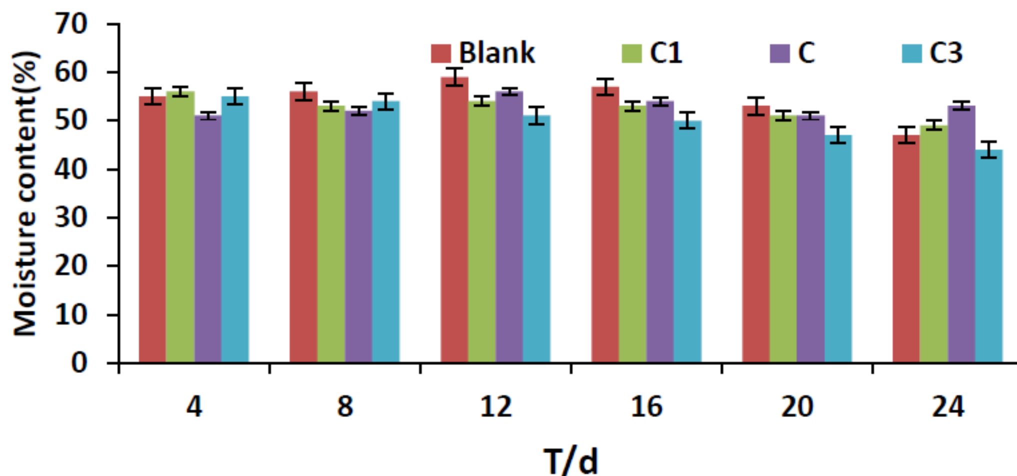


**Fig (2): different moisture in compost types**

**pH:**

During the following of pH, it was found that it ranged between 7.1 and 7.8 in both control and composts samples as shown in Figure 3, and this was identical to what he said (El-Sayed G. Khater 2015).

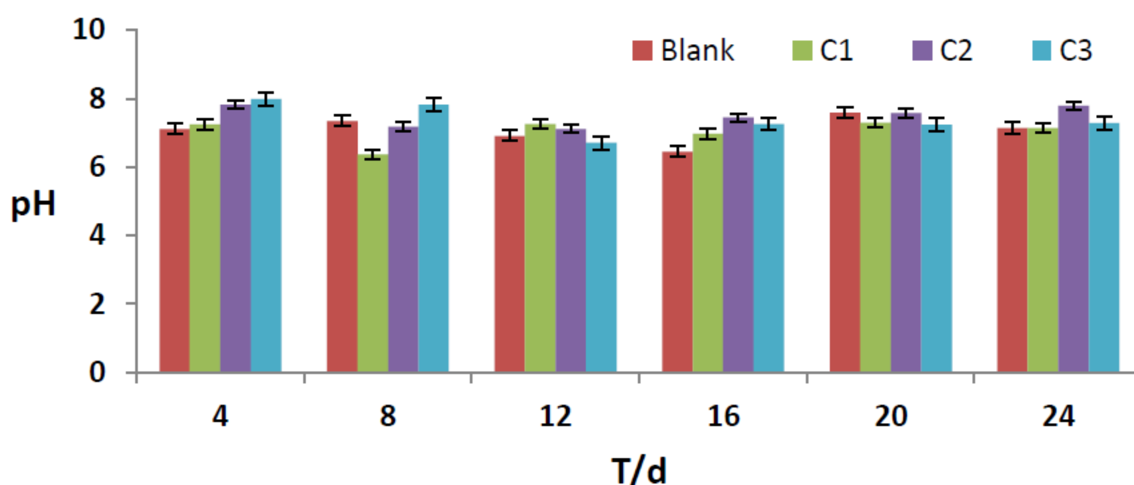


**Fig (3): different pH in compost types**

**Chemical Composition of Compost:**

The chemical analyzes of the compost were performed during two periods of the beginning of the process where the analyzes of the plant's major nutrients were performed and it was found that they are different from those mentioned by (El-Sayed G. Khater 2015).

As shown in Table (2), C2 exhibited the highest N, P and K content (1.6, 3.86 and 1.13, respectively). On the other hand this compost exhibited low organic carbon and organic matter as well 20.7 and 26.1% , respectively. It was noticed that, C3 showed low pH value and C/N ratio.

This difference is due to the increase in the amount of cane sucker that was used. Both organic matters, organic carbon and carbon to nitrogen ratio were analyzed and were found to correspond roughly with those reported in (El-Sayed G. Khater 2015). These results are shown in Table (2).

**Table 2:** Chemical composition of different types of composts

| Time (days) | N (ppm) | P (ppm) | K (ppm) | pH | C/N (%) | Organic C (%) | Organic matter (%) |
|---|---|---|---|---|---|---|---|
| **Blank** | 1.4 | 2.74 | 0.43 | 7.98 | 1:35.1 | 49.2 | 84.8 |
| **C1** | 1.0 | 2.86 | 0.57 | 7.82 | 1:32.6 | 32.6 | 56.2 |
| **C2** | 1.6 | 3.86 | 1.13 | 7.82 | 1:17.0 | 20.7 | 26.11 |
| **C3** | 1.6 | 1.84 | 0.46 | 7.26 | 1:14.6 | 23.4 | 40.3 |

The following results of the mature compost (curing) were compared with the commercial compost and there was a positive difference in the results of the compost made from the cane sucker as shown in Table 3. The results showed that, the organic carbon of curing compost was three-fold higher than the commercial. While the C/N ratio of the commercial compost is obviously higher than the curing one. the content of phosphorous, potassium and nitrogenous of curing compost was much higher than the commercial. There were no clear differencebetween pH values of the two composts.

**Table 3:** comparative between commercial compost and curing

| | ratio N / C | Organic C (%) | Organic matter (%) | pH | K | P | N |
|---|---|---|---|---|---|---|---|
| **Commercial compost** | **37.4** | **9.55** | **16.62** | **7.98** | **1.46** | **1.142** | **1.0785** |
| **Curing compost** | 14.9 | 39.5 | 26.11 | 7.82 | 18.9 | 28.7 | 3.32 |

**Isolation of Microorganisms:**

**1 - Microorganisms Isolated from Bagasse:**

The microorganisms that naturally grow on the bagasse were isolated and classified into bacteria, fungi, and yeasts, and each of them was defined as six bacteria isolates, five fungi and three strains of yeasts were defined, and each of these varieties was defined according to the established methods and these strains are shown in Table (4).

**Table 4:** Microorganisms isolated from bagasse:

| The organism | Bacteria | Fungi | Yeast |
|---|---|---|---|
| **Fresh Bagasse** | *Bacillus subtilis* | *Aspergillus flavus* | Y-01 |
| | *B. macerans* | *A. niger* | Y-02 |
| | *B. pumilus* | *A. fumigates* | Y-03 |
| | *B. polymyxa* | *Trichoderma* | - |
| | *Echerichia coli* | *Longibrachiatum* | - |
| | *Bacillus sp.* | - | - |

**2- Microorganisms Isolated from Compost: -**

Microorganisms that grow during the various stages of compost maturity have been isolated and divided into bacteria, fungi and yeasts, and each of them has been defined as five bacterial isolates, noting that *Echerichia coli* and three fungi have not been observed, noting that *Trichoderma* sp and *Longibrachiatum* have not appeared as the temperature increases as mentioned. (Cahyani, VR 2004) and five strains of yeasts due to the use of a black honey waste solution, as several strains of yeasts that grow naturally, were isolated on these sugars (Gerke, Chen and Cohen 2006).

Each of these varieties was determined according to the methods used and these strains in Table (5).

**Table 5:** Microorganisms isolated from compost

| The organism | Bacteria | Fungi | Yeast |
|---|---|---|---|
| Blank | *Bacillus subtilis* | *Aspergillus flavus* | Y-04 |
| | *B. macerans* | *A. niger* | Y-05 |
| | *B. pumilus* | *A. fumigates* | Y-06 |
| | *B. polymyxa* | ==== | Y-07 |
| | *Bacillus sp.* | ==== | Y-08 |
| Compost | *Bacillus subtilis* | *Aspergillus flavus* | - |
| | *B. macerans* | *A. niger* | - |
| | *B. pumilus* | *A. fumigates* | - |
| | *B. polymyxa* | === | - |
| | *Bacillus sp.* | === | - |

**Identification of Isolates: -**

**1 – Morphological Identification:**

It was found that all isolates are similar in all apparent traits, as the genus *Bacillus* is comprised of Gram-positive bacteria, as he said (Bruce *etal*., 2010; Maughan and Van der Auwera, 2011).

These isolates and their morphological characteristics have been collected in Table (6).

**Table 6:** Morphological identification microorganisms isolated

| Source | Gram stain | Morphological shape | Endospors forming | Motility test | Name isolate |
|---|---|---|---|---|---|
| Fresh Bagasse | +Ve | Bacillus | +Ve | Facultative | *B. subtilius* |
| | +Ve | Bacillus | +Ve | Facultative | *B. macerans* |
| | +Ve | Bacillus | +Ve | Facultative | *B. pumilus* |
| | +Ve | Bacillus | +Ve | Facultative | *B. polymyxa* |
| | +Ve | Bacillus | +Ve | Facultative | *Bacillus sp* |
| Blank | +Ve | Bacillus | +Ve | Facultative | *B. subtilius* |
| | +Ve | Bacillus | +Ve | Facultative | *B. macerans* |
| | +Ve | Bacillus | +Ve | Facultative | *B. pumilus* |
| | +Ve | Bacillus | +Ve | Facultative | *B. polymyxa* |
| | +Ve | Bacillus | +Ve | Facultative | *Bacillus sp* |
| Compost | +Ve | Bacillus | +Ve | Facultative | *B. subtilius* |
| | +Ve | Bacillus | +Ve | Facultative | *B. macerans* |
| | +Ve | Bacillus | +Ve | Facultative | *B. pumilus* |
| | +Ve | Bacillus | +Ve | Facultative | *B. polymyxa* |
| | +Ve | Bacillus | +Ve | Facultative | *Bacillus sp* |

**2 - Biochemical Tests:**

After the isolates were similar in morphological characteristics, the definition was performed by means of biochemical tests in which isolates such as indol, catalase, citrate, methyl red, nitrate, pigment and urease tests.

All isolates were similar in the catalase test and urease test as mentioned (Park J. *et al.,* 2003) While found a difference between isolates in the rest of the tests as mentioned by (Park J *et al.,* 2003) and the results of these tests were limited as shown in Table (7).

**Table 7:** Biochemical tests of microorganisms isolated

| Source | Catal-ase | Citrate test | Indol Test | Methyl Red | Nitrate test | Pigme Test | Urease Test | Name isolate |
|---|---|---|---|---|---|---|---|---|
| **Fresh Bagasse** | +Ve | +Ve | -Ve | -Ve | +Ve | -Ve | -Ve | *B. subtilius* |
| | +Ve | Ve | -Ve | -Ve | +Ve | -Ve | -Ve | *B. macerans* |
| | +Ve | -Ve | -Ve | -Ve | -Ve | -Ve | -Ve | *B. pumilus* |
| | +Ve | Variable | -Ve | -Ve | +Ve | -Ve | -Ve | *B. polymyxa* |
| | +Ve | +Ve | -Ve | -Ve | +Ve | -Ve | -Ve | *Bacillus sp* |
| **control** | +Ve | +Ve | -Ve | -Ve | +Ve | -Ve | -Ve | *B. subtilius* |
| | +Ve | Ve | -Ve | -Ve | +Ve | -Ve | -Ve | *B. macerans* |
| | +Ve | -Ve | -Ve | -Ve | -Ve | -Ve | -Ve | *B. pumilus* |
| | +Ve | Variable | -Ve | -Ve | +Ve | -Ve | -Ve | *B. polymyxa* |
| | +Ve | +Ve | -Ve | -Ve | +Ve | -Ve | -Ve | *Bacillus sp* |
| **Compost** | +Ve | +Ve | -Ve | -Ve | +Ve | -Ve | -Ve | *B. subtilius* |
| | +Ve | Ve | -Ve | -Ve | +Ve | -Ve | -Ve | *B. macerans* |
| | +Ve | -Ve | -Ve | -Ve | -Ve | -Ve | -Ve | *B. pumilus* |
| | +Ve | +Ve | +Ve | -Ve | +Ve | +Ve | -Ve | *Azotobacter* |
| | +Ve | +Ve | -Ve | -Ve | +Ve | -Ve | -Ve | *Bacillus sp* |

**Isolation Of Cellulolytic Bacteria:**

CMC aqueous microorganisms were isolated and tested for their ability to degrade cellulose on CMC agar plates. Three bacterial strains showing the ability to dissolve cellulose in bagasse showed as different strains emerged after treatment to raise the levels of nutrients (Sadhu S. *et al*., 2013) and this was explained in Table (8).

**Table 8:** Isolation of cellulolytic bacteria

| Source | Cellulase Test | Gram stain | Oxygen requirement | Name isolate |
|---|---|---|---|---|
| **Fresh Bagasse** | + Ve | + Ve | Facultative | *Bacillus sp* |
| | + Ve | + Ve | Facultative | *B. macerans* |
| | + Ve | + Ve | Facultative | *B. polymyxa* |
| **Control** | + Ve | + Ve | Facultative | *Bacillus sp* |
| | + Ve | + Ve | Facultative | *B. macerans* |
| | + Ve | + Ve | Facultative | *B. polymyxa* |
| **Compost** | + Ve | + Ve | Facultative | *Bacillus sp.* |
| | + Ve | + Ve | Facultative | *B. circulance* |
| | + Ve | + Ve | Facultative | *B. subtilius* |
| | + Ve | + Ve | Facultative | *Azotobacter* |

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