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KINETICS OF INOCULATION COMPOSTING OF SOME BIOWASTES

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ABSTRACT

A method was used to improve the composting efficiency by seeding with inoculum (a blend of *Bacillus megaterium, Bacillus lichenformis, Trichoderma harzianum* and *Streptomyces cellulosae*). In this study, blends of tree trimmings and sawdust, in ratio 2.6:1, were treated with the inoculum or were not treated. The effect of inoculation was evaluated in terms of time required to obtain a stable product and product quality based on physicochemical variables of temperature, water retention capacity (WRC), organic carbon and microbial concentration were investigated to study the efficiencies of inoculation composting. In order to understand the mechanisms of inoculation composting process, two stages kinetics equations were developed from the viewpoint of microbial kinetics.

The inoculated composting process didn't only reach a relatively high temperature (62° C) but also maintained the high temperature for long time (15 days). The highest value of WRC was obtained with inoculated composting process (2.54 g g^{-1}). The high values of THB, thermophilic bacteria, total fungi and total actinomycetes were recorded with inoculated composting process (12, 8, 7 and 8.5 $\log_{10}$ CFU g^{-1} dry wt, respectively). The equations of two- stage composting showed that microbial concentration, in the first stage, was the main limiting factor of the degradation rate. The degradation rates in uninoculated and inoculated were 21.93 and 32.15 g kg^{-1} d, respectively. In second stage, the degradation rate was mainly affected the half velocity coefficient Km and in turn stabilize the composting products efficiently. Therefore, inoculation could improve the efficiency of the composting process.

Key words: Biowaste, inoculation composting, two- stage kinetics.

INTRODUCTION

The composting process always occur in nature, however, many artificial measures have been developed to improve composting efficiency. Over the past decades, effective inoculation has been reported by several searchers (Gaur et al, 1982; Matthur et al, 1986; Shin *et al* 1999; Beiy *et al* 2000; Baheri & Meysami, 2002; Acevedo *et al* 2005 and Xi *et al* 2005). Various specialized inocula have been applied in practice. For example Hatakka (1994) studied lignin-modifying enzymes from selected white- rot fungi and found that white- rot fungi played an important role in lignin degradation.

Nakasaki *et al* (1994) reported that a thermophilic bacterium *Bacillus licheniformis* could effectively decompose protein and prevent the drop of initial pH values during composting, thus, it could stimulate proliferation of other thermophilic bacteria.

Ohtaki *et al* (1998) revealed that inoculations could increase the microbial population, formulate beneficial microbial communities, improve microbiological quality and generate various desired enzymes, and thus enhance the conversion of organics and reduce odorous gas emissions. The studies of Lei and Gheynst (2000) indicated that the inoculated microbial population and indigenous populations would evolve continuously, leading variations during different composting stages.

The inoculants consist of naturally-occurring, aerobic microorganisms that can be founding any heating soil. The function of these microorganisms is to (a) decompose the readily- available organic materials under controlled conditions, (b) increase the time of composting at high temperature remained, and (c) stabilize the residual material into finished compost within 6 to 8 weeks. Another role of the inoculated microorganisms is to detoxify certain products that may be harmful to soils, plants and animals, including people (Xi *et al* 2005).Shin *et al* (1999) studied the enhancement of composting efficiency by adding solid and liquid inoculants.

Generally, previous studies indicated that the inoculated microbial populations and indigenous populations would keep evolving continuously, leading to variations during different composting stages.

Little effects have been made to example the kinetics of inoculation processes within a multi-stage context. Because of the

existence of two clearly different stages of composting process, it is indispensable to understand the kinetics corresponding to these stages, when investigating the efficiency of inoculation.

The purpose of this study was to determine the effects of seeding inoculums during the composting process. Meanwhile, to better understand the kinetics of inoculation composting, two- stage kinetics equations were developed by composting the intrinsic rate equation which fundamental microbial kinetics. In this study, maximum degradation rate and half velocity coefficient were used to determine the effects of inoculum on the compost efficiency.

MATERIALS AND METHODS

Composting materials

Tree trimmings were collected after pruning the trees, stored in a shadow place and minced manually to 2.5- 3.5 cm in size and 1 cm in thickness before using as raw material. They were mixed with sawdust as a bulking agent.

Physical and chemical characteristics of the materials are reported in Table (1)

Table (1): The characteristics of composting materials at the beginning

Material	Moisture content%	Organic carbon%	Total nitrogen%	C:N ratio	Weight (kg)
Tree trimmings	70	50	3.10	016	550
Sawdust	10	55	0.11	500	210

The inoculum was made by mixing microorganisms which were isolated from different composted materials that substantially biodegrade easily decomposed organic substrates. They were *Bacillus megaterium, Bacillus licheniformis,* and that strongly biodegrade cellulose and lignin substrates, *Trichoderma harzianum* and *Streptomyces cellulosae.* The characteristics of inoculant are listed in Table (2).

The equations of Richard and Nancy (1996) were used to adjust the desirable moisture content (60 %) and proper level of C: N ratio (30:1) without excessive change of moisture content.

Table (2): The characteristics of seed inoculant

Microorganisms	Optimum temperature	Counts in inoculum CFU/ml
Bacillus megatrium	30 °C	9.2×10^{9}
Bacillus licheniformis	60 °C	7.1×10^{10}
Trichoderma harzianum	30 °C	1.3×10^{6}
Sterptomyces cellulosae	60 °C	4.7×10^{8}

Composting windrow and method

Two separate windrows were constructed by dimensions 5m in length. 1m in width and, 0.6 m in height. Four layers of compost materials, each layer about 15 cm thick of compost material consists of tree trimmings and sawdust, each both of windrows contained tree trimmings and sawdust in ratio 2.6 :1.

One of the windrows, the inoculum (about 2.5 g kg^{-1}) was added to the pile and spread evenly on top of each compost layer (windrow B), while another windrow left without inoculation (windrow A). The moisture content of the initial waste mixture in all tests was adjusted to about 600g kg^{-1} by feeding water to the mixture. The windrows were turned manually every two days during the first 15 days and weekly during the rest of composting process. If needed, water was added during turning. Samples were obtained from the homogenized material after each turning. Composting processes lasted 3 months.

Analytical procedures

Samples taken from three sites of the piles were assembled, mixed and used to determine moisture content, water retention capacity and total ash.

The moisture content was determined through the weight loss at 105 ° C for 3 h. pH was determined in water extracted (1:5 w/v) from samples after shaking for 2h. Water Retention Capacity (WRC) and was determined according to Acevedo *et al*, 2005.

The temperature was continuously measured at three depths (top, middle and bottom) in the composting piles, using measuring lances to monitor the process. Total ash was measured by burning the samples at 550 °C for 4 h (AOAC, 1990).

Organic matter of the samples was determined as follows:

Percentage of organic carbon = % volatile solids (VS) ÷ 1.8,

where %VS =100-%ash (Adams *et al* 1951).

The percentage of organic matter = % organic carbon x 1.72 (assuming organic matter contains 58% organic carbon), (Pluske *et al* 1960).

Microbiological analysis

Total aerobic heterotrophic bacteria (THB) were determined by the plate counting method as described by Strom (1985). Thermophilic bacteria counts were determined on nutrient agar where the plates were incubated at 60 ° C for 3 days (Hassen et al, 2001) . The number of fungi was measured on Sabouroud- Dextrose agar supplemented with 30 μ g /ml streptomycin, incubated at 30 ° C for 2 days (Booth, 1971). The number of actinomycetes was measured on ISP medium 4 and incubated at 30° C for 7 days (Lacey, 1973).

Two-stage microbial kinetics of the inoculation composting

There are two different in terms of the limiting factors in the inoculation composting process. At the beginning, enough organic matter was sufficient to be decomposed while the microbial populations was insufficient and in turn limiting the composting process. With the process ongoing, the organic matter concentration decreased while the microbial populations increased, therefore, the substrate become the main limiting factor of the composting process. Based on the above analysis, two- stages kinetics equations derived from the intrinsic rate equations and fundamental microbial kinetics were developed and then used to examine the efficiency of inoculation composting process.

In the first stage, the degradation rate follows the following expression (Haug, 1993).

$$V = -dS/dt = KAv\ X / Kx + X \quad \text{..... (1)}$$

From Eq. (1) we have:

$$1/V = (Kx/KAv)\ (1/X+1)/KAv = (Kx\ Vm)(1/X) + 1/Vm \quad \text{.....(2)}$$

In the second stage, the degradation rate was measured as follows:

$$V = (Vm\ S) / Km + S \quad \text{..... (3)}$$

From Eq. (3) we have

$$1/V = (Km/Vm)(1/S) + 1/Vm \quad \text{..... (4)}$$

Nomenclature

Av	the available surface area per unit volume
K	the maximum rate of solid substrate hydrolysis which occurs at high microbial concentration
Km	Michaelis constant (g /kg)
Kx	the half velocity coefficient (equal to the microbial concentration when dS/dt= V/2)
S	the soluble faction substrate concentration (g/ kg).
T	composting time (day).
V	the hydrolysis rate of solid substrate (degradation rate).
X	the microbial concentration (g/kg).
Vm	the maximum degradation rate (g/ kg.d).

RESULTS AND DISCUSSION

Temperature profile

Catabolic processes are associated with exothermic reactions and the temperature of a pile in a composting is estimated to be a function of the heat generated in the bio- oxidation and the system dissipative capacity. Provided that the superficial area remains constant, system temperature varies with direct proportionality to the metabolic activity and temperature kinetics is an indication of system bio-oxidative behavior. Temperature profiles for the two piles and the ambient temperature are illustrated in Fig 1. The ambient temperature only fluctuated within a very narrow rang (around 25 °C) during the composting period. However, the composting rose up gradually after the moisture content was adjusted to 60% and air follow was introduced.

The temperature in the uninoculated composting process increased to a peak of about 56 ° C on the 5th day and remained at a peak temperature for about 7 days (thermophilic stage). The temperature dropped thereafter and remained at a lower level from 12 to 30 days (cooling stage) and then further dropped to the ambient temperature from 30 to the end of composting period(maturing stage). Although the temperature profile of inoculation composting process was similar to those of conventional non- inoculated composting, however, the peak was about 62 ° C on the 3rd day and remained at a peak temperature for about 15 days (Fig 1).

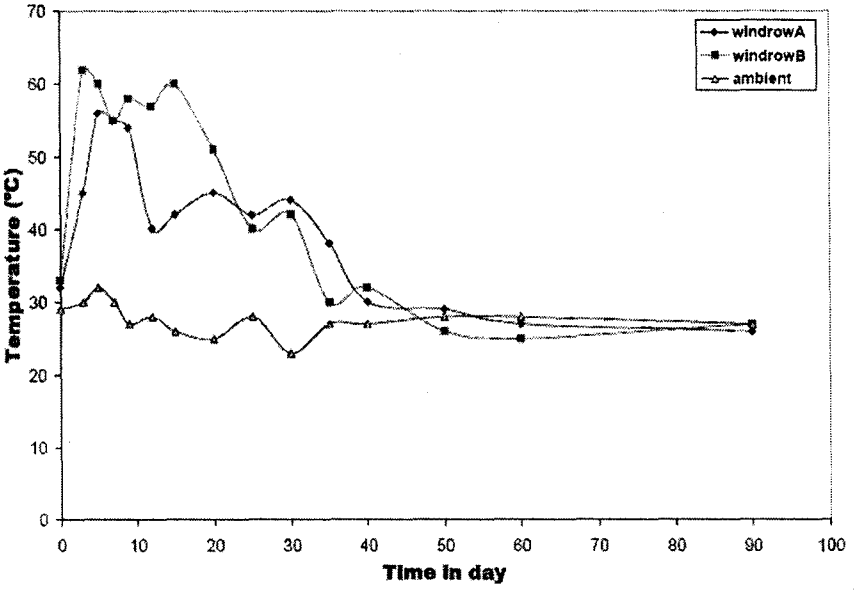

Fig (1) Changes in temperature during composting processes

It was noted from Fig 1 that in the first 3 days, the temperature in inoculated composting process was higher than those in the uninoculated. This is perhaps because of the inoculum contained a large quantity of microorganisms and could quickly decompose soluble and biodegradable organic matter such as monosaccharide, starch, lipids and proteins and increase the temperature up to a high value.

In the inoculated composting process, the inoculation of pile with selective microorganisms could not only reach a relatively high temperature but also maintain the high temperature for relatively long

time. Moreover, it delay period could be effectively shortened by inoculating using proper microorganisms (Tiquia *et al* 1997).

Microbial dynamics of composting processes

To investigate the effects of inoculum on composting processes, total aerobic heterotrophic bacteria counts in the composting system were examined. The THB profiles are shown in Fig 2.The THB population in the inoculated composting process was much higher that those in the uninoculated process. At the beginning of the composting, the $\log_{10}$ CFU g^{-1} dry wt varied from 6.5 and 8.8 in the inoculated and inoculated composting processes, respectively (Fig2).The corresponding peak $\log_{10}$ CFU g^{-1} dry wt values were 8 at day 7 and 12 at day 3, respectively This suggested that inoculation composting would require a shorter time to reach the maximum population compared with uninoculated composting. It is clear that counting total heterotrophs is considered a good measure of microbial activity during composting process period.

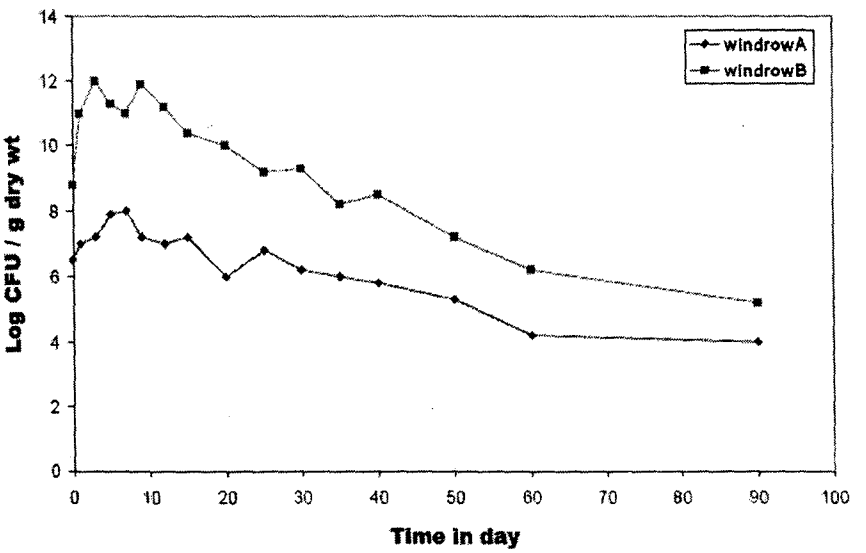

Fig. (2) Total aerobic heterotrophs (THB) profile during composting processes

As high temperature favor cellulose degradation, thermophilic bacteria demonstrated a high count at day 9 and day 3 reaching 6.5 and 8 $\log_{10}$ CFU g^{-1} dry wt for uninoculated and inoculated composting processes, respectively. While, a peak count in inoculated

composting process remained until day 20, the high count in uninoculated gradually decreased (Fig 3).These results are accordance to the temperature profile (Fig 1).

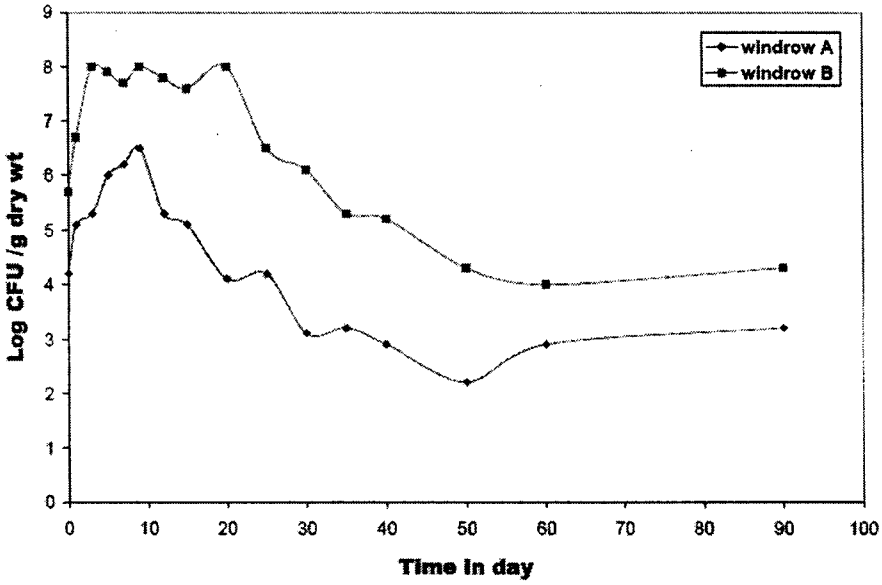

Fig (3) Thermophilic bacteria counts during composting processes

Fungi are believed to be involved in the decomposition of cellulose and lignocelluloses compounds of the compost (Ryckeboer *et al* 2003). In this study, they peaked a high count on day 40 and day 30 (6.3 and 7 $\log_{10}$ CFU g^{-1} dry wt) for uninoculated and inoculated composting processes, respectively, and remained at this level thereafter (Fig 4).

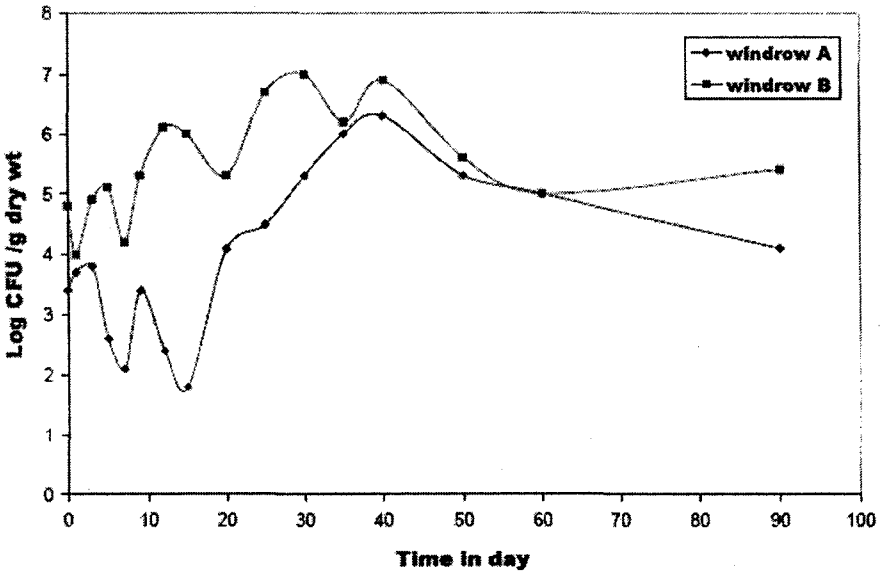

Fig (4) Total counts of fungi during composting processes

The numbers of actinomycetes increased gradually recording a peak value at day 35 and day 20 (7 and 8.5 $\log_{10}$ CFU g^{-1} dry wt) for uninoculated and inoculated composting processes, respectively, and remained high throughout curing phase(Fig 5). Actinomycetes utilize complex organic compounds and their population tends to increase in the latter stages of composting. Their appearance as a grey-white growth at the surface of the material is often considered as an indication of compost maturing (Gazi *et al* 2007).

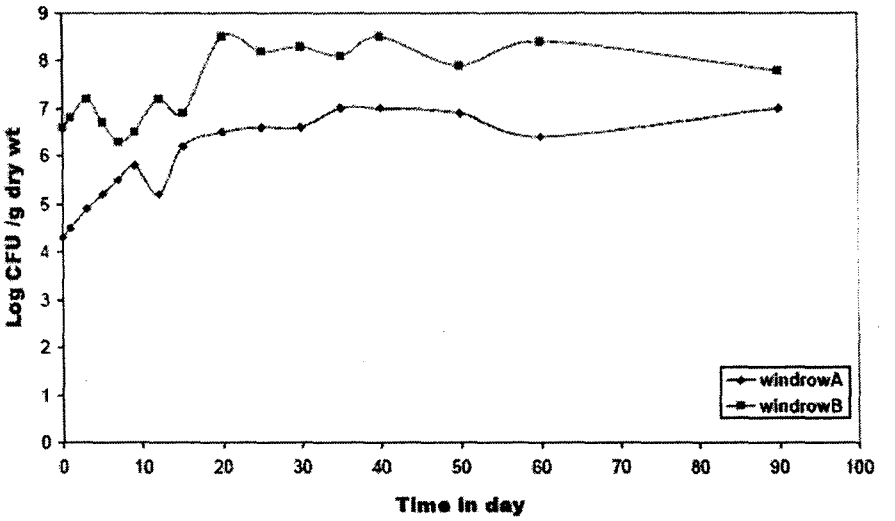

Fig (5) Total counts of actinomycetes during composting processes

Ecological study of microbial population of THB, thermophilic bacteria, fungi and actinomycetes during composting have shown that there was an ecological succession of microorganisms.Microbial biomass was also seen as a significant indicator to illustrate the composting process. High quantities of microbial biomass shorten the composting time and stabilize the extent maturity (Hassen *et al* 2001).

At the end of composting, the inoculated wastes were well mature, it was loose, fine and dark brown, had obvious humic smell and did not attack by flies. However, the wastes without inoculum were loose, coarse and brown, had strong smell.

Two-stage microbial kinetics of the inoculation composting

Although most organic wastes and residues are decomposed by the indigenous microbial flora (Tuomela *et al* 2000). This does not mean the microbes concentration is limiting factor, particularly in the first stage of the composting process. In terms of Eqs 5 and 6 unless X >> Kx,the degradation rate could be improved with the increase of the microbial concentration (X). It was noted the inoculation would be an effective way to improve the microbial concentration and in turn increase the composting rate. In addition, by inoculating mixture of selective microorganisms, maximum degradation rates, KAv varied from 21.93 to 31.15 g kg^{-1} d in the uninoculated and inoculated

composting processes, respectively(Equations 5 & 6 and Figs 6 and 7).

V^1uninoculated = 21.93 X / 4.65 + X ..... (5)

V^1inoculated = 31.15 X / 13.03 + X ..... (6)

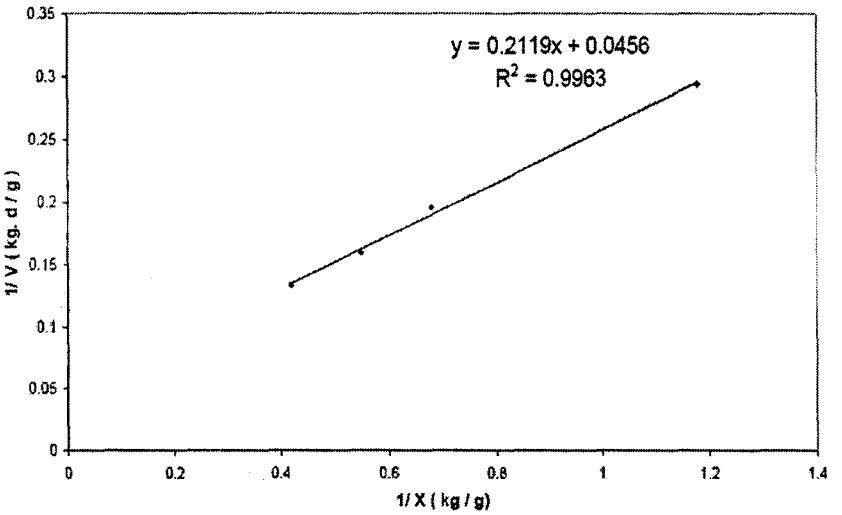

Fig (6) 1/ X vs 1/ V in the first stage of uninculalted- composting process

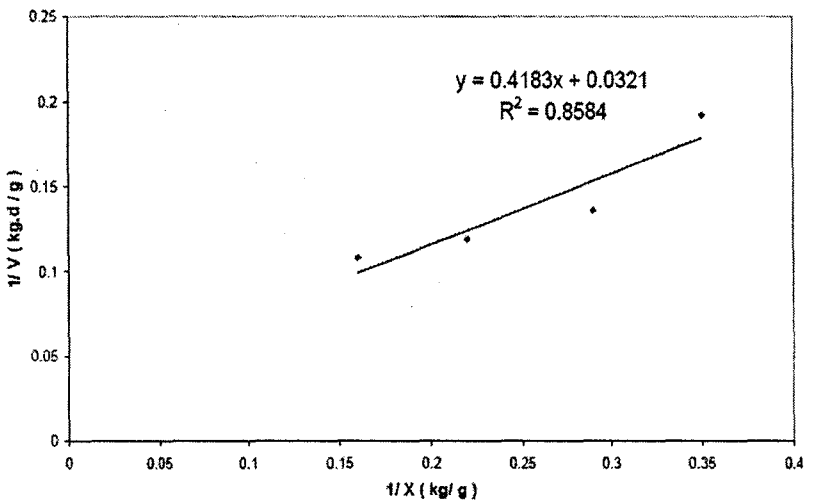

Fig. (7) 1/X vs 1/V in the first stage of inoculated composting process

As the composting goes on, the soluble substrates become less, thus the substrates may become the significant limiting factor to the degradation rate. At this time, the composting process would change to the second stage. During this stage, cellulose fiber and lignin became the main substrates. In this study, KAv (i.e. Vm) in the uninoculated and inoculated composting process were 52.08and 27.86 g kg $^{-1}$ d, respectively(Equations 7 & 8 and Figs 8 & 9). Obviously, the improvement of Vm was significant. However, these values, in this stage, were much higher than those in the first stage. This further suggested that the substrate was the main limiting factor during the second stage. Usually, Km value is an indicator of the composting products stability. The lower the Km, the more stable the composting products. From Esq. 7 and 8 , inoculation could reduce the half degradation rate coefficients Km which were 293.9and 153g kg^{-1} in the uninoculated and inoculated composting processes, respectively(Equations 7 & 8 and Figs 8 & 9). These results indicated that inoculum containing cellulolytic strains could efficiency stabilize the composting process.

V^2uninoculated = 52.8 S / 293.9+ S ..... (7)

V^2inoculated = 27.8 S / 153+ S ..... (8)

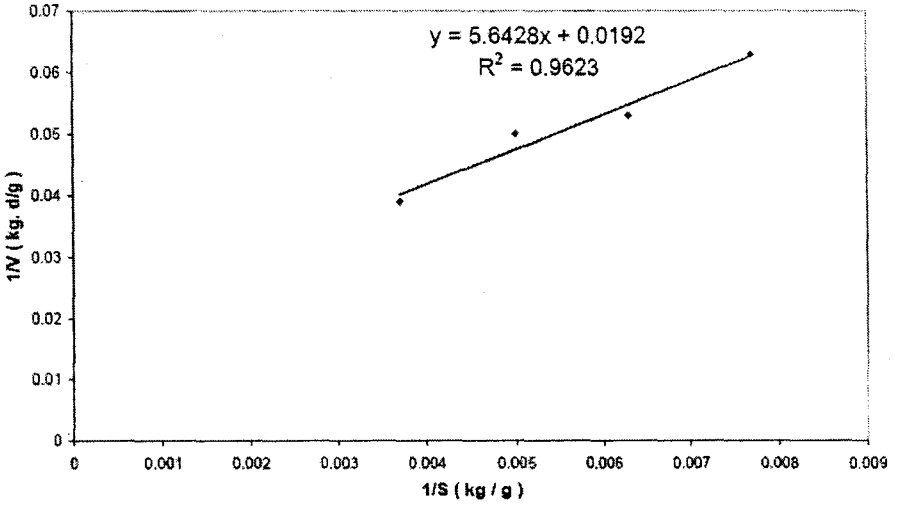

Fig. (8) 1/S vs 1/V in the second of uninoculated composting process

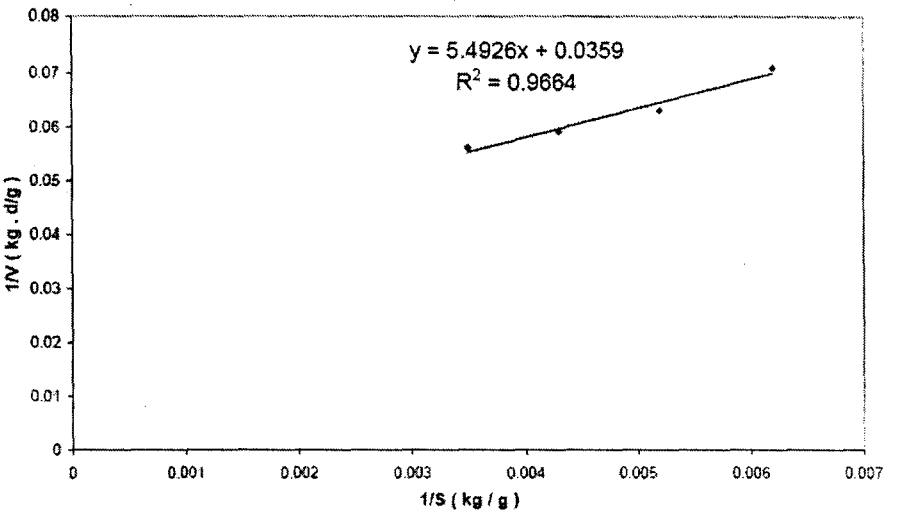

Fig. (9) 1/S vs 1/ V in the second stage of inoculated composting process

According to current Colombian regulations (ICONTEC 2003) the water retention capacity (WRC) for a material suitable for agricultural use has to be equal toils own weight. This means that one gram of organic material should retain at least one gram of water. Table (3) shows the average WRC values for ten repetitions of inoculated and uninoculated. It is established that for both cases the averages were very high with respect to the minimum required by the Colombian regulation and the highest value was obtained for the inoculated composting process with respect to uninoculated (2.54 and 1.8 g g^{-1} , respectively) (Acevedo *et al* 2005).

Table (3) Water retention capacity (WRC) for inoculated and not-inoculated composting processes

Day	non-inoculated		inoculated
	g water /g compost		
40th	1.86		1.44
50th	2.45		1.62
60th	2.32		2.12
90th	3.32		2.33

Conclusion

A composting process by seeding with an inoculum containing *Bacillus megaterium ,Bacillus licheniformis, Trichoderma harzianum* and *Stretomyces cellulosae* ,was found viable for improving the composting efficiency. Two-stage kinetics equations were developed to explain the inoculation composting process. In the first stage, the composting process was limited by microbial concentration, thus, the degradation rate could be improved effectively by seeding inocula. Meanwhile, the delay period could be shortened. In the second stage, inoculation could reduce the half degradation rate coefficient, Km, and in turn to stabilize the composting products and improve the composting quality.

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حركية التلقيح لبعض المخلفات الزراعية لانتاج سماد عضوى

احمد عبيد عبد الحافظ
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لتحسين فعالية انتاج السماد العضوي،استخدمت طريقة اضافة لقاح مكون من باسيللس ميجاتريم ، باسيللس ليشنفورميس ، ترايكودرما هارذيانم ، استربتوميسس سلليلوزي الى خليط مكون من نواتج تقليم الاشجار و نشارة الخشب بنسبة 2.6 :1 علي التوالي مع اضافة اللقاح السابق او بدون اضافة (مقارنة) .

اثر استخدام اللقاح هو تقييمه من حيث الوقت اللازم للحصول علي منتج مستقر و ذات جودة عاليةمن خلال قياس التغيرات الفيزوكيميائية مثل الحرارة ، القدرة علي الاحتفاظ بالمياه ، الكربون العضوي بالاضافة الي الحمل الميكروبي .

من اجل فهم اليات تلقيح كومة السماد العضوي ، اشتقت معادلات حركية انتاج السماد العضوي ذات المرحلتين من خلال الحركية الميكروبية . ولقد اوضحت النتائج ان عملية تلقيح كومة السماد، لم تسجل اعلي درجة حرارة فقط (62° م) بل استمرت علي هذه القيمة لفترة 15 يوم, كما سجلت.النتائج اعلي قيمة لقدرتها علي الاحتفاظ بالماء مع كومة السماد الملقحة بالميكروبات سالفة الذكر(2.54 جرام ماء لكل جرام سماد عضوي طازج) مقارنة مع تلك الغير ملقحة، علاوة علي ذلك ، اظهرتالنتائج ان عملية تلقيح كومة سماد سجلت اعلي قيم من البكتريا الهوائية الهيتيروفية ، البكتريا المحبة للحرارة ، الفطريات و الاكتينوميستات بقيم 12 و 8 و7 و 8.5 لو10 وحدة خلايا لكل جرام وزن جاف علي التوالي .

اظهرت نتائج معدلات حركية انتاج السماد العضوي انه في المرحلة الاولي ، كان التركيز الميكروبي هو العامل المحدد في معدل تحلل المادة العضوية و التي سجلت قيم 21.93 و 32.15 جرام لكل كيلو جرام في اليوم لكلا من الكومة الغير الملقحة (كنترول) و الكومة الملقحة باللقاح علي التوالي , اما. في المرحلة الثانية كان معدل التحلل متاثرا بقيمة (Km) و بالتالي ثبات و استقرار الناتج من السماد العضوي ، لذلك يمكن القول ان استخدام لقاحات محضرة سلفا في انتاج السماد ا لعضوي يحسن من فاعلية و كفاءة انتاج السماد العضوي ويقصر فترة انتاجه ونضجه.