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Microbial Population Dynamics and Exoenzyme Activities in Semidecomposed Organic Substrate Exposed to Low Intensity Colour Lights during Vermicomposting

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A B S T R A C T

During vermicomposting, decomposition of organic matter is mostly carried out by the aerobic microbes, facilitated by the earthworms. Light, an important ecological factor can penetrate up to 3-4 cm into surface soil or organic substrates and therefore is expected to influence the biological function of microbes during decomposition. This study reports the effect of low intensity colour lights on microbial population and exoenzyme activities along with changes in certain soil chemical parameters such as pH and organic carbon in semi-decomposed organic substrate (cattle dung) inoculated with the earthworm *Eudrilus eugeniae* over an incubation period of 42 days. The results indicated that darkness provide the most suitable environment for maximal bacterial population and activities of the enzymes, invertase, amylase and cellulase. The least population of bacteria was observed in the substrate under white light and red lights whereas blue and green lights indicated relatively higher bacterial population and enzyme activities. The maximal depletion in organic carbon was recorded in substrate in dark and the minimal in white light. Soil pH indicated noticeable decline in the substrate exposed to white and red lights. The study thus indicated that darkness provides the most favourable environment for maximal bacterial growth, exoenzyme activities and percent organic carbon reduction in earthworm inoculated organic substrate.

Introduction

Microbial population, biomass and activities determine the rate of decomposition of organics and consequently fertility of soil by regulating bioavailability of nutrients. A number of ecological factors influence soil microbial population and enzyme activities (Liu *et al.*, 2008; Araujo *et al.*, 2009). Exo-enzymes, chiefly excreted by microorganisms in soil and organic substrates play a vital role in decomposition and nutrient cycling (Pavel *et al.*, 2004; Shi *et al.*, 2006). Temperature and moisture have been considered to be important limiting factors to influence microbial growth and metabolism during decomposition (Li and Sarah, 2003). Information are very limited on how visible light could influence bacterial growth and activities (Lucca *et al.*, 2012; Kamel *et al.*, 2016) in soil and other organic

substrates. Since light can penetrate up to 3-4 cm in soil, it is likely to certain extent influence the soil biota especially those which thrive in the surface region.

It is known that UV light kills bacteria, but the bactericidal effects of UV may not be unique since recent studies indicate that visible light also produces a somewhat similar effect. The 405 nm light wave length produce a dose dependent bacterial effect on *Pseudomonas aenuginosa* and *Staphylococcus aureus* at about 95.1% and nearly 90% rate. The 470 nm light effectively killed *P. aenugina* at all dose level but killed *S. aureus* at 10-15 cm² (Guffey *et al.,* 2006, Todars, 2012). Fontana *et al.,* (2015) reported the effect of light on bacterial growth and reported that certain bacteria need light to grow and survive. Many bacteria harbour sufficiently high concentration of endogenous photo synthesizers which are destroyed by intensive irradiation with visible light especially by violet and blue light of wavelength 405nm and 470nm (Ashkenazi *et al.,* 2003; Guffey and Willborn, 2006; Maclean *et al.,* 2008). The irradiation at 625nm (Red light) did not affect *S. aureus*, *E.coli*, and *P. gingivalis*, whereas wavelength of 425nm (Blue) and 525nm (Green) had deleterious effects. *S aureus* can get killed at 525nm (Kim *et al.,* 2013).

Mueller *et al.,* (2010) reported that inactive compounds like auto fluorescent proteins can absorb visible day light (500-700nm) and can emit active electrons producing reactive oxygen species (ROS) leading to an increase in photo killing processes in bacteria. Kamel *et al.,* (2016) examined the influence of non-coherent polarized light upon growth of bacteria *S. aureus, E. coli, P aeruginosa* and found that blue and green spectra reduced the growth of the bacteria relative to other colours. Therefore it is apparent that different colour lights have variable impact on bacterial growth and metabolism.

Vermicomposting of organic substrates is conventionally carried out in dark with the epigeic earthworms *Eudrilus eugeniae* and *Eisenia fetida*. No information is available on the bacterial activity in the substrate in response to light exposure. Therefore the present study was undertaken to observe the effects of low intensity colour lights on the bacterial population and certain exoenzyme activities in semidecomposed cattle dung. The changes in pH and percent organic carbon during vermicomposting were also assessed.

Materials and Methods

Eudrilus eugeniae was procured from the vermiculture unit of Government Quality Control Laboratory, Bhubaneswar, India. The worms were acclimatized in semi-decomposed cattle dung taken in rectangular earthen pot (40 cms × 40 cms) in dark for 7 days. Five treatment pots of size (30cms × 30cms) in triplicate were labelled as T1 (Dark), T2 (White), T3 (Blue), T4 (Green), T5 (Red) respectively and each pot was filled with 1 kg of semi decomposed cattle dung. Twenty clitellated earthworms of identical size were transferred to each treatment pot. Thermo cool sheets were used to cover the pots. The moisture level in the substrate was maintained at 40-50% by intermittent sprinkling of distilled water. LED lights (0.5 watt) of white, blue, green and red colours were fixed at the center on the inner side of the thermocool sheets for treatments T2 toT4. T1 was taken as control with no light provision. Substrate samples were collected from 3-4cm depth from each pot at an interval of 7 days for chemical and microbiological and enzymatic studies. The pH of the samples was measured by a digital pH meter (Systronics). Organic carbon (OC) and organic matter (OM) were measured as per Walkley and Black (1934). Bacteria were isolated by serial dilution and spread plate method (Parkinson *et al.,* 1971). The activities of amylase, invertase and

cellulase were measured as per Ross and Robert (1970). Statistical analysis of data was done for ANOVA using SPSS 6.0 software.

Results and Discussion

The percent change in pH, organic carbon and total organic matter have been depicted in Figure 1a. Changes in the microbial population and enzyme activities with respect to the control have been presented in Figure 1b to 1e. The highest percent increase in pH (1.8%) of the substrate was observed in T1 and the highest decrease (6.5%) in T2. Soil pH ranged from 7.61 on the 42nd day in T1 to 5.94 on 28th day in T2. Statistical analysis indicated non-significant variation in the substrate pH between different colour light exposures over the experimental period. The maximum decrease (63.88%) in OC in the substrate was observed in T1 followed by 50% in T2. The highest OC (5.6 g/kg) was noted on the 42nd day of T5 and lowest (1.3 g/kg) OC on the same day in T1. The highest (9.65g/kg) OM was recorded on 42nd day in T5 and the lowest (2.24 g/kg) OM on the same day in T1. Statistical analysis indicated non-significant variation in OC and OM of the substrate between treatments.

The maximum percent increase in bacterial population (96%) was observed in T1. The maximum decrease (34.11%) was observed in T2. The colony forming units (CFU) ranged from 25-49 x10^4 CFU/g soil and was highest on 14th day in T3. The lowest microbial population was observed in T5. Statistical analysis did not indicate significant variation in the microbial population between treatments.

For exoenzyme activities, the maximum percent increase in amylase activity was observed in T1 (75.92%) during the experimental period. The enzyme activity decreased by 18.18% in T2 over the period. The enzyme activities in T3 and T4 did not show appreciable variations. The highest amylase activity (0.190 mg glucose/g soil/h) was observed on the 42nd day in T1 and lowest (0.074 mg glucose/g soil/h) on the 1st day in T1. Cellulase activity indicated maximum increase of 24% in T1. The enzyme activities decreased by 15% in T2 over the experimental period. The highest cellulase activity (0.087 mg glucose/g soil/h) was observed on the 42nd day in T1 and lowest (0.048 mg glucose/g soil/h) on this day in T2. T3 and T4 did not show much variations. Invertase activity varied between treatments. The highest (7.4%) increase in the enzyme activity was observed in T1. The enzyme activity decreased by 16% in T2. The highest invertase activity (2.693 mg glucose/g soil/h) was noted on the 42nd day in T1 and lowest (1.978 mg glucose/g soil/h) on the 21st day in T1. Statistical analysis of data indicated non-significant variation in the activities of the enzymes between treatments.

In the present study variations were observed in the bacterial population and exoenzyme activities in the organic substrate exposed to different low intensity light colours and it was observed that white light exerted the maximum inhibitory effect on bacterial growth and enzyme activities relative to other colour lights and darkness. Guffey *et al.*, (2006) have reported that the 405 nm light produce a dose dependant bactericidal effect on *P aenuginxa* and *S aureus*. They also observed that different wave lengths of light had differential effects on certain bacteria. For example, 470 nm light effectively killed *P. aenugina* at all doses but killed *S, aureus* at 10-15Jcm².conditions. Fontana *et al.,* (2015) observed that blue light eliminates the black pigments in *Porphyromonas gingivalis* and has inhibitory effects on these bacteria. The results obtained in the present study support the earlier findings that light inhibits bacterial activity and darkness favours bacterial growth and multiplication.

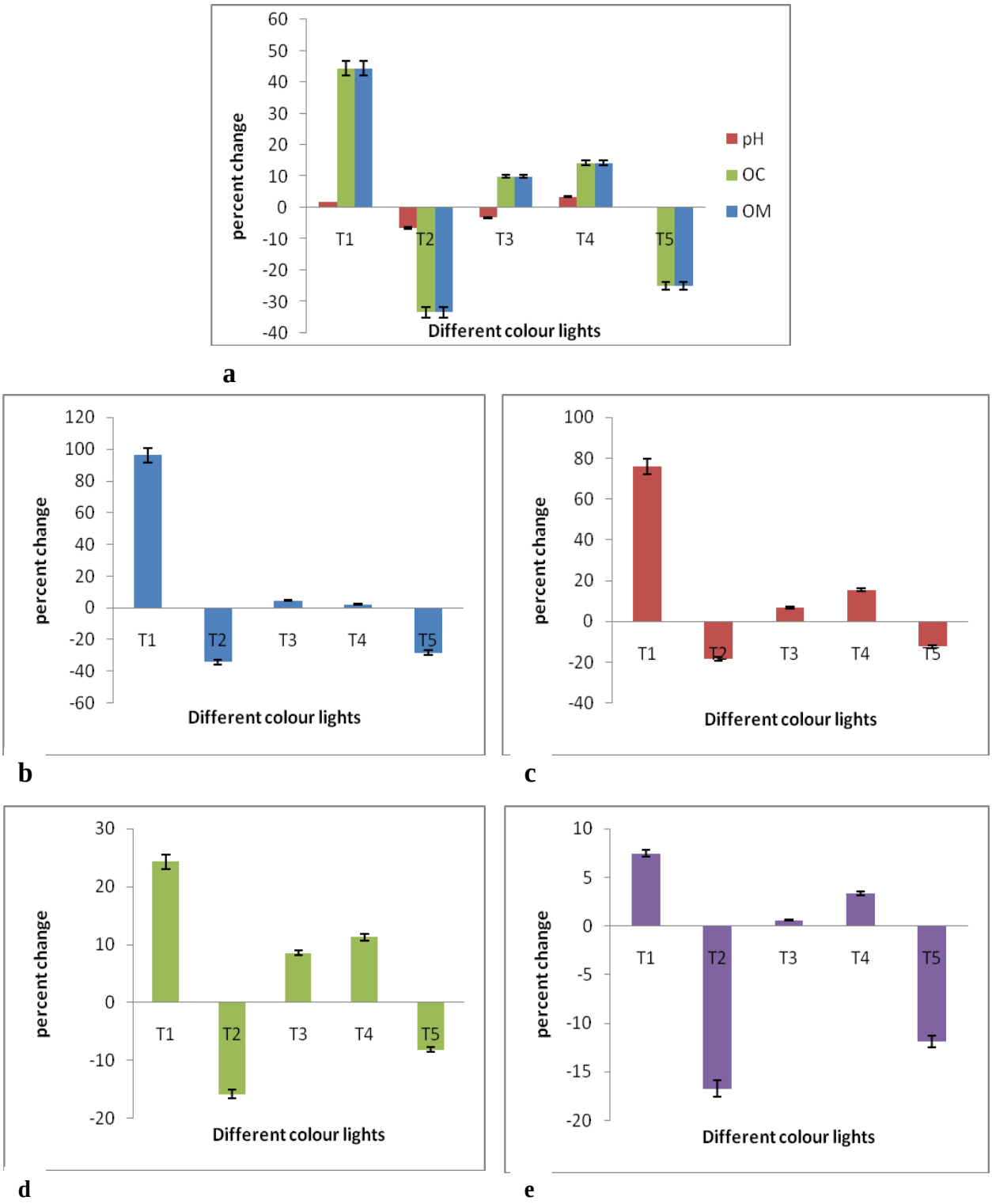

Figure.1: Percent change of a) pH, OC, OM, b) bacterial population, c) amylase activity, d) cellulase activity, e) invertase activity in the organic substrate in control and exposed to different colour lights. T1-Dark,T2-White,T3-Blue,T4-Green,T5-Red

However, contradictory results have been reported by Kamel *et al.,* (2016) who observed increase in bacterial count of *S. aureus*, *E. coli* and *P. aeruginosa* in laboratory conditions with exposure to non-coherent polarized white light relative to blue, red and green lights. Ashkenazi *et al.,* (2003); Guffey and Willborn (2006) and Maclean *et al.,* (2008) observed that many bacteria harbor sufficiently high concentration of endogenous photo-synthesizers which are destroyed by intensive irradiation with visible light especially by violet and blue light of wavelength 405nm and 470nm. Kim *et al.,* (2013) observed that irradiation at 625nm (Red light) was not bactericidal to *S. aureus, E. coli,* and *P. gingivalis*, whereas wavelength of 425nm (Blue) and 525nm (Green) had deleterious effects. *S. aureus* was also killed at 525nm. Mueller *et al.,* (2010) reported that inactive compounds like auto fluorescent proteins can absorb visible day light (500-700nm) and emit active electrons producing reactive oxygen species (ROS) leading to an increase in photo killing processes in bacteria. Lucca *et al.,* (2012) reported that blue LED light (470 nm) effectively inhibits growth of bacteria and filamentous fungi.

In the present study, all the treatments were inoculated with the earthworm *E. eugeniae* and exposed to different coloured lights at low intensity. The variation in the bacterial population and enzyme activities was most likely due to the dual effects of light exposures and earthworm activities. The results also suggest that darkness provides the most favourable environment for maximal bacterial growth and white light exerts an inhibitory effect. The relatively high percent reduction in organic carbon in T1 with the highest population of bacteria and enzyme activities support this hypothesis. Other low intensity colour lights apparently do not influence the bacterial growth and exoenzyme excretion substantially in the organic substrate during the process of decomposition. The study thus indicated that bacterial population and exoenzyme activities in semi-decomposed cattle dung are influenced by variation in light colours during vermicomposting but this variation was not significant. White light had the inhibitory effect on bacterial growth and enzyme excretion. Darkness provides the most favourable environment for maximal bacterial growth and exoenzyme activities in the organic substrate and consequent decomposition of organics.

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