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BIODEGRADATION EVALUATION OF POLY (3-HYDROXYBUTYRATE-CO-3-HYDROXYVALERATE (PHBV)/NANOCRYSTALLINE CELLULOSE (NCC) BIO-NANOCOMPOSITE RESINS

RINGKASAN: Satu bahan bio-nanokomposit poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV)/Nanocrystalline cellulose (NCC) bercampur dengan 10 % (w/w) NCC yang permukaannya telah dimodifikasikan, disediakan dengan nisbah berat 90/10 menggunakan kaedah "melt mixed". Di sebalik kelebihan polimer ini, PHBV juga mempunyai kelemahannya yang tersendiri disebabkan oleh sifat semula jadinya sebagai poliester hablur yang mudah rapuh dan keras. Ciri-ciri ini membataskan aplikasi polimer ini dalam banyak bidang. Oleh sebab itu, polimer campuran (*nanofiller*) merupakan salah satu cara untuk mengatasi masalah ini secara ekonomikal. Polimer campuran ini terdiri daripada polimer-polimer yang berbeza seperti NCC. Polimer campuran ini dapat meningkatkan sifat mekanikal polimer tersebut berbanding dengan polimer asal serta meningkatkan kekuatan terhadap impak pada suhu rendah dan juga meningkatkan kekuatan terhadap rintangan haba. Walau bagaimanapun, berdasarkan pengetahuan kami, kadar biodegradasi PHBV/NCC tidak dikaji secara meluas. Artikel ini membentangkan pengujian biodegradasi PHBV/NCC menggunakan kompos terkawal pada skala makmal dengan menggunakan alat *Microbial Oxidative Degradation Analyser* (MODA) berpandukan ISO 14855-2: 2007. Pengujian biodegradasi dijalankan pada suhu 58 °C menggunakan kompos yang diperolehi daripada sisa pertanian. Tahap biodegradasi PHBV/NCC ditentukan dengan menyukat kandungan karbon dioksida yang terhasil. Hasil pengujian ini mendapati kadar biodegradasi adalah 77.10 % selepas penggeraman selama 180 hari. PHBV/NCC juga disimpulkan sebagai polimer yang boleh terbiodegradasi dengan peratusan degradasi sebanyak 94.76 % apabila dibandingkan dengan bahan rujukan (selulosa) berdasarkan ISO17088:2008 dan EN13432:2000.

ABSTRACT: A bio-nanocomposite poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV)/Nanocrystalline cellulose (NCC) blends with inclusion of 10 % (w/w) of surface modified NCC was prepared with the mass proportion of 90/10 using a melt mixed technique. However, despite its superior properties, PHBV also has its disadvantages due to its nature of being a highly crystalline polyester which exhibits stiffness and brittleness. This less desired traits restricts their application in many fields. To overcome this, the most efficient and economical approach is to incorporate polymer blends (nanofiller) of different polymers, such as NCC that have could enhance the mechanical properties compared to the original polymers as well as improve the impact strength at low temperature and heat resistance. However, the biodegradation rate of PHBV/NCC based to our best knowledge, has not been widely studied. In this paper, the biodegradability of PHBV/NCC was tested under a laboratory-scale controlled composting using Microbial Oxidative Degradation Analyser (MODA) according to ISO 14855-2:2007. Biodegradation evaluation was performed at 58 oC using compost derived from agricultural waste. The degree of biodegradation of PHBV/NCC was measured by measuring the carbon dioxide evolved. It was observed that the biodegradable rate was 77.10 % after 180 days incubation. PHBV/NCC was also concluded to be a biodegradable polymer with the degradation percentage of 94.76 %, relative to cellulose as a reference material based on ISO 17088:2008 and EN13432:2000.

Keywords: Bio-nanocomposite, PHBV/NCC, Biodegradation, Laboratory-scale composting

INTRODUCTION

Recently, there is an increase concern on the extensive use of plastic materials in many applications due to its superior properties. However, these materials are made from non-renewable fossil fuel that are difficult to be degraded and stays in the environment for a very long time. Furthermore, most of these waste will eventually end up in landfill, disposed in incinerators for energy recovery or finally ends up in our environment when recycling practices are not properly implemented (Singh *et al.*, 2017). Therefore, a significant alternative is needed to overcome this. One viable option is to develop a bio-based and biodegradable plastics that are readily accessible to microbial degradation (Funabashi *et al.*, 2009). These biodegradable plastics inclusive of polymers derived from biomass such as agro resources of corn, sugarcane, rice hull and etc.; polymers that are conventionally and chemically synthesised and the monomers are derived from plant such as poly(lactic acid) (PLA) and polycaprolactone (Shah *et al.*, 2008). Among the various types of biodegradable plastics being developed is polyhydroxyalkanoates (PHAs). PHA has attracted attention from many researchers and industries due to its mechanical and thermal properties which are found to be similar to conventional thermoplastics despite having

good biodegradability (Arcos-Hernandez et al., 2012; Iggui *et al.*, 2015; Sedničková *et al.*, 2018). A typical example of PHA is poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) which can be produced either by yeast and fungi, but mostly by bacteria i.e. pure wild type or genetically modified cultures obtained from various agricultural raw materials (Dasan *et al.*, 2017). Figure 1 shows the chemical structure of PHBV. The hydroxyvalerate (HV) content of the PHBV determines its physical properties and can be manipulated by controlling the carbon source supplied during the production stage (Weng *et al.*, 2011). Due to this, PHBV can be synthesised to be used in a wide range of plastic applications and products (Koller, 2017).

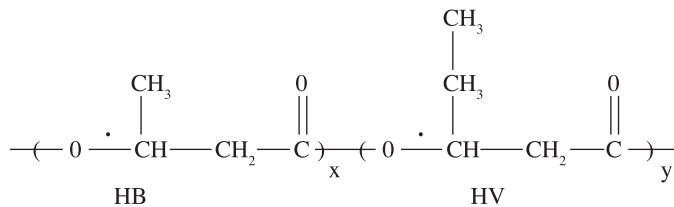


Figure 1. Chemical structure of PHBV

However, despite its superior properties, PHBV also has its disadvantages due to its nature of being a highly crystalline polyester which exhibit stiffness and brittleness (Passos, 2015). This less desired traits restricts their application in many fields (Ismarul *et al.*, 2017). To overcome this, the most efficient and economical approach is to incorporate polymer blends (homogenous or heterogeneous mixtures) of different polymers that could have enhance the mechanical properties compared to the original polymers as well as improve the impact strength at low temperature and heat resistance (Dasan *et al.*, 2017). One example is blending PHA with a ductile biodegradable polymer i.e. polybutylene succinate (PBS) and polycaprolactone (PCL). However, like most polymers, PBS and PCL are found to be thermodynamically immiscible with PHA. This will lead to the decrease in the mechanical properties of the blend, thus defeats the purpose altogether (Yu *et al.*, 2011).

Nonetheless, to produce a fully renewable and environmental friendly nanocomposites, both the polymer matrix and the nano-reinforcement have to be derived from renewable resources. Nano-fillers has found to be an excellent compatibilizers of immiscible blends. Nanocrystalline cellulose (NCC) is a type of "green" nanofiller developed from cellulose fibre by acid hydrolysis. Cellulose on the other hand is a one of the major component of natural fibre that are commonly used as an organic filler material for the composite preparation due to their high levels of strength and stiffness per unit weight (Asari @ Mansor *et al.*, 2016). Furthermore, its high aspect ratio plays a structural role in which they act as a reinforcement to improve the mechanical and barrier properties of the matrix. Due to this, NCC has been widely tested to reinforce many polymer matrices, such as PHBV and shows promising reinforcing characteristic (Ferreira *et al.*, 2018).

Understanding the biodegradation properties of bio-composites like PHBV/NCC is important as it will primarily affect the areas of application of this biopolymer. One major areas of application are in agricultural sector, whereby, in the past, PHBV is being applied as plastics films for crop protection as well as plastic mulch replacing conventional non-degradable plastics (Brunel *et al.*, 2014). Currently, many investigation has already been conducted in studying the degradation behaviour of PHAs (Altaee *et al.*, 2016; Emadian *et al.*, 2017; Rosa *et al.*, 2003). Weng *et al.* investigated the biodegradation of PHBV films in a pilot-scale composting condition (Weng *et al.*, 2010). They found that the biodegradation behaviour of PHBV is similar when compared in both the pilot-scale composting and laboratory-scale. Acros-Hernandez *et al.* studied the degradation of mixed culture (activated sludge) PHAs produced in waste water treatment and confirms its degradation under controlled conditions in soil (Arcos-Hernandez *et al.*, 2012). They also found that biodegradation occurs primarily at the polymer surface. This is further supported by Numata *et al.* who studied the enzymatic degradation of PHA materials and Hermida *et al.* who observes the degradation of PHBV via surface erosion mechanism in liquid mineral salt medium (Hermida *et al.*, 2009; Numata *et al.*, 2008). Goncalves and Franchetti investigated the biodegradation of different blends of PHBV (GonÇalves *et al.*, 2013).

However, the biodegradation rate of PHBV/NCC based to our best knowledge, has not been widely studied. This paper investigates the biodegradation rate of PHBV/NCC resin produced from filter paper under composting condition on a laboratory scale according to ISO 14855-2 (ISO14855-2:2018, 2018).

MATERIALS AND METHOD

Preparation of PHBV/NCC Bio-nanocomposite

The PHBV (ENMAT Y1000), (Average viscosity molecular weight, $M_n = 40-50 \times 10^3$ and HV content = 1-2 %) was supplied from Tianan Biologic Materials Co., Ltd. Meanwhile, the NCC was prepared from waste filter papers and washed to remove impurities. Acid hydrolysis was performed at concentration of 55 % sulphuric acid (H_2SO_4) at 50 °C followed by stirring at 300 rpm for 1 hour. The NCC solution was immediately diluted to 10 times fold with distilled water and cooled in an iced water bath before being centrifuged (10,000 rpm for 10 minutes). The solution was then washed three times with distilled water through centrifugation until pH 6-7 was reached. The final suspension was then subjected to ultrasonication (10 minutes at 40 Hz) and oven dried (50 °C for 12 hours). The resulting NCC obtained was then subjected to chemical surface modification via solvent exchange using acetic acid (CH_3COOH) supplied from Sigma-Aldrich. The solvent exchange procedure was performed according to (Ismarul *et al.*, 2017)

The PHBV/NCC bio-nanocomposite blends with inclusion of 10 % (w/w) of surface modified NCC was prepared with the mass proportion of 90/10 using a melt mixed

technique to prepare the blend. The surface modified NCC powder was heated up using a circulating oven (60 °C for 2 hours) to sufficiently remove excess moisture. The PHBV-NCC powder was then pre-mixed using a mechanical stirrer at low speed. A twin screw extruder was used to produce the PHBV/NCC pellets with the following temperature settings; feeding zone (130 °C), mixing zone (180 °C) and die (200 °C). The extruded pellets were cooled in water bath and oven dried (50 °C for 2 hours).

Materials for Biodegradation Test

Cellulose powder with a particle size of less than 20 µm was used as reference material (cellulose microcrystalline; Sigma-Aldrich, USA). Fig. 2 shows the chemical structure of cellulose. Other chemicals used for the biodegradation test by the Microbial Oxidative Degradation Analyser (MODA) (Saida FDS, Japan) instrument are as follows; sodium hydroxide on a talc support in granulated form with 1.6-3 mm size (Merck, Germany), soda lime in small granules with 3-5 mm size (R&M Chemicals, Malaysia) and sea sand (acid washed) with size less than 850 mm (R&M Chemicals, Malaysia).

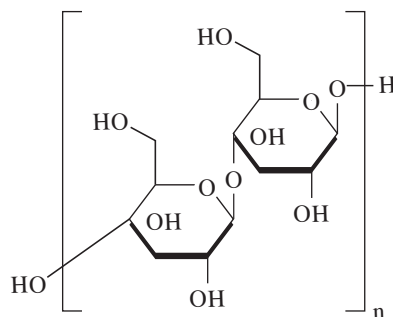


Figure 2. Chemical structure of cellulose

Preparation of Samples for Biodegradation Test

The PHBV/NCC resin was prepared by crushing it using a rotating mechanical mixer (15,000 rpm, 5 L) for 10 minutes (interval of 2 minutes) to prevent overheating of the motor. The crushed PHBV/NCC powder is then separated using a sieve vibrator (250 µm) for 10 minutes using standard sieves. The particle distributions of PHBV/NCC powder was determined using Particle Size Laser Diffraction (model Coulter LS 100Q). The particle size distribution was within 0.4 to 864 µm. Table 1 showed the particle distributions of the crushed PHBV/NCC powder.

Table 1. Particle size distribution of PHBV/NCC

Size (µm)	Percentage of distribution (%)
<5	0.94
< 20	3.47
<50	4.82
< 100	11.8
<1000	100

Preparation of compost as inoculum

A well-aerated compost was used as inoculum taken from a composting plant (Pusat Biomass Bersepadu MPSJ, Bandar Bukit Puchong). Agricultural waste (fallen leaves, hedge trimmings and dead plant matter) were composted for approximately 4-6 months to obtain a mature compost. The matured compost was sieved through a 5 mm standard sieve and any inert materials like glass, stones and metals were removed manually. At this stage, the compost is stable enough to be stored (up to 3 years) under cool temperature to prevent from excessive drying. The characteristic of the compost are shown in Table 2. However, prior to usage, a pre-incubation step is necessary to help acclimatize the microorganism to the actual test condition (Kunioka *et al.*, 2006). This step also helps the reactivation of the biological activities of the microorganisms that are in a dormant stage due to storage time. To further enhance the composting microorganism and adding more source of mineral salts, livestock excretion (fresh cow dung) was added to the sieved compost. The carbon/nitrogen ratio was adjusted to 15 and the carbon/phosphorus ratio was adjusted to 30.

The compost was prepared by mixing sea sand (0.1-0.315 mm, 320 g) to the pre-incubated compost (60 g of dry solid). Sea sand was added to facilitate good aerobic condition as well as increasing the water-holding capacity and homogeneity between the compost and test material (Funabashi *et al.*, 2009). Water was added to achieve a moisture content of 65 %. The compost mixture was then re-activated (at 58 oC) and gently mixed once a day for 1 week with the moisture content maintained at 65 % before initiation of test.

Table 2. Characteristics of compost used for biodegradation testing

Parameter	Concentration
Total solid, TS (%)	41.6
Volatile solid, VS (%)	58.6
Carbon (%)	37.4
Hydrogen (%)	4.7
Nitrogen (%)	2.7
Sulphur (%)	0.4
Oxygen (%)	23.7
C/N ratio	13.8
pH (10% solution)	7.1

Biodegradation evaluation under laboratory-scale composting conditions

The biodegradation test was conducted according to ISO 14855-2 test method using MODA instrument. This respirometric system is equipped with an air supply system, composting bioreactor and a complete system for the quantification of the evolved carbon dioxide (CO₂) during aerobic biodegradation. The composting bioreactor was maintained at a constant temperature of 58 °C ± 2 °C to simulate the optimum condition for thermophilic composting. Fig. 3-5 shows the MODA instrument.



Figure 3. Microbial Oxidative Degradation Analyser (MODA) instrument



Figure 4. Absorption Column for trapping evolved carbon dioxide



Figure 5. Incubation chamber for the six composting vessels

The PHBV/NCC powder (10 g) was added to the re-activated compost (compost with cow dung and sea sand; 400 g; moisture content 65 %) and carefully mixed before transferring to a composting vessels. The same amount of reference material (cellulose, 10 g) was prepared in the same way to monitor the inoculum (compost) activity. A blank control was prepared by using only the inoculum as a mean to

monitor the respiration activity of the compost as the CO₂ evolved from microbial respiration does not contribute to the mineralisation of the test sample. Duplicates of each blank control, reference material and test material were prepared. The composting vessels were incubated at 58 °C ± 2 °C for 180 days with the air flow rate of 30 ml/min of CO₂ free air being supplied to each vessels. The ammonia released from the reaction vessels was trapped with 1 M sulphuric acid. The inoculum in the control blank composting vessel produces 41 mg CO₂/g volatile solid (VS) during the first 10 days of the test which indicates that the compost used as inoculum was stable.

During the biodegradation test, the amount of evolved CO₂ and water was determined from the weight increment of the absorption column. The absorption column was measured daily (once a day; excluding weekend and public holidays). The compost from each composting vessel were mixed gently once a week to ensure uniform contact between the test material and compost and also to prevent extensive channelling from occurring inside the vessel. The water content were carefully monitored and were kept in the range of 80 % to 90 % throughout the incubation period.

Calculation

The amount of CO₂ evolved was compared with the theoretical amount of CO₂ (ThCO₂) of PHBV, which is the degree of biodegradation. The ThCO₂ of the PHBV were calculated based on the carbon content obtained from elemental analysis.

$$\text{ThCO}_2 = m \times w_c \times \frac{44}{12} \quad (1)$$

where, m is mass of test material introduced in the composting vessel (grams) and w_c is carbon content of test material (expressed as mass fraction). Meanwhile, the value 44 and 12 are the molecular and atomic masses of carbon dioxide, respectively.

The percentage of biodegradation of PHBV was calculated from the amount of CO₂ evolved during each measurement interval.

$$= \frac{\Sigma(\text{CO}_2 \text{ produced})_{\text{Test Vessel}}^{\text{time}} - \Sigma(\text{CO}_2 \text{ produced})_{\text{Blank}}^{\text{time}}}{(\text{ThCO}_2)_{\text{PHBV}}} \times 100 \quad (2)$$

Where the degree of percentage of biodegradation of PHBV was calculated from the cumulative amount of evolved CO₂ (grams) during the test period and was subtracted with the mean cumulative amount of evolved CO₂ (grams) obtained from the control blanks. This is then compared with respect to the maximum ThCO₂ that could be released.

RESULTS AND DISCUSSION

Visual observation of physical degradation

The visual observation of the changes of cellulose and PHBV/NCC during and at the end of the biodegradation test was observed and the photos are shown in Fig. 6. It can be observed that PHBV/NCC powder became smaller to the extent of non-visible with increasing composting time. The same observation can be seen with cellulose. After 40 days of composting, it was difficult to observe the presence of PHBV/NCC in the compost. Actinomycetes (white filamentous hyphae) growth was observed during the first 2 weeks of incubation. These actinobacteria play a key role of microbial community during composting as the ability of actinobacteria to decompose lignocellulose into soluble carbohydrate and can accelerate the degradation of cellulose in composting (Steger, 2007).

Biodegradation rate and mechanism of degradation

The carbon content of cellulose (calculated based on its chemical structure) and PHBV/NCC (calculated by elemental analysis) is 44.44 % and 54.67 % respectively. The ThCO₂ and biodegradation rate of cellulose and PHBV/NCC is shown in Table 3. Cellulose are commonly used as the reference material in many biodegradation analysis due to their hydrophilic characteristic and that their biodegradation occurs relatively quickly. The biodegradation behaviour of cellulose is also well studied (Castro-Aguirre *et al.*, 2017; Funabashi *et al.*, 2009; Gonçaves & Franchetti, 2013; Kunioka *et al.*, 2006, 2009; Snook, 1994). Cellulose requires cellulases for its degradation, which is an inducible enzyme that are synthesised by large numbers of microorganisms, either cell-bound or extracellular during their growth on cellulosic materials.(Kunioka *et al.*, 2006). Furthermore, cellulases has the ability to degrade cellulose to oligomers or glucose (monomer unit), facilitating the biodegradation process. Therefore, the ISO method is considered to be valid if the biodegradation degree of the reference material is greater than 70 % after 45 days, indicating good quality compost with active cellulases activity.

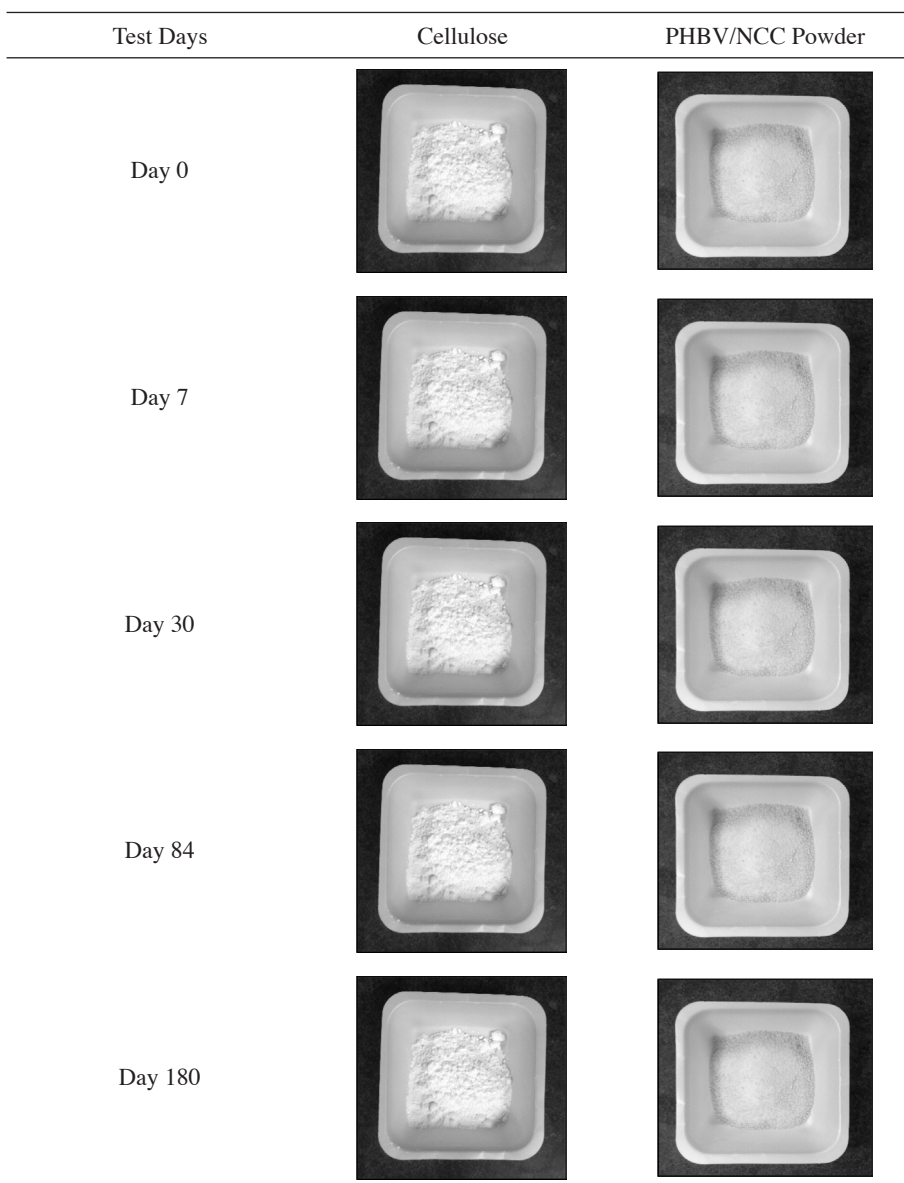


Figure 6. Visual observation of inoculum for Cellulose and PHBV

The incubation period was extended to the maximum of 180 days since both the cellulose and PHBV/NCC did not reach plateau phase of biodegradation within the standard time of 45 days as stated in the ISO method. At the end of the biodegradation test, the biodegradation curves of cellulose and PHBV/NCC were determined and shown in Fig. 7. The cumulative CO₂ of each composting bioreactor were also calculated and shown in Fig. 8. It was observed that during the initial stage of degradation (first 30 days) the CO₂ evolution of cellulose and PHBV/NCC

was identical, suggesting a similar biodegradation mechanism occurring. Weng et al. had proposed that PHBV/NCC degradation process starts with the biodeterioration stage, i.e. the microorganism erodes the surface PHBV/NCC resulting in the formation of many filament-like residues (Weng *et al.*, 2011). This is then followed by the depolymerisation stage in which the filaments will start to degrade in three dimension reducing their molecular weight forming many cavities. The cavities will then undergo microbial assimilation as food sources and finally will be completely mineralised and consequently CO₂ gas will be increasingly generated (Gonçaves & Franchetti, 2013; Kijchavengkul *et al.*, 2006). This mechanism of degradation was in agreement with the finding from (Numata *et al.*, 2008)

The biodegradation curve starts to plateau after 70 days with the average degree of biodegradation of cellulose and PHBV/NCC were 81.36 % and 77.10 % respectively at the end 180 days of incubation. The relative degree of biodegradation of PHBV/NCC with relative to cellulose as a reference material is 94.76 %, which indicates that is as a biodegradable material in accordance to ISO 17088:2008 (ISO17088:2008, 2008). ISO 17088 stipulates that a truly biodegradable material should have a relative degree of biodegradation of not less 90 %.

However, the resulting compost of PHBV/NCC obtained at the end of the test could not be confirmed to comply as compostable as no ecotoxicological test was conducted to evaluate its final quality.

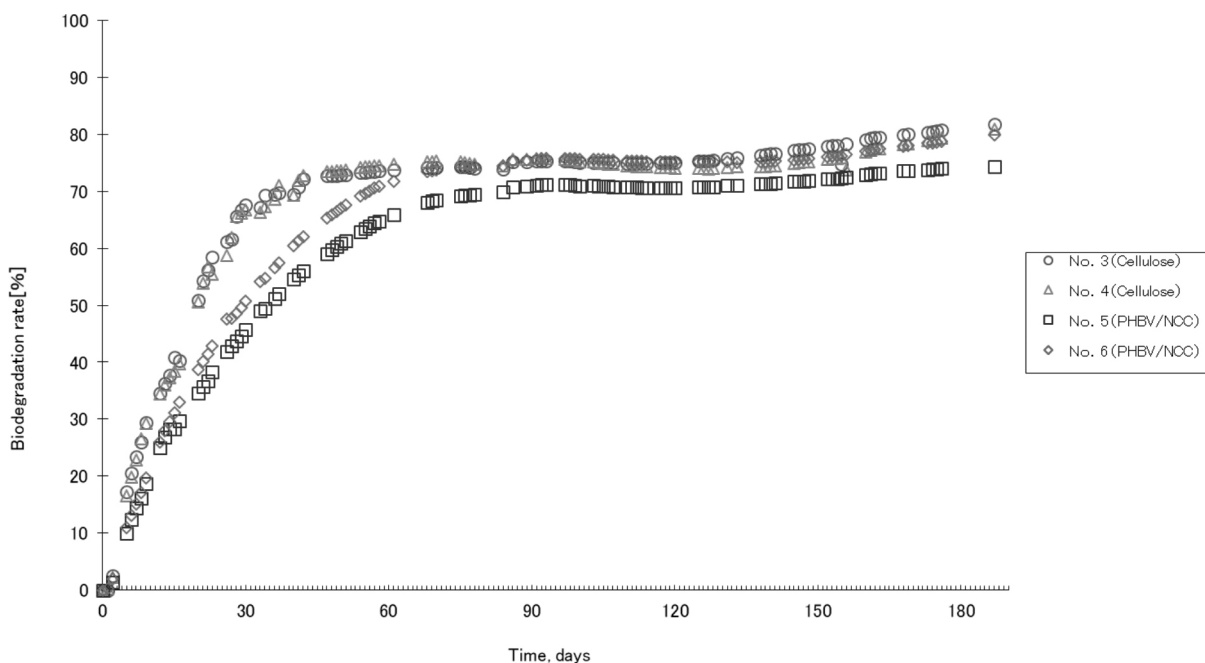


Figure 7. Biodegradation rate for Cellulose and PHBV/NCC

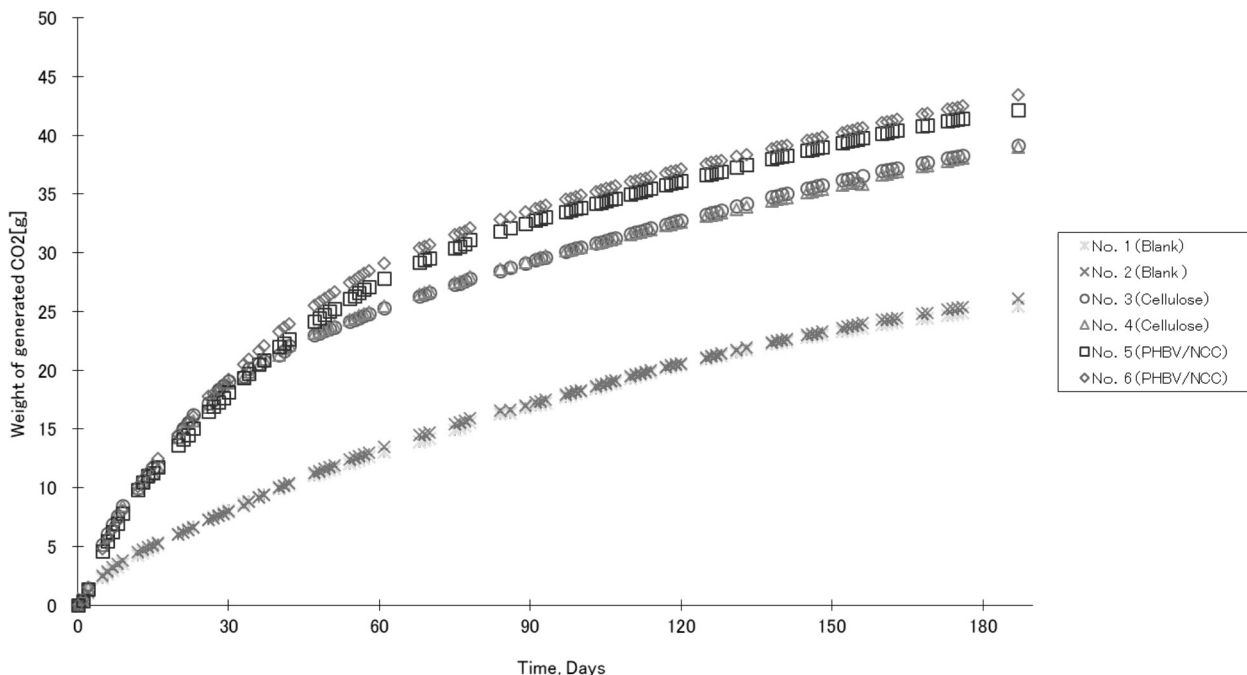


Figure 8. Amount of cumulative carbon dioxide evolution of Cellulose and PHBV/NCC

Table 3. Theoretical amount of CO₂ and biodegradation rate of cellulose and PHBV/NCC

Composting Bioreactor	Total CO ₂ generated (g)	Theoretical Amount of CO ₂ (g)	Biodegradation (%)	pH compost after 180 days
Blank	1	25.48	–	7.08
	2	26.12	-	7.13
Cellulose	3	39.14	81.78	7.37
	4	39.00	16.30	80.93
PHBV	5	42.18	74.31	7.29
	6	43.41	22.05	79.88

CONCLUSION

It was determined that PHBV/NCC was biodegradable with 77.10 % mineralisation when tested under a controlled compost at 58 °C based on the ISO14855-2 method using Microbial Oxidative Degradation Analyser (MODA) for 180 days. PHBV/NCC

was also concluded to be a biodegradable polymer with the degradation percentage of 94.76 % with relative to cellulose as a reference material based on ISO 17088:2008 and EN13432:2000 (EN13432, 2000).

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