Institut für Nutzpflanzenwissenschaften und Ressourcenschutz (INRES)

Lehr- und Forschungsbereich Pflanzenernährung

der Rheinischen Friedrich-Wilhelms-Universität zu Bonn

Vermicomposting of Organic Solid Wastes as a Biological Treatment for Sanitation

Inaugural – Dissertation

zur

Erlangung des Grades

Doktor der Agrawissenschaften

(Dr.agr.)

der

Hohen Landwirtschaftlichen Fakultät

der

Rheinischen Friedrich-Wilhelms-Universität

zu Bonn

Vorgelegt am

06.08.2012

von

Phương-Nam Nguyễn

aus

Hanoi, Vietnam

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Date of the Oral Test: **06. 08. 2012**

Year of Publication: **2012**

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Phương-Nam Nguyễn

Bonn, den 14.06.2012

Acknowledgements

I would like to give my gratitude to:

PD. Dr. Joachim Clemens, my supervisor for his support, guidance and patience throughout my studies;

Prof. Heinrich W. Scherer for giving me the opportunity to pursue my PhD thesis in INRES institute;

Prof. Heiner Goldbach, the Dean of Institute of Plan Nutrition (INRES) for supporting my scientific career;

Prof. Luu Van Boi, Dean of Chemistry Faculty, Hanoi University of Sciences, Vietnam for his advice and support of my application for a scholarship to study for a PhD in Germany;

the Vietnamese Ministry of Education & Training (MoET Vietnam) and the Deutscher Akademischer Austausch Dienst (DAAD) for awarding me the scholarships which allowed me to pursue this study;

the KOMPLETT project for supporting my experimental equipment and chemicals, especially Mr. Axel Heck and Dr. Yen-Phi for their scientific discussions;

Dr. Andrea Rechenburg in Hygienic und Öffentliche Gesunheit Institute, Bonn University for hygienic parameters testing of my experimental samples;

the international office of Bonn University, especially Ms. Sandra Gröger, for the extra scholarship funding to support me during my dissertation writing;

the TA staff within INRES, especially Ms. Waltraud Koeler, Ms. Angelika Weits, Ms. Lilli Wittmaier, Ms. Angelika Glogau, Ms. Deborah Rupprech and Ms. Brigitte Überbach for their assistance in the lab as well as Ms. Waltraud Schäfer, the secretary of INRES, for her help with my documents;

Ms. Evi Windisch and Ms. Carina Fuhs known as assistants for my experimental remaining and sampling;

Mr. Tran Manh Canh, Vice President of Hanoi Food Corporation agreeing to my application for a scholarship to study aboard;

Dr. Le Van Hoat and Mrs. Hoang Thi Nguyet, my good friends, for advising on my scientific career aboard;

Dr. Thomas Eirchert, Dr. Nguyen Trung Thanh and Mr. Mohammad Abdel-Razek Mohammad for their helps in statistics;

my writing editors: Mr. Jimmy Grimes, Ms. Katie Kinstedt, Ms. Mary McAfee and Ms. Lisa Gygax;

and last but not least Dr. Ute Arnold, and Mrs. Katrin Spoth for supporting and helping me to adapt to the German way of life for the five years I spent in Bonn, Germany.

I am very grateful to my family and friends in Vietnam and also in Germany, especially in Bonn/Köln for their patience, support and belief in me.

To all those who are not mentioned by name but have always been there, know that you are always in my heart. Thank you all.

Dedication

This work is dedicated to:

My parents (**bố Oanh** & **mẹ Dung**) who have always believed in me

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LIST OF ABBREVIATIONS

AB-DTPA	Ammonium bicarbonate diethylene triamine pentaacidic
AT_4	Respiration activity at four days
BGK	The Bundesgütegemeinschaft Kompost
Bs	Separated solids of digested biogas slurry
Bw	Biowaste
Ca	Calcium
CAP(s)	Cast-associated process(es)
CH_4	Methane
Cm	Cattle manure
C_{tot}	Total carbon
CO_2	Carbon dioxide
CUS	Centralized urban sanitation system
D. rubida	*Dendrobena rubida*
D. veneta	*Dendrobena veneta*
DM	Dry matter
DRI	Dynamic respiration index
DSOUR	Specific O_2 uptake rate for solid sample
E. andrei	*Eisenia andrei*
E. eugeniae	*Eudrilus eugeniae*
E. fetida	*Eisenia fetida*
EC	Electric conductivity
EcoSan	Ecological sanitation
EU	European union
Fm	Non-urine faecal matter
FS	Fresh substrate
GAP(s)	Gut-associated process(es)
GHG	Greenhouse gas
Gw	Garden waste
HCl	Chloride acid
Hm	House manure
ISO	International organization for standardization
IWA	International water association
K_{tot}	Total potassium
KCl	Potassium chlorite
KH_2PO_4	Potassium dihydrogen phosphate
Kw	Kitchen waste
L. mauritii	*Lampito mauritii*
L. rubellus	*Lumbricus rubellus*
LC_{50}	Lethal concentration
LFG	Landfill gas
MDG(s)	Millennium Development Goal(s)
MDG7	Millennium Development Goal 7
Mg	Magnesium
MSW	Municipal solid waste
N_{Kj}	Total Kjeldahl nitrogen
N_{tot}	Total nitrogen
Na_2HPO_4	Disodium hydrogen phosphate
NaOCl	Sodium peroxide chloride
NH_3	Ammonia
NH_4^+	Ammonium
NH_4HCO_3	Ammonium hydrogen carbonate

NO_3^-	Nitrate
NOEC	No observed effect on concentration
OD_{20}	Cumulative O_2 uptake in 20 hours
oDM	Organic dry matter
P_{exc}	Exchangeable phosphorus
P_{tot}	Total phosphorus
P. elongata	*Pheretima elongate*
P. excavatus	*Perionyx excavates*
Pm	Pig manure
Pom	Poultry manure
Ps	Paper pulp solid
Pw	Potato waste
RI	Respiration index
S	Sulphur
SEI	Stockholm environmental institute
SOUR	Specific O_2 uptake rate
SRI	Static respiration index
TAN	Total ammonium nitrogen
TOC	Total organic carbon
TS	Total solid
UN	United Nations
VS	Volatile solid
WHO	World health organization
Ws	Wastewater sludge

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SUMMARY

Vermicomposting of Organic Solid Wastes as a Biological Treatment for Sanitation

More than 38 billion m^3 of organic solid wastes (OSW) are produced annually from human, livestock and crop activities worldwide. To achieve the Millennium Development Goal for sanitation (MDG7), several alternatives for OSW treatments have been introduced, some of which follow the concept of ecological sanitation (EcoSan). Based on the EcoSan approach, one biological treatment which is now being widely developed, is vermicomposting.

This thesis examines monoculture (*Eisenia fetida*) and polyculture (*Eisenia fetida* and *Dendrobaena veneta*) vermicomposting of three OSW materials, separated solids of digested biogas slurry (Bs), non-urine faecal matter (Fm) and bio-waste (Bw), to produce hygienically safe products.

Preliminary suitability tests of the three fresh materials for vermicomposting showed that *E. fetida* did not accept these materials, most likely due to unsuitable levels of either high pH (~8.5), EC (~9.0 mS cm^{-1}), or total ammonium nitrogen (TAN) (>10 g kg^{-1}), or low pH (<6.0).

Based on the finding that a small TAN amount (<30 mmol L^{-1}) at alkaline pH (>7.0) dramatically affected the earthworms, the fresh Bs were pre-treated for 2 weeks by composting to eliminate some TAN. With that pre-treatment, the low pH of fresh Bw (<5.0) increased up to 7.5. Consequently, two single pre-treated Bs and Bw were accepted by earthworms for vermicomposting, whereas without pre-treatment, 100% mortality of earthworms was found in single Fm due to increased TAN content (from 25.7 to 526 mmol kg^{-1}) during storage. However, three mixtures of Fm, Bs and Bw (namely BsBw, FmBw and BsFmBw) were accepted and digested by both worm species due to the less toxic influence of TAN (<30 mmol kg^{-1}).

There were no significant differences in physico-chemical properties of all vermicomposted substrates and qualities of products between monoculture and polyculture treatment. After 12 weeks of vermicomposting plus 12 weeks of stabilisation storage for maturing, all 24-week vermicomposts of five OSW materials seemed to be mature (the respiration index for four days (AT_4) <10 mg O_2 g^{-1}), and met the European Union (EU) threshold for compost maturity.

From positive occurrences in 10.0 g fresh OSW, *Salmonella* spp. was not found in 10.0 g of the final products. In addition, numbers of *Enterococcus* spp. and *E. coli* in vermicomposts were reduced strongly (2.7-5.8 and 3.3-6.4 log_{10} units, respectively). According to WHO guidelines for sanitation, these products could be applied safely in agriculture.

ZUSAMMENFASSUNG

Vermikompostierung organischer Abfälle als biologische Maßnahme zur Wiederverwertung und Hygienisierung

Jährlich werden weltweit 38 Milliarden Kubikmeter organischer Abfall produziert, der sich aus menschlichen und tierischen Abfällen sowie Ernterückständen zusammensetzt. Um das Millennium Entwicklungsziel für Sanitation (MDG7) zu erreichen, wurden verschiedene Alternativen zur Behandlung von organischen Feststoffen eingeführt. Einer der in jüngster Zeit stärker weiterentwickelten Ansätze ist die Vermikompostierung.

In dieser Arbeit wurden mittels Monokultur (*Eisenia fetida*) und Polykultur (*Eisenia fetida* und *Dendrobaena veneta*) drei verschiedene organische Feststoffgemische vermikompostiert um hygienisierte Substrate zu erhalten: Feststoffe aus der Biogas-Vergärung (Bs), Fäkalien ohne Urin (Fm) und Bioabfall (Bw).

Erste Eignungstests mit den drei frischen Materialien (Bs, Fm und Bw) zeigten, dass *E. fetida* diese nicht akzeptiert, aller Wahrscheinlichkeit nach aufgrund von extremen pH (<6,0 bzw. >8,5), hoher elektrischer Leitfähigkeit (~9,0 mS cm^{-1}) und hohem Ammonium Gehalt (>10 g TAN kg^{-1}).

Die Ergebnisse zeigten auch, dass geringe TAN Konzentrationen (<30 mmol L^{-1}) bei alkalischem pH (>7.0) starke Auswirkungen auf die Regenwürmer hat. Daher wurde frisches organisches Abfallmaterial (Bs) 2 Wochen durch „Präkompostierung“ vorbehandelt um einen Teil des TAN als NH_3 zu eliminieren. Mit der gleichen Vorbehandlung stieg der pH von frischem Bioabfall von 4,4 auf 7,5 an. Danach wurden die beiden Substrate von den Regenwürmern zur Vermikompostierung akzeptiert. Ohne Vorbehandlung führte die Vermikompostierung zu 100% Sterblichkeit aufgrund der hohen TAN Konzentration (526 mmol kg^{-1}). Aufgrund der geringeren Toxizität bei TAN Konzentrationen <30 mmol kg^{-1} wurden die Mischungen aus BsBw, FmBw, and BsFmBw von beiden Regenwurmarten akzeptiert.

Es wurden keine signifikanten Qualitätsunterschiede zwischen Behandlung mit Monokultur und Polykultur beobachtet. Nach 12 Wochen Vermikompostierung plus 12 Wochen Reife-Stabilisierung erreichten alle Vermikomposte, die aus fünf verschiedenen organischen Feststoffen stammten, eine niedrige Respirationsrate (AT_4 <10 mg O_2 g^{-1}) und damit eine ausreichende Stabilisierung die EU-Richtlinie für Kompost. *Salmonella* spp. wurde -nach positiven Befunden in den Rohmaterialien- nicht mehr im Vermikompost nachgewiesen. Zudem konnte mit Regenwürmern eine stärke Reduzierung von *Enterococcus* spp. und *E. coli* erreicht werden (2.7-5.8 log_{10} bzw. 3.3-6.4 log_{10}). Entsprechend der WHO-Richtlinien für die Verwertung von können diese Produkte als sicher in der Landwirtschaft angewendet werden.

1. INTRODUCTION

More than 38 billion m^3 of organic solid wastes (OSW) are produced annually from human, livestock and crop activities worldwide (Surthar, 2010). Three categories that represent a major part of OSW materials include bio-waste from households, 'waste water' substrate (separated faecal matter from toilets), and the solids from biogas plant effluent (digested animal slurries and by-products from crops). Many areas where an intensive agriculture and dense population go hand in hand have a variety of the substrates available, although they are not controlled by centralised sanitation systems (wastewater treatment centres and OSW treatment facilities). In order to achieve the Millennium Development Goal for sanitation (MDG7), these OSW materials must be treated to avoid pathogen spread and to convert the material into valuable products for agriculture (Christensen, 2010a).

1.1. THE MILLENNIUM DEVELOPMENT GOAL FOR SANITATION

The Millennium Development Goals (MDGs) were established by the United Nations (UN) in the year 2000 (Fig. 1.1). They require all countries to focus on global problems linked to issues of the environment and public health (Donat-Castello *et al.*, 2010). The urgency for action in the sanitation sector is obvious, considering the 2.6 billion people worldwide who still lack access to any kind of sanitation. Moreover, 2.4 million annual deaths, consisting mostly of children under the age of five, are caused mainly by sanitation-related diseases and poor hygiene conditions (WHO, 2009).

Figure 1.1. Eight goals for the MDGs to be achieved by 2015 (*www.undp.org*)

The MDG for sanitation (MDG7) was introduced at the Johannesburg World Summit on Sustainable Development in 2002. This set an objective of halving the number of people without adequate sanitation in the 1990s by the end of 2015 (Fig. 1.2). Public health is at the heart of the MDGs. It is central to MDGs 4, 5 and 6 and a feature of the MDG7. The global mission of ensuring environmental sustainability is one way of setting aims to achieve the MDG7 quickly as well as the MDGs in general. However, with the rapid expansion of the global economy and correlated effects within

countries, the global mission may not be successfully implemented at a regional or country level (Wagstaff & Claeson, 2004).

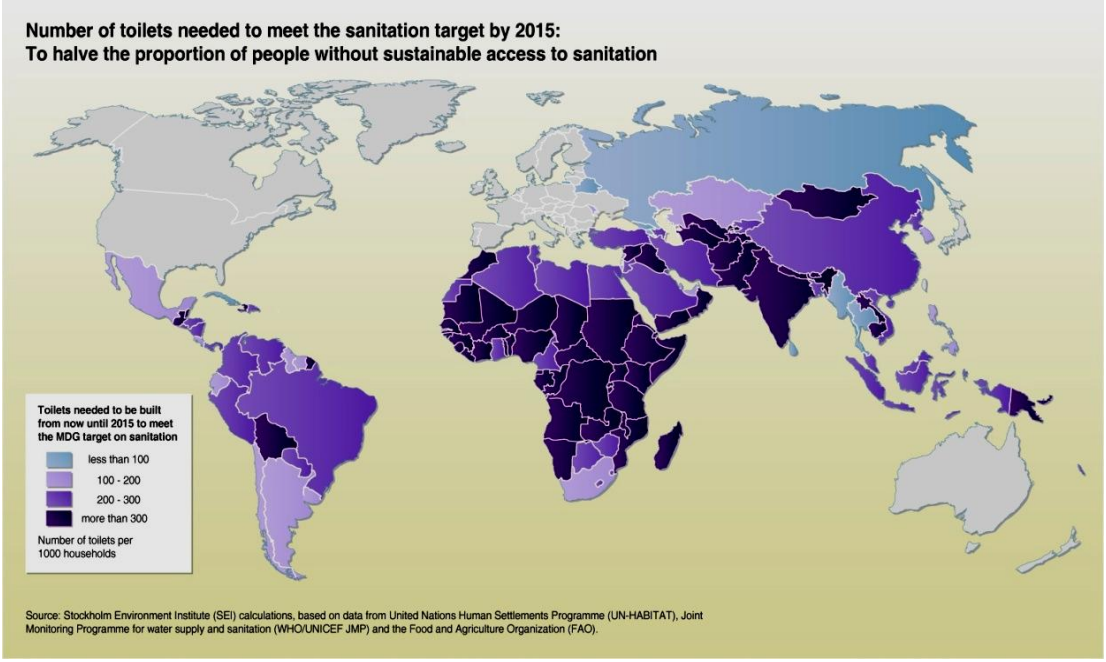

Figure 1.2. Number of toilets (per 1000 habitants) needed for different countries to meet the MDG for sanitation (MDG7) by 2015 (SEI, 2004)

To achieve the MDG7, access to new sanitation for ~0.4 million people day^{-1} must be provided from 2001 to the end of 2015 (Shalabi, 2006). WHO (2009) statistics showed that there were an estimated 9.0 million child deaths in 2007, significantly fewer than the 12.5 million estimated in 1990. The report also gave a global estimate of 37% of deaths of <5-year-old children occurring in the first month of life because of sanitation-related diseases. Moreover, most of them died in the first week in some developing countries in Africa or Asia (Donat-Castelló *et al.*, 2010).

Under poor hygiene conditions, even a small-scale animal farm in those areas could become a dangerous potential source of serious pathogens. Reasons for the widespread occurrence of diseases in a community could be thoroughly poor sanitary treatment of human excreta and animal manure contaminated by harmful pathogens (Monto & Fendrick, 2000).

1.2. ECOLOGICAL SANITATION CONCEPT

The conventional sanitation concept is neither an ecological nor economical solution for either industrialised or developing countries (IWA, 2009). These water-based sewage systems were designed and established on the premise that human excreta is a waste, suitable only for disposal, and that the environment is capable of assimilating this waste (Langergraber & Muellengger, 2005).

The Ecological Sanitation (EcoSan) concept underlines sustainable, closed-loop systems which close the gap between sanitation and agriculture. The EcoSan

concept can be understood as both a systematic approach and an attitude. These single technologies are only a mean 'to-an-end' and may range from near-natural waste water treatment techniques to compost toilets. The completed cycle for waste treatments ranges from simple household installations to complex and mainly decentralised systems (Langergraber & Muellengger, 2005). In addition, the technologies employed are not only defined as ecologically sound development but also conductive to the local environment. EcoSan systems combine a wide range of conventional, modern and traditional technologies (Shalabi, 2006).

The objective of EcoSan is to offer economically and ecologically sustainable and culturally acceptable systems that aim to close the natural nutrient and water cycles (Langergraber & Muellengger, 2005). Key features of this approach are the prevention of pollution and diseases caused by human excreta (Liu *et al.*, 2009; Larson & Duarte, 2001). In addition, rather than treating the excreta as a waste, nutrients from it are recycled (Sangwan *et al.*, 2008; Yadav *et al.*, 2010). The EcoSan projects can follow an idealised scheme of the general mass flows (Otterpohl *et al.*, 1997) (Fig. 1.3).

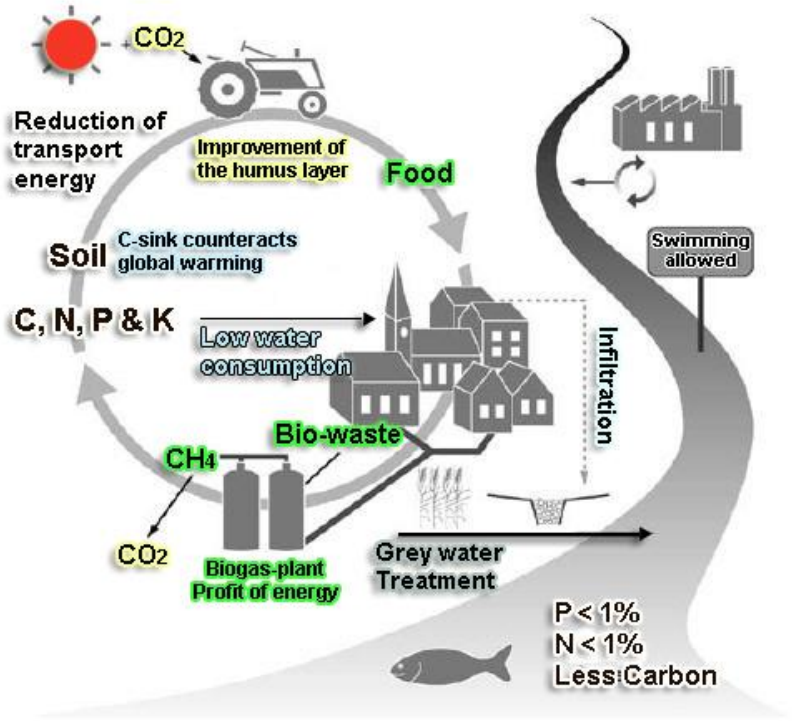

Figure 1.3. Scheme of the mass flows in EcoSan concept (Otterpohl *et al.*, 1997)

The EcoSan systems can be designed and constructed to be more efficient using both modern and classic technologies which are applied in source control systems. EcoSan is also considered to be a production unit that produces high quality reusable water, safe fertilisers and soil-improving material, including processed organic wastes (Otterpohl *et al.*, 1997). For example, a conservative estimate states that human excreta from one person could replace 50% of fertilisers needed for essential agricultural production for one person (Elmitwalli & Otterpohl, 2007). For this reason,

the EcoSan systems can be defined under the term 'resource management' technology as they can produce an organic-rich fertiliser rather than a waste (Bakema *et al*., 2002).

The primary aim of an EcoSan system is the collection and treatment of human excreta, which are separated from the rest of the wastewater (Shalabi, 2006). The faecal matter is the smallest fraction in the wastewater, but it contains the highest portion of pathogens and organic matter in comparison with other wastewater streams. However, if the faeces is mixed with a large amount of other wastewater streams and they are treated together to avoid pathogenic contamination of people from the water cycle. The separated faecal matter from the black water which is part of to the nutrient cycle, can provide renewable energy and restore degraded soils (Otterpohl *et al.*, 1997). However, almost all previous studies have focused on hygiene treatments of human excreta for targets of sanitation, while only a few studies report on them as a nutrient source.

1.3. THE MANAGEMENT OF ORGANIC SOLID WASTES IN SANITATION

Generally, sustainable waste management is considered to be a prerequisite to protect the environment and to conserve natural resources (Costi *et al*., 2004). Sustainable waste management is understood as an ecological and efficient method to manage 'the secondary resources', including solid, liquid, gaseous and radioactive substances in the different ways and fields of expertise appropriate for each (Edwards, 2007; Yadav *et al*., 2010). Besides the environmental pollution and damage caused by the gaseous, liquid and radioactive substances, OSW materials are recognised as a serious problem because they contain many pathogens which are relatively close to animal and human diseases (Costi *et al.*, 2004).

Focusing on OSW materials, OSW management means a process of collecting, transporting, separating, recycling/disposal and monitoring solid organic wastes (El-Fadel *et al.*, 1997). The OSW management concept suggests an opportunity to enhance the quality of life by effectively improving provisions of the urban basic service (Baud *et al*., 2001). The term OSW management usually relates to managing materials produced by livestock and human activities, and is generally undertaken to reduce the potentially effects these materials may have on health (IWA, 2009; Langergraber & Müllengger, 2005). The sustainable practice of OSW management could not only prevent the infectious potential of poor sanitation, but also recycle a huge amount of reusable materials (Edwards, & Bohlen, 1996; Somani, 2008). Moreover, OSW management is carried out to transform the OSW materials to valuable products as compost, heat and bio-fuel. Consequently, the efficient and sustainable management of OSW must be established as the main objective for targeting environmental problems (Shalabi, 2006).

Early OSW management simply consisted of burying any given waste in a dug hole. When human cities began to become more urbanised and OSW management became a serious issue, it developed into a more complex procedure including three main steps: collecting, separating and treatment (Fig. 1.4).

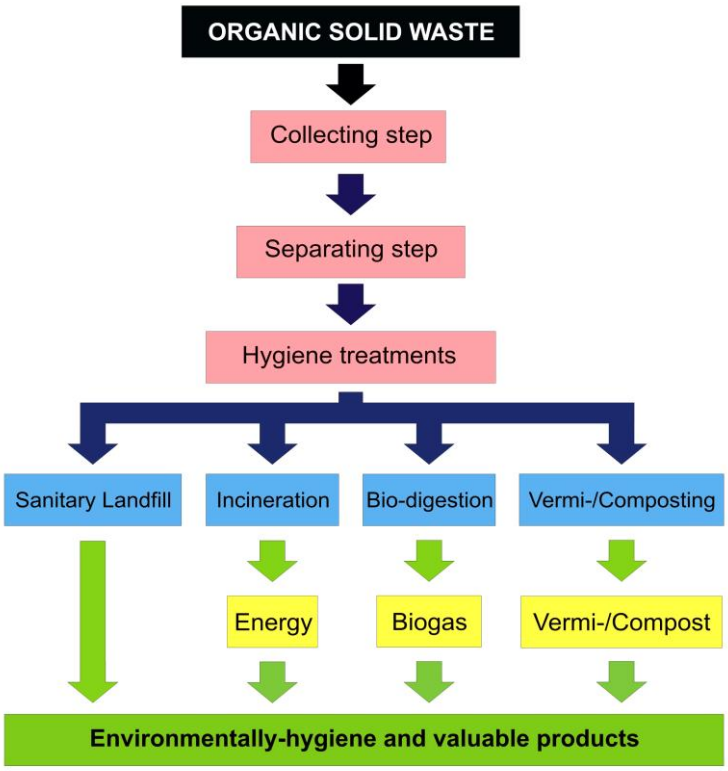

Figure 1.4. Four hygiene treatments and outputs of the OSW management procedure (*own creation*)

Depending on the different types of OSW materials and their respective components, various methods have been developed for OSW management purposes (Christensen, 2010a). The last steps in the OSW management procedure are hygiene treatments for sanitisation. Four methods are widely used for the purposes of sanitisation and nutrient recycling (Christensen, 2010b). In several hygiene systems such as anaerobic digestion (AD) or incineration, OSW materials are considered as input substrate for renewable energy production. Moreover, combinations of these methods are also often employed to treat the wastes more efficiently than using only a single method.

1.4. THE NEED OF BIOLOGICAL TREATMENT FOR THE MANAGEMENT OF ORGANIC SOLID WASTES

Biological treatments (AD, composting and vermicomposting) have been widely recognised as the most efficient, sustainable and environmentally friendly methods for converting OSW materials to hygienically safe and valuable products (Garg *et al*., 2005). Like controlled anaerobic processes in biogas plants, composting and vermicomposting treatments do not lead to other environmental problems (e.g. leakage, air pollution) as seen in the classical treatment facilities (sanitary landfill, incineration). However, the optimisation of these biological methods for decentralised systems still needs to be investigated further (Edwards, 2007).

By vermicomposting, OSW materials can be digested biologically and converted to

earth-like, soil-building substances based on the growth of earthworms (Edwards & Bohlen, 1996). In addition, this biological treatment seems to be a flexible process facilitating any scale of procedure, especially if the treated amounts of OSW are quite small and 'heat sanitising' composting cannot be achieved (Zhenjun, 2004). Because of the above reasons, vermicomposting has been established widely all over the world, especially in developing countries with low adaptive ability to new technologies for nutrient cycling.

Despite the advances in vermicomposting, a number of factors which limit the process are not well understood. The aim of this thesis is therefore to identify the negative factors which can affect vermicomposting, so that by eliminating them can become an efficient method for sustainable OSW management as regards achieving the MDG target for sanitation.

1.5. HYPOTHESIS AND OBJECTIVES OF THE STUDY

1.5.1. Hypothesis

Vermicomposting is a biological method to efficiently treat various organic solid wastes (OSW) and to transform them into valuable & hygienically safe products.

Vermicomposting can be optimised by pre-treatments of fresh substrates and using combinations of different earthworms (polyculture).

1.5.2. Objectives of the study

Objective 1: To study the suitability of vermicomposting in treatment of fresh separated solids of digested biogas slurry (Bs), non-urine faecal matter (Fm) and biowaste (Bw) as feed for *E. fetida*.

Objective 2: To study the single- & multi-factor effects of chemical parameters (pH level, EC and Total Ammonium Nitrogen-TAN) on the survival rate of *E. fetida*.

Objective 3: To study the changes in physico-chemical properties, nutrient content, maturity and pathogenic reductions in vermicomposted substrates during monoculture (only *E. fetida*) and polyculture treatment (within the presence of *E. fetida* and *D. veneta*).

2. LITERATURE REVIEW: VERMICOMPOSTING OF ORGANIC SOLID WASTES

Vermiculture (derived from the Latin '*vermis'* meaning worm) involves the mass production of earthworms for the degradation of organic matter and the production of compost with worm casts or 'vermicompost' (Edwards, 2007). Vermicomposting is known as one of the biological processing possibilities for OSW treatment (Edwards & Bohlen, 1996). The method is an accelerated process of bio-oxidation and stabilisation of the OSW materials that involves interactions between earthworms and microorganisms. In the process, organic matter can be digested and converted to earthworm biomass and vermicompost (Edwards, 2007). To date, many vermicomposting studies have been published and contributed new perspectives on the OSW treatment (Domínguez & Edwards, 2004; Somani, 2008; Zhenjun, 2004).

2.1. VERMICOMPOSTING PROCESS

2.1.1. Development of vermicomposting research

The role of earthworms has been recognised since the end of the 19th century regarding the break down of organic matter and release of nutrients (Edwards, 2007). Darwin's publication, '*Formation of vegetable mould through the action of worms with observations on their habits*', published in 1881, is known as the first research to mention earthworms (Feller *et al.*, 2003). During the last decade of the 20th century, more research focused on the ecology and biology of earthworms and the different uses of earthworms. Starting from only six publications in the period 1870-1889, there are now over 5000 publications dealing with earthworms (Feller *et al.*, 2003; Zhenjun, 2004).

Edwards (2007) roughly divided the 130-year development of earthworm ecological research into four stages focusing on:

(i) Morphology and ecological habits (1881-1949)

(ii) Natural earthworm ecology (1950-1969)

(iii) Vermiculture and utilisation for humans (1970-1992)

(iv) Bioactive proteins from earthworms and vermicomposting (after 1992).

Since 1970, due to the proven success of vermicomposting, this method and other similar treatments using earthworms to produce valuable soil-like additives and proteins for animal feed from organic matter have been expanded rapidly (Edwards & Bohlen, 1996). The increase in researchers' interest in vermicomposting accompanied the interests of commercial organisations (Edwards, 2007).

Recently, vermicomposting has been discussed as a key step in sustainable OSW management. In developed countries, a variety of vermicomposting studies have been conducted in England (Gunadi & Edwards, 2003; Gunadi *et al.*, 2002; Morgan, 2004); Germany (Elmitwalli & Otterpohl, 2007; Ernst *et al.*, 2008), Spain (Aira & Domínguez, 2008; Elvira *et al.*, 1997; Monroy *et al.*, 2009) and the USA (Arancon *et al.*, 2008; Edwards, 2007; Hartenstein *et al.*, 1980). Researchers have also increasingly focused on this method in developing countries, for example, in China (Liu *et al*., 2009; Zhenjun, 2004), India (Ghosh, 2004; Kale *et al*., 2004; Yadav *et al.*, 2010), Vietnam (Fuchs *et al.*, 2005) and Brazil (Pereira *et al.*, 2009), considering it as an efficient and sustainable alternative for local OSW treatment (Snel, 1999).

Vermicomposting facilities for domestic and industrial applications are already commercially available in Canada, the USA, Italy, Japan and India. From a few-ton system started in Canada in 1970, there are now some processes with a capacity of more than 2500 tons month^{-1}. Since 1978, the American Earthworm Company has operated a farm with ~500 tons capacity month^{-1}. The largest vermicomposting facility to date was built in California, the USA, in 1993 (Sherman-Huntoon, 2000). In this facility, about 22.5 tons of earthworms process 75 000 tons of material annually. Other examples are treatment facilities with ~2000 tons of sugarcane waste month^{-1} in India and ~3000 tons of OSWs month^{-1} in Japan (Ghosh, 2004).

2.1.2. Processes during vermicomposting

According to Ndegwa & Thomson (2000), vermicomposting can be divided into two main processes:

Physical and mechanical processes: Organic matter is aerated, mixed and homogenised by earthworms. In the case of composting, the process usually requires large tools/units that are associated with high cost. Vermicomposting avoids these operation costs (Ndegwa & Thomson, 2000; Ndegwa *et al*., 2000).

Ecological and biochemical processes: Vermicomposting is a process with several interactions between earthworms and microorganisms. In the worm intestine (or gut), there are many biochemical processes among bacteria, protozoa, actinomycetes and fungi (Edwards & Fletcher, 1988). In addition, some digestive enzymes which are known as catalytic reagents for biochemical reactions exist in the worm gut (Atiyeh *et al*., 2000).

In summary, Edwards (2007) gave a new definition of vermicomposting as an innovative strategy which includes two successive processes: Gut-associated processes (GAPs) and Cast-associated processes (CAPs), with several specific sub-processes (Fig. 2.1).

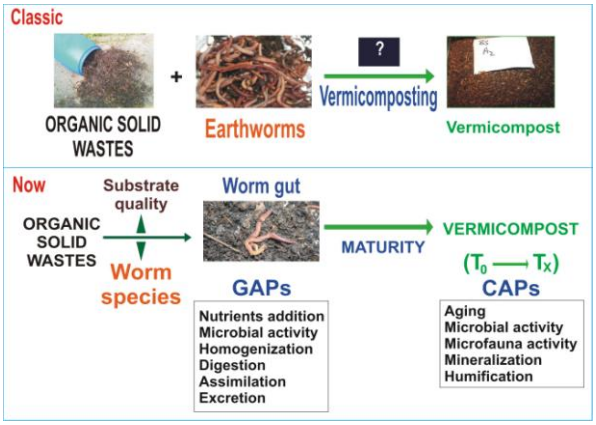

Figure 2.1. Strategies for vermicomposting: (**A**) classical approach and (**B**) split approach in two steps: Gut-associated processes (GAPs) & cast-associated processes (CAPs); adapted from Edwards, 2007

2.1.3. Interaction between earthworms and microorganisms

The interactions between earthworms and microorganisms in soil and OSW materials seem to be of major importance in the degradation of organic matter and the release of mineralised nutrients (Aira *et al.*, 2007a; Edwards & Bohlen, 1996; Loh *et al.*, 2005).

Edwards (2007) described earthworm-microbial community interactions in natural soils on three spatial scales: micro-, meso-, and macro-scale. Similar interactions can be expected in vermicomposting processes, but mainly at micro- and meso-scale, which are mentioned briefly below.

Micro-scale: This scale represents that of the worm's intestine, burrow lining or casts. At this scale, earthworm ecologists describe food preferences of earthworms, the fate of microorganisms in the intestines of earthworms, and the chemical and biological composition of casts and successful processes within them.

Meso-scale: This scale represents an integration of the drilosphere with surrounding matter. The term drilosphere was coined by Lavelle (1988) and the drilosphere includes five main components (Brown *et al.*, 2000): the internal micro-environment of the earthworm gut; the earthworm surface in contact with the soil; surface and belowground earthworm casts; middens; and burrows, galleries, or diapause chambers. At meso-scale, ecologists consider the ways in which earthworm activity influences whole soil characteristics and functions, such as the distribution of microorganisms, soil respiration, microbial biomass, bacterial/fungal ratios, and the way these processes alter soil fertility and the incidence and severity of root diseases. These can be analysed using microcosms or in small-scale field trials.

In the context of the current research, the importance of interactions at the micro- and meso-scale lies in the elimination of pathogenic microorganisms, particularly human pathogens (Edwards, 2007). However, almost nothing is known about the relative contribution of these interactions to the observed changes in microbial populations during the decomposition of organic matter (Monroy e*t al.*, 2009). The adverse and benefical interactions between earthworms and microorganisms were summarised by Edwards & Fletcher (1988) (Fig. 2.2).

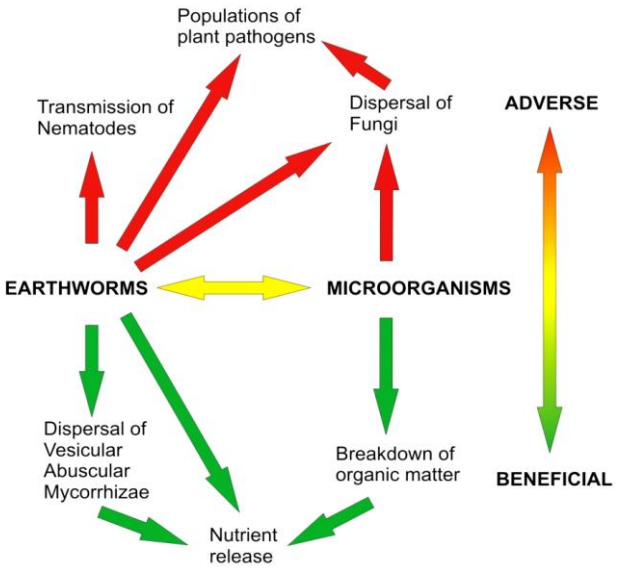

Figure 2.2. Adverse and beneficial effects of interactions between earthworms and microorganisms, adapted from Edwards & Fletcher (1988)

2.1.4. The role of earthworms in organic matter breakdown

The most important role of earthworms in biological processes includes breaking down solid organic matter (Atiyeh *et al.*, 2000) and releasing a portion of the organic matter into earthworm biomass and to respiration products (Domínguez *et al.*, 2001) rendering nutrients available to plants (Somani, 2008).

The role of earthworms in the breakdown of organic matter is attributed to three processes in the worm gut: interaction with gut microorganisms, digestive enzymes, and physical grinding. The latter two are discussed in more detail here.

The digestive system of different species, genera and families of worms differs in detail, but the worm gut has a common basic structure. Little information is available about the process of worm digestion and the quality of materials that pass through the worm gut (Edwards & Bohlen 1996). *E. fetida* appears to extract its nutrients from finely fragmented organic matter which is mixed with soil.

The digestive system of earthworms consist of a buccal cavity, pharynx, oesophagus, crop, gizzard and intestine. Feed adheres to mucus extruded by the buccal epithelium. Later, pressure in the wall of the buccal cavity is released which establishes a partial vacuum to transport materials through the crop, the gizzard and the intestine (Zhenjun, 2004). The time taken for food to pass through the worm gut can vary from 3-5 hours in the case of *E. fetida*, to 12-20 hours for *L. terrestris* (Edwards, 2007). Laverack (1963) observed that there are commonly six types of digestive enzymes in the worm gut (protease, lipase, amylase, lichenase, cellulase and chitinase). However, a variety of other enzymes have been also reported depending on species (Edwards & Fletcher, 1988). There is an increase in the absorption of nutrients along the length of the worm gut. The posterior gut, with its characteristic peritrophic membrane, appears to be a major zone of absorption (Edwards & Fletcher, 1988).

2.2. BREAK DOWN OF ORGANIC SOLID WASTES

Different earthworm species exist in almost all regions of the world except those with extreme climates, such as deserts and glaciers. These species have quite different life cycles, behaviours and environmental requirements. They are classified into three major ecological categories based primarily on their feeding and burrowing strategies: epigeic, endogeic and anecic (Maboeta *et al.*, 1999; Zhenjun, 2004). Only epigeic earthworms seem to be relevant for vermicomposting (Edwards, 2007).

About 8000 species of earthworm worldwide have been described as epigeic from ~800 genera belonging to the order Oligochaetae. Of those, seven earthworm species are used in vermicomposting, namely *Eisenia fetida*, *Dendrobaena veneta*, *Dendrobaena rubida*, *Lumbricus rubellus*, *Perionyx excavatus*, *Eudrilus eugeniae* and *Pheretima elongata* (Edwards, 2007; Zhenjun, 2004). These species show good growth on organic wastes compared with other species (Edwards, 2007). Edwards (2007) provides a comparative summary of the life cycle and cocoon production of the seven earthworm species suitable for vermicomposting (Fig. 2.3). These seven species are described in more detail in the following sections.

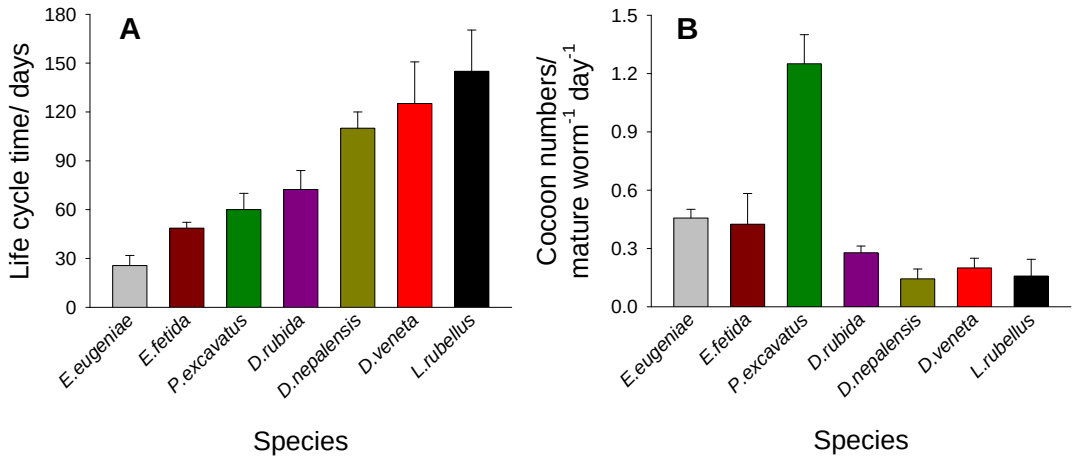

Figure 2.3. (**A**) Length of life cycle and (**B**) mean cocoon production of the seven earthworm species suitable for vermicomposting; error bars indicate standard error, adapted from Edwards (2007)

2.2.1. Eisenia fetida

Most studies of vermicomposting focus on the species *Eisenia fetida* (Fig. 2.4) (Reinecke *et al.*, 1992; Tripathi & Bhardwaj, 2004).

Figure 2.4. *Eisenia fetida* (brandling or tiger worm), the most common earthworm for vermicomposting

It occurs worldwide and naturally colonises many organic solid wastes, e.g. garden waste and animal slurry (Gunadi *et al.*, 2003). *E. fetida* has a rapid growth rate, good temperature tolerance (up to 35^{o}C) and accepts a large range of moisture (60-90%) (Reinecke *et al.*, 1992). Moreover, *E. fetida* can be handled easily and it is tolerant to other species (Aira *et al.*, 2002; Gunadi *et al.*, 2003; Loehr *et al.*, 1985).

Under optimal conditions, the life cycle of *E. fetida* ranges from 45 to 51 days (Domínguez & Edwards, 2004). The time for hatchlings to reach sexual maturity is in the range 21-30 days. Cocoon laying begins 48 hours after copulation and the rate of cocoon production ranges from 0.4 to 1.3 cocoon day^{-1}. Hatching viability is around 80% and the incubation period of the *E. fetida* cocoon varies from 18 to 26 days (Edwards, 2007). On average, approx. 2.5-3.8 young earthworms hatch out from an *E. fetida* cocoon depending on temperature. The maximum life expectancy of *E. fetida* is 4.5-5.0 years (Domínguez & Edwards, 2004), but the average survival rate is ~20 months at 18-28^{o}C (Aira *et al.*, 2007b).

2.2.2. Dendrobaena veneta

Dendrobaena veneta has been reclassified by taxonomists into the genus and species instead of the older classification of *Eisenia* hortensis, and is commonly referred to as the European night-crawler (Edwards, 2007; Muyima *et al.*, 1994). Although *D. veneta* does not grow rapidly, the worms have been used widely in vermicomposting and vermiculture systems, especially in industrial-scale processes. Because of their large size, they can be handed easily (Fayolle *et al.*, 1997). With the advantage of larger size in comparison with the other species, *D. veneta* is also the earthworm species subjected to the most detailed ecological studies (Reinecke *et al.*, 1992). Reproduction, maturity rate and environmental requirements of this species were studied in the early 1990s (Loehr *et al*., 1985; Viuoen *et al*., 1992). *D. veneta* has a low reproduction rate and a slow maturity rate compared with other vermicomposting worms such as *E. fetida*, *P. excavatus* and *E. eugeniae* (Edwards, 2007) (Fig. 2.3).

D. veneta prefers temperatures in the range 9-30^{o}C for vermicomposting (Fayolle *et al*., 1997; Munima *et al.*, 1994). Munima *et al.* (1994) also reported that *D. veneta* performs better in high moisture conditions (65-85%) than other species for vermicomposting of materials with lower moisture content such as paper sludge. The life cycle of the species is quite long (100-250 days). About 65 days is the average time for this worm to reach sexual maturity (Fayolle *et al.*, 1997). The species produces ~0.28 cocoons day^{-1} but the hatchling viability is only 20%. The incubation period of *D. veneta* is ~42 days and its hatchling rate from a viable cocoon is approximately 1.1 earthworms (Edwards, 2007).

2.2.3. Dendrobaena rubida

Dendrobaena rubida is also suitable for vermiculture as well as for vermicomposting treatment, although it has not been commonly used (Elvira *et al.*, 1997a). This is a temperate species with a clear preference for organic soils, and it inhabits substrates

such as decaying rotting wood and straw, pine litter and compost (Edwards, 2007).

The life cycle of the species is completed in 75 days and its rapid maturation and high reproductive rate could make the worm suitable for vermicompost processing (Edwards, & Bohlen, 1996). *D. rubida* grows well in the range of 15-25^{o}C and needs 54 days to reach sexual maturity after hatching (Domínguez & Edwards, 2004). The rate of cocoon production is about 0.46 cocoons day^{-1} and the hatching viability is up to 75% (Elvira *et al.*, 1997a).

2.2.4. Lumbricus rubellus

Lumbricus rubellus is found in moist substrates, such as animal manure and sewage solids (Elvira *et al.*, 1997a). *L. rubellus* has a long maturation time and a low reproductive rate, which suggests that the species is not an ideal earthworm for vermicomposting, although its size, vigour and ability to survive in soils could make it interesting as fish bait or for soil improvement (Adi & Noor, 2009). Moreover, the species is not an opportunistic species with obligatory bi-parental reproduction (Sims & Gerard, 1985), which contributes to its low reproductive rates (Zhenjun, 2004).

L. rubellus has a relatively long life cycle in the range of 120-170 days, with a slow growth rate and a long maturation time of 74-91 days (Edwards, 2007). Edwards (2007) also reported that the optimum temperature of this worm is 15-18^{o}C and the optimum moisture is 80-85%. The net reproductive rate is 0.05 hatchlings day^{-1} because of the low cocoon production rate of ~0.1 cocoons day^{-1}, with only one hatchling emerging from each cocoon.

2.2.5. Perionyx excavatus

Perionyx excavatus is a tropical earthworm which has been widely used in vermiculture and belongs to the *Megascolecidae*, commonly found over a large area of tropical Asia and Australia (Khwairakpam & Bhargava, 2009; Maboeta *et al.*, 1999). This is an ideal species which can be used to break down organic wastes under high temperatures in tropical countries (Reinecke *et al.*, 1992). High moisture contents and adequate amounts of suitable organic material are required for populations of the *P. excavatus* to become fully established and to process organic wastes efficiently (Hallatt *et al.*, 1992).

Under optimal conditions, the life cycle of *P. excavatus* takes 40-71 days from hatching to maturity (Maboeta *et al.*, 1999). This species prefers high temperatures (20-30^{o}C) with an optimum of 25^{o}C and may die at temperatures <9^{o}C (Loehr *et al.*, 1985). With about 90% hatching rate and 1.1 worms cocoon^{-1}, the species has a high net reproductive rate from nearly 6.7 cocoons day^{-1} (Reinecke *et al.*, 1992).

2.2.6. Eudrilus eugeniae

Eudrilus eugeniae is the other of the two tropical species used commonly in vermicomposting (Domínguez *et al.*, 2001; Reinecke *et al.*, 1992). Belonging to the *Eudrilidae*, the earthworm is a native African species that lives in both soils and

organic wastes. The species is large and grows rapidly and is relatively prolific when cultured (Loehr *et al.*, 1985). *E. eugeniae* could be considered an ideal species for vermicomposting because it is handled and harvested more easily than the others (Domínguez *et al.*, 2001). However, its disadvantage is that it has a relatively narrow temperature tolerance, from only 20 to 28^{o}C (Domínguez *et al.*, 2001).

E. eugeniae can be highly reproductive when living un ideal conditions (Kale *et al.*, 1992). The earthworm is capable of decomposing large quantities of organic wastes rapidly and incorporating them into the topsoil (Loehr *et al.*, 1985). *E. eugeniae* has a life cycle of 43-122 days depending mainly on temperature. The maximum life expectancy of the worm is up to three years (Edwards, 2007). The time requirement of *E. eugeniae* for maturity is 40 days and for cocoon incubation is ~16 days. The average number of hatchlings is 2.7 earthworms cocoon^{-1} (Domínguez & Edwards, 2004). Up to now, the species has been bred extensively in the United States for the fish-bait market, where it is known commonly as the African night-crawler (Khwairakpam & Bhargava, 2009).

2.2.7. Pheretima elongata

Pheretima elongata is a Megascolecid earthworm which has been tested for vermicomposting of OSW including household wastes and animal slurries (Kale *et al.*, 1992; Somani, 2008). The species is indigenous to India. Some Indian researchers have claimed that *P. elongata* is used for a commercially viable processing facility for the vermicomposting of eight tons of substrates per day (Edwards, 2007). Loehr *et al.* (1985) also reported that the species is well-suit to complete processes including vermicompost and reusable water production from sewage sludge, manure slurries and wastewater from food processing.

P. elongata survives by dwelling deeply in low organic matter and tolerates in a temperature range from 19 to 30^{o}C (Somani, 2008). The duration of its reproductive life is longer than 200-400 days and the period of maturity from hatchling to adult seems to be in the range of 120-150 days (Domínguez & Edwards, 2004). The average time for cocoon production of *P. elongate* is ~20-24 weeks and its cocoon incubation period is 28-31 days. The incubation period of this species seems shorter than for the others but the number of worms hatchling is limited (1.0 worm cocoon^{-1}).

2.2.8. Polyculture treatment of different earthworm species

In nature, several different earthworm species may co-exist together, but each lives in a different niche and uses different organic materials for food (Khwairakpam & Bhargava, 2009). Therefore, it is possible to mix more than one earthworm species in vermicomposting (mixed- or poly-culture) treatment. This combination can accomplish a greater stabilisation of vermicompost than a single species (pure- or mono-culture) treatment. Suthar (2008) reported that earthworm communities may influence the spatial variability of resources, altering their availability to microorganisms, thereby regulating nutrient cycling processes. Because mixing more than one species can be beneficial for the rapid decomposition of organic solid

wastes, relationships between different earthworms themselves need further study (Suthar, 2008).

Mixtures of *D. veneta*, *E. fetida*, *E. eugenuae*, *P. excavatus* and *P. hawayana* have been used to study polyculture vermicomposting of anaerobic sludge (Loehr *et al*., 1985). *D. veneta* has been co-incubated with *E. fetida* in polycultures of faecal matter (Shalabi, 2006). Similar biological properties (tolerance to temperature and moisture) of these two species, is perhaps one of the reasons for combining them for polyculture treatment in vermicomposting of various OSW materials.

In contrast, Elvira *et al.* (1997a) reported that polyculture treatment tests did not show any advantage over monoculture treatment. The growth rate of a *L. rubellus* and *D. rubida* mixture decreased slightly in polyculture treatment compared with monoculture treatment with. Analyses showed that *E. eugeniae* and *P. excavatus* did not co-exist comfortably in polyculture treatment, probably due to food competition.

2.2.9. Earthworm suitability test for vermicomposting

The use of earthworms for eco-toxicological evaluation has been developed widely since the first International Workshop on Earthworm Eco-toxicology held in Sheffield in 1991 (Edwards, 2007). Actually, there are many tests available to investigate the relationship between earthworms and their environment. The parameters tested, can be physico-chemical impacts, chemical toxicity, earthworm reproduction or suitability of feed materials for vermicomposting (Table 2.1).

Table 2.1: Testing methodologies using earthworms as an indicator of environmental impact, adapted from Edwards (2007) and Kaplan *et al.* (1988)

Method	Medium	Test objective	Reference
Contact filter paper test	Wet filter paper	Chemical toxicity	Heimbach (1984)
Artisol test	*Aqueous medium	Chemical toxicity	Heimbach (1984)
Artisol test	Aqueous soil leachate	Physico-chemical impacts	Galli *et al.* (1997)
Test with **NOEC	***Artificial soil	Subtle effects & teratogenic effects	Ma (1984); Jensen *et al.* (2007)
Aquatic toxicity test	***Artificial soil	Worm growth & reproduction	Lowe & Butt (2007); Jensen *et al.* (2007)
****LC_{50} test	***Artificial soil	Immediate effects of chemicals	Spurgeon *et al.* (1994)
Chronic toxicity test	***Artificial soil	Growth & reproduction of earthworm	Rinke & Wiechering (2001)
Suitability test for vermicomposting	Animal slurry or vegetable waste	Worm survival & material acceptability	Kaplan *et al.* (2008); Edwards (2007)

*: Including silica mixed with glass balls and water

**: NOEC: No observed effect concentration standard

***: Artificial soil (by DM) = 70% sand + 20% clay (kaolin) + 10% organic matter

****: LC_{50}: Lethal concentration

As previously mentioned (*see 2.2.1*), *E. fetida* is not only the most commonly used species for vermicomposting but it is also used to study chemical toxicity and other factors such as worm reproduction, soil quality, etc. Tests focus on this species because of its worldwide distribution and great tolerance to high or low moisture levels and temperature (Aira *et al.*, 2007a; Edwards, 2007; Kaplan *et al.*, 1980; Neuhauser *et al.*, 1980). Artificial soil (mixture of sand, clay and organic matter) is often used to test the toxicity of chemicals to earthworms (Lowe & Butt, 2007; Van-Gestel e*t al.*, 1992).

Nevertheless, almost all of the tests performed to date focus only on the aspects of the environmental conditions of earthworm ecology or biology in soils. Thus, there is less information about methods to test the suitability of earthworms in different OSW materials for vermicomposting. Perhaps the limited investigation into such methods is due to the fact that each species requires different ecological and physico-chemical conditions for its optimal development, growth and re-production (Domínguez & Edwards, 2004).

Several physico-chemical properties of feeding materials are reported to have a strong effect on earthworm activity (Table 2.2). The most important parameters are moisture (Edwards & Bohlen, 1996), temperature (Fayolle *et al.*, 1997; Loehr *et al.*, 1985; Viuoen *et al.*, 1992), pH (Domínguez & Edwards, 2004; Kaplan *et al*., 1980), salt content (Hughes *et al.*, 2009; Owojori *et al*., 2009) and TAN content (Domínguez & Edwards, 2004; Kaplan *et al.*, 1980). The suitability test of various substrates for earthworms requires further study to investigate the correlation of earthworm acceptability with feeding materials for vermicomposting.

Table 2.2: Comparison of main physico-chemical parameters among seven earthworm species used for vermicomposting; these limit intervals use the highest and lowest limits in previous studies

Species	Moisture/ %		Temp./ ^{o}C		pH		Salt content / %	TAN/ mmol L^{-1}
	Survival	*Optima*	Survival	*Optima*	Survival	*Optima*		
E. fetida	60-90	*70-80*	0-35	*15-25*	4.0-9.0	*5.0-8.0*	<0.5	<71.4
D. veneta	65-85	*80-85*	9-30	*15-25*	>4.5	na	<0.5	<31.2
D. rubida	79-90	*73-75*	15-25	*18-25*	>4.1	na	<0.5	<31.2
L. rubellus	70-90	*80-85*	5-25	*15-18*	5.5-9.0	*6.0-7.0*	na	na
E. eugeniae	70-85	*80*	9-30	*22-25*	>4.5	*7.0*	<0.5	<31.2
P. excavatus	76-85	*81*	9-30	*25*	>4.5	*7.0*	<0.5	<31.2
P. elongata	60-85	*60-75*	25-37	*26*	na	na	na	na

na: data not available

2.3. SUITABLE FEEDING MATERIALS FOR VERMICOMPOSTING

2.3.1. Organic solid waste materials for earthworm feeding

Animal slurry is cited as the most favourable material for feeding earthworms (Aira *et al*., 2007b; Garg *et al*., 2005) (Table 2.3). This material is widely used as a medium when earthworms are used as an eco-indicator to investigate environmental or

chemical impacts of different soils (Dominguez & Edwards, 1997). Slurry is also considered a nutrient-rich feed for earthworms (Atiyeh *et al.*, 2000b; Kaplan *et al.*, 1980; Lazcano *et al.*, 2000).

Table 2.3: Physico-chemical properties and nutrient content of animal manures used as a favourable feed in vermicomposting, all data is given in dry weight (DM)

Animal manure	DM/ %	pH	EC/ mS cm^{-1}	TAN/ g kg^{-1}	oDM/ %	C/N ratio	N_{tot}/ %	P_{tot}/ %	K_{tot}/ %	Reference
Cow	22.0	6.7	na	200	93.8	36.5	1.3	na	na	Atiyeh *et al.* (2000)
Poultry	46.4	8.2	na	na	na	44.0	0.7	na	na	Garg & Kaushik (2005)
Horse	46.0	8.5	2.0	na	na	49.1	1.0	0.6	0.9	Sangwan *et al.* (2008)
Donkey	45.6	8.1	3.9	na	48.5	97.1	0.5	0.5	1.3	Garg *et al.* (2005)
Sheep	26.6	8.2	0.9	na	32.3	88.9	0.4	0.3	0.7	Garg *et al.* (2005)
Goat	~20	7.0	na	na	na	48.9	1.0	0.6	0.5	Loh *et al.* (2005)
Cattle	~20	7.1	na	na	na	51.0	1.1	0.3	0.2	Loh *et al.* (2005)
Buffalo	27.7	8.4	2.6	na	na	93.0	0.6	0.5	1.1	Garg *et al.* (2005)
Pig	24.0	8.3	0.3	2.4	86.0	19.0	2.4	na	na	Aira *et al.* (2007)

na: data not available

Furthermore, by-products from crop harvesting such as vineyard waste (Nogales *et al.*, 2005), potato waste (Dominguez *et al.*, 2001) and fruit and vegetable waste (Gunadi & Edwards, 2003) are acceptable feed materials for earthworms. However, to produce good vermicompost, they should be mixed with other materials which are known to be worm-favourable feeds before being used for vermicomposting (Garg & Kuashik, 2005; Sangwan *et al.*, 2008). Sewage sludge or digested solids from wastewater (Alidadi *et al*., 2005; Alvira *et al*., 1997b), paper waste (Arancon *et al*., 2008; Alvira *et al*., 1997b) and cotton industrial waste (Albanell *et al.*, 1988) could also be considered good vermicomposting substrates when mixed with other OSW materials.

However, there are many other OSW materials which cannot be directly used as feed for earthworms because of the presence of some toxic chemicals in the components (Kaplan *et al.*, 1980). The toxic factors in the feed can be urine, inorganic salts, ammonium, ammonia, alkali, acids, alcohol, methane gas, etc. (Dominguez & Edwards, 2004; Edwards, 2007; Gunadi & Edwards, 2003). Examples are urine-contaminated slurry (Kaplan *et al.*, 1988), fresh human faeces (Yadav *et al.*, 2010), anaerobic digestion waste (Elvira *et al.*, 1997; Garg *et al.*, 2005) and digested solids of biogas slurry (Garg *et al.*, 2006). However, these OSW materials can be pre-treated by different methods before being used for earthworms (Gunadi & Edwards, 2003; Suthar, 2010).

2.3.2. Pre-treatment of OSW materials for vermicomposting

The main idea behind pre-treating fresh OSW materials is to keep a balance between three factors: the time of processing, the survival of earthworms and the possible decrease in the amount of food available for earthworm growth (Gunadi *et al*., 2002). These authors also reported that the pre-treatments of OSW materials could have the benefit of eliminating human pathogens and weed seeds from vermicompost. However, several OSW materials may decrease in quality in terms of nutrient availability for earthworm growth when they are pre-treated for a long time. This may inhibit the rate of growth and number of cocoons and hatchlings produced by earthworms (Nair *et al.*, 2006).

Generally, the pre-treatment of feeding materials for vermicomposting includes two methods: thermophilic pre-composting to reduce toxic factors and mixing some different types of OSW materials together to achieve an acceptable feed (Gunadi *et al*., 2002).

The thermophilic pre-composting could make OSW materials more acceptable and cause less worm toxicity factors (e.g. excess heat, anaerobic gases and ammonia) and too low or high pH level (Frederickson, 1997). However, depending on the types of OSW materials, the optimal time of pre-composting differs (Table 2.4).

Table 2.4: Comparison of pre-treatments of some organic substrates used for vermicomposting

Substrate	Purpose	Method	Time/ days	Reference
Green wastes	Excess heat release, pathogen reduction	Composting	14	Frederickson *et al.* (1997)
*Cm	Earthworm toxic factors & pathogen reduction	Composting	7	Gunadi *et al.* (2002)
*Cm	Earthworm toxic component reduction	Composting	14	Gunadi & Edwards (2003a)
**Kw & cow dung	Experimentation & decomposition	Composting	15	Tripathi & Bhardwai (2004)
**Kw	Moisture & pathogen reduction	Composting	9	Nair *et al.* (2006)
***Pw	Earthworm toxic gas elimination	Composting & turning	21	Garg *et al.* (2009)

*: Separated cattle manure solids (Cm)
**: Kitchen waste (Kw)
***: Non-recyclable post consumer paper waste (Pw) contains office paper trimmings, food-soiled paper, napkins, brown paper, cardboards, torn-up corrugated boxes, paperboard packaging material and other paper scraps

Although the pre-composting process may reduce the amount of food available for the growth and reproduction of earthworms, the process should be adopted and adjusted as the first step of vermicomposting is to eliminate the earthworm toxicity factors in the feeding substrates and to be tolerated by different species (Frederickson *et al.*, 2007). Pathogen reduction is an additional benefit that can be obtained from the process (Gunadi *et al*., 2002; Nair *et al.*, 2006).

Another pre-treatment option is to mix two or more fresh OSW materials together.

This mixing has been used quite commonly for pre-treatment of vermicompost substrates, especially when the main waste cannot be accepted directly as feed by earthworms (Yadav & Garg, 2009). Several OSW materials such as paper-pulp mill sludge, filter cake, etc. have unsuitable properties for earthworm feeding but they can be ideal materials in a mixture with other OSW materials (Yadav & Garg, 2009). Normally, when mixing OSW materials, some types of green waste are mixed with animal residues (Somani, 2006). For examples, it is advisable to combine yard and garden wastes with other animal manures, such as pig or cow slurry (Somani, 2006). A huge amount of crop residues from farm activities such as roots, branches, vegetable and fruit peelings (containing much more cellulose compounds) and similar derivatives could be mixed with animal slurries which have a high N amount to make a favourable feed for earthworms (Bansal & Kapoor, 2000; Frederickson *et al.*, 2007). According to Sangwan *et al.* (2008), horse manure and spiked filter cake in a 1:1 ratio could become the favoured substrate for *E. fetida*.

Yadav & Garg (2009) made a summary of suitable OSW materials which could be mixed with some industrial wastes as a pre-treatment before using them as feed materials for vermicomposting by several earthworm species (Table 2.5). Yadav & Garg (2009) also reported that vermicomposting of food industry sludge could produce a lot of *E. fetida* biomass and that the substrate was converted to good quality vermicompost when it was mixed up to 30% with cow dung.

Table 2.5: Industrial wastes used as feed materials for vermicomposting after mixing with other favourable OSW materials, adapted from Yadav & Garg (2009)

Industrial waste	OSW amendment	Species
Filter cake, trash	Cow dung	*E. eugeniae*
Distillery sludge	Cow dung	*P. excavatus*
Ligno-cellulosic waste from olive oil industry	Municipal bio-solids	*E. andrei*
Solid textile mill sludge	Cow dung, poultry, biogas plant slurry & agricultural wastes	*E. fetida*
Paper-pulp mill sludge	Cow dung, *Mangifera indica* & saw dust	*E. fetida, E. eugeniae & L. mauritii*
Wood chips	Sewage sludge	*E. fetida*
Filter cake	Horse manure	*E. fetida*
Sewage sludge	Cow dung	*E. fetida*

2.4. OUTPUT PRODUCTS OF VERMICOMPOSTING

2.4.1. Nutrient content in vermicompost

Besides its use as an alternative to other sanitary forms of OSW management, vermicomposting treatment can reap other benefits, such as producing vermicompost that is a soil improver for agriculture (Suthar, 2010). Through the action of earthworms, nutrients may be more available in the vermicompost produced as compared to those in the parent material or in non-vermicomposted materials.

The main advantage of vermicompost over its parent material is its available nutrients and high level of organic matter humidification (Edwards, 2007). Edwards (2007) also reported that vermicompost can have positive effects on soil and its physico-mechanical properties. After application to soil of vermicompost as a balanced bio-fertiliser, crop yields can increase by up to 30-50%.

Because of the close relationship between parent material and the final product, the type of fresh vermicomposting substrate determines the properties of the vermicompost. According to Edwards (2007), N_{tot} concentration in vermicompost of animal wastes varies within the range 1.8-3.0% of total dry matter, whereas P_{tot} and K_{tot} concentration are in the range of 0.2-2.7% (Table 2.6).

Table 2.6: Major plant nutrient elements in animal vermicomposts, adapted from Edwards (2007)

Vermicompost	N_{tot}/ %	P_{tot}/ %	K_{tot}/ %	Ca/ %	Mg/ %	Mn/ %
Separated cattle solids	2.2	0.4	0.9	1.2	0.3	0.1
Separated pig solids	2.6	1.7	1.4	3.4	0.6	0.1
Cattle solids on straw	2.5	0.5	2.5	1.6	0.3	0.1
Duck solids on straw	2.6	2.9	1.7	9.5	1.0	0.1
Chicken solids on shavings	1.8	2.7	2.1	4.8	0.7	0.1
Metro-Mix 360	1.8	0.2	0.5	0.9	2.2	0.9

Elemental contents in products can change through the activity of earthworms by the adding earthworm biomass such as mucus, excreta or dead bodies (Ernst *et al.*, 2008; Monroy *et al.*, 2009). Edwards (2007) also reported that earthworm activities in fresh materials resulted in the plant available nutrients in vermicompost being present in higher amounts compared with the same material un-treated (Table 2.7).

Table 2.7: Effects of *E. fetida* on nutrient in vermicomposts, adapted from Edwards (2007)

Nutrient	Cattle vermicompost		Pig vermicompost		Potato vermicompost	
	Control	Treatment	Control	Treatment	Control	Treatment
$N\text{-}NO_3^-$/ g kg^{-1}	8.8	259.4	31.6	110.3	74.6	1428.0
P/ %	0.1	0.2	1.1	1.6	0.2	0.2
K_{exc}/ %	0.2	0.4	1.5	1.8	1.9	3.1
Ca_{exc}/ %	0.4	0.6	1.6	2.3	0.9	1.4
Mg_{exc}/ %	0.1	0.1	0.5	0.7	0.2	0.3

2.4.2. Maturity of vermicompost

The vermicompost is ready as soon as it is considered to be mature. Mature vermicompost contains acceptable concentrations of phytotoxic compounds or short-chain organic acids (Brewer & Sullivan, 2003). The maturity level of vermicompost is determined as an important parameter for vermicompost quality assessment. Respiration can be directly related to the metabolic activity of a microbial population

in soils (Gómez *et al*., 2006). It is used as a useful parameter of the microbial activity in compost. Microorganisms respire at higher rates in the presence of large amounts of bio-available organic matter while the respiration rate is lower if this type of material is less (Aira & Domínguez, 2009). The respiration index (RI) defines the rate of O_2 uptake or CO_2 evolution of a sample under controlled conditions of the assays (Gómez *et al*., 2006). Methods to determine RI are based on three mechanisms: (i) self-heating, (ii) CO_2 production and (iii) O_2 uptake (Adani *et al*., 2001). The O_2 uptake can be analyzed mainly by two assays either by total solid or volatile solid (Iannatti *et al*., 1993) (Table 2.8).

Table 2.8: Comparisons of different stability thresholds recommended for static respiration activity of compost by several institution references, data was given by total solids (***a***) or volatile solids (***b***)

Institution reference	Test period/ hours	Threshold/ mg O_2 g^{-1}	Reference
Austria & Germany (*AT_4)	96	<5.0***a***	Gomez *et al.* (2006)
European Union	96	<10.0***a***	Gomez *et al.* (2006)
**ASTM	96	<35-50***a***	Gomez *et al.* (2006)
US Department of Agriculture & Composting council	1	<0.5***b***	Iannotti *et al.* (1993)
Italy (Regione Veneto, I)	1	<0.6***b***	Gomez *et al.* (2006)
**SOUR	1	<1.0***b***	Lasaridi & Stentiford (1998)

*: Respiration activity at four days parameter
**: The American Society for Testing and Materials
***: Specific O_2 uptake rate

2.4.3. Pathogens in organic solid wastes and the reduction after vermicomposting

One of the most important goals for OSW management is pathogen reduction because of the health hazard they represent to human beings if they are not treated carefully (WHO, 2004). There are more than 120 different types of viruses and bacteria that may be excreted in human and animal faeces (Schönning & Stenström, 2004). At the least, consideration of bacteria such as *Salmonella*, *Escherichia coli* and *Enterococcus* spp*.* are generally important when evaluating microbial risks from various OSW sources, including human faeces, sludge and animal manures (Jönsson *et al*., 2004; WHO, 2004).

Microorganisms present in fresh materials for vermicomposting often include many sanitation-related pathogens such as intestinal-faecal micro-flora, bacteria, protozoa, parasites and viruses (Liu *et al*., 2009). Pathological findings indicate that many animals appear to harbour bacteria such as *Salmonella* app.*, E. coli, Enterococcus* spp. and coliphages in the intestinal tract (Murry & Hinckley, 1992). Excreted in animal manures, they can survive for months in the soil, and then enter the food chain via groundwater, thereby causing human diseases (e. g. diarrhoea, typhoid).

Many previous studies have mentioned that there are large numbers of pathogens, bacteria and viruses in OSW materials due to organic matter-rich substrates, especially animal and human excreta (Aria & Domiguez, 2008; Edwards, 2007;

Tripathi & Bhardwaj, 2004). WHO (2004b) reported that pathogens in human excreta are mainly related to the solid part rather than the urinary fraction. The faecal indicators in greywater systems show numbers of coliform bacteria up to 8.1 log_{10} units, *E. coli* up to 6.1 log_{10} units and *Enterococcus* in a range of 3.0-5.1 log_{10} units (WHO, 2004b). For the other types, pathogens are also present in the separated solids of digested biogas, although they are inactivated by a slight reduction during the thermophilic stage in the biogas reactor (Yen-Phi *et al*., 2009). The main organic part of municipal residues is also reported to contain many pathogens, especially in the form collected from CUS systems (Vaz-Moreira *et al*., 2008). Therefore, the European Eco-label Standards Applicable to Compost requires that *E. coli* <1000 MPN g^{-1} and *Salmonella* spp. must not be detected in 25 g of compost (Brinton, 2000; Siebert, 2007b).

In sewage sludge, earthworms are reported to cause a maximum decrease in *Salmonella enteritidis* of 29% per day in contrast to a maximum decrease of 14% in their absence (Brown & Mitchell, 1981). Murry & Hinckley (1992) also reported that the number of *Salmonella enteritidis* decreases ~3.0% after 48 hours of inoculation of horse manure in the presence of *E. fetida*, whereas the bacteria increases by 2.0% in the absence of the earthworm. *E. fetida* can remove pathogens including faecal coliforms <1000 MPN g^{-1}; *Salmonella* spp. <3.0 MPN g^{-1} and helminth ova< one viable ova g^{-1} from septic tank sludge after 60 days (Rodríguez-Canché *et al.*, 2010).

2.4.4. Plant growth in vermicompost

The OSW materials can be an especially rich nutrient supply source for soil improvement. Dehydrated or composted products should be applied and mixed into the soil before cultivation starts (Somani, 2008). As a result, vermicompost of organic wastes supplies nutrients but also improves the physical structure and the water-holding capacity of the soil (Arancon *et al*., 2008). There is some well-substantiated evidence that vermicompost can significantly promote the growth of plants. Faster growth of *Vinca rosea* and *Oryza sativa* was observed after addition of vermicompost to soils (Reddy & Ohkura, 2004). Germination testing is a parameter for plant media where plants are tested for their rates of growth in different types or ages of compost product (Fig. 2.5).

Figure 2.5. Germination test of different composted materials with grass (*photo: camvan*)

Normally, the test plants include peppers (Arancon *et al*., 2005), beet vinasse (Tejada *et al.*, 2009), cucumbers (Atiyeh *et al.*, 2002), maize (Gutiérre-Miceli *et al*., 2008), wheat (Sharma & Madan, 1988), petunia (Arancon *et al*., 2008) and tomatoes (Atiyeh *et al.*, 2000a). A variety of bedding plants such as *Alyssum, Antirrhinum*, *Aster*, *Campaluna*, *Cineraria*, *Coleus*, plumose asparagus and sweet peas are also recommended for the germination test. Moreover, ornamental shrubs such as *Eleagnus pungens*, *Cotoneaster conspiacua* and *Pyracantha sp.* have been used to test the growth of a wide range of plants in a variety of vermicomposts (Edwards, 2007).

Germination of plant seedlings tends to be significantly better in vermicompost than in other commercial plant growth media (e.g. Metro-Mix 360) (Atiyeh *et al.*, 2001). In addition, there is evidence that vermicompost added to plant media increases growth, flowering and fruiting of vegetables (Arancon *et al.*, 2008). For example, tomatoes showed the highest yield when Metro-Mix360 was mixed with 20% pig manure vermicompost (Fig. 2.6). Some field crops also show an increase in vegetable growth at low application rates of vermicompost (Arancon *et al.*, 2008).

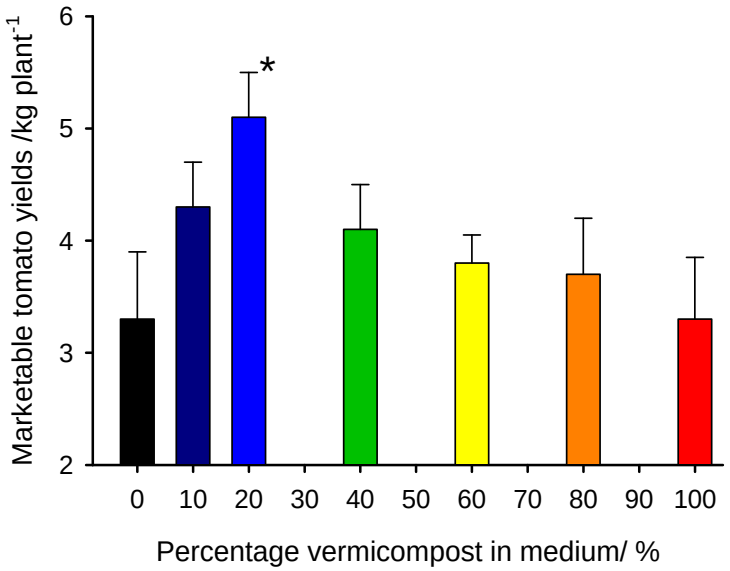

Figure 2.6. Tomato yields produced in the standard commercial media (Metro-Mix 360) supplemented with different vermicompost ratios (10-100%); '*' is significantly different compared with control (0%) (P<0.05), adapted from Arancon *et al.* (2008)

2.4.5. Benefit of earthworm biomass

When vermicomposting is established on an industrial scale, besides the vermicompost product there is a large amount of earthworm biomass that may be used as a protein source (Aranda, 1992). Earthworms can be used as a protein supplement for fish (Chan & Griffiths, 1988) as well as for animals such as poultry or pigs (Edwards, 2007). According to Hartenstein *et al.* (1980), the DM content in *E. fetida* is ~18% of body weight (Table 2.9). However, other studies report that the amount could be up to >34% (Aguilar & Escobar 2004; Rodríguez-Canché *et al.,* 2010).

Table 2.9: Comparison of chemical analyses of *E. fetida* components, % of DM

Component/ %	Chan & Griffiths (1988)	Aranda (1992)	Aguilar & Escobar (2004)	Rodríguez-Canché *et al.* (2010)
DM	na	20-25	41	34-36
Crude fibre	na	3-5	na	5-6
Protein	60-61	62-64	41	56-61
Carbohydrates	3	15-21	12	na
Fat	7-10	7-10	8	13-14
Ash	8-10	8-11	3	19-21

na: data not available

Total N-P-K concentrations in an earthworm body is ~10; ~1.0 and ~1.0% respectively and is independent of body weight during biomass increase (Chan & Griffith, 1988; Rodríguez-Canché *et al.*, 2010). Losses of Ca, Mg and Na during a doubling of the biomass are in the order of 0.8%, 0.4-0.7% and 0.4%, respectively. The percentage of protein in the tissues of *E. fetida* ranges from 40% to 64% by DM (Aguilar & Escobar, 2004; Rodríguez-Canché *et al.*, 2010).

2.5. VERMICOMPOSTING OF FAECAL MATTER

The EcoSan concept regards human excreta as a resource to be recycled rather than as a waste for disposal. The idea that human excreta (urine and faecal matter) are a waste with no useful purpose can be viewed as a modern construct (Kirchmann *et al*., 2005). The usage of human excreta for crop fertilisation has been widely practised in many regions of the world throughout history (Heinonen-Tanski *et al.*, 2005). The Chinese have been composting human and animal excreta for a few thousand years and Japan introduced the practice of recycling human excreta for agriculture in the 12th century. In Europe, until modern times, it was common for farmers to recycle human and animal excreta (Yadav *et al.*, 2010).

However, human excreta are the root of the pollution problems that result from conventional approaches to good sanitation, particularly flush-and-discharge toilets (Kirchmann *et al.*, 2005). Besides urine and faeces, greywater is also important to consider as it occupies the greatest volume in the household wastewater stream (Otterpohl *et al*., 1997) (Table 2.10).

Faecal matter actually represents the smallest volume as a household waste component (~50 kg person^{-1} year^{-1}) in comparison with urine (~500 L person^{-1} year^{-1}) or grey water (75000 L person^{-1} year^{-1}), but it contains much more pathogenic bacteria than the other fractions (Shalabi, 2006). Because of hygiene and sanitation requirements, systems of faeces treatment should sanitize this material adequately. The purpose of the systems should not be only good hygiene, but also recycling of nutrients (Kirchmann *et al*., 2005; Sinton *et al.*, 1998).

Table 2.10: Main components of household wastewater and their potential reuse options, adapted from Otterpohl *et al.* (1997) and Yadav *et al.* (2010)

Parameter	Grey water	*Urine	Faecal matter
Volume/ L P^{-1} $year^{-1}$	~75000	~500	**~50
N_{tot}/ %	3	80-87	10
P_{tot}/ %	10	50-60	40
K_{tot}/ %	34	20-54	12
Methodology	Water treatment	Water treatment	Vermi-/composting, anaerobic digestion
Reuse option	Water cycle	Fertiliser	Soil conditioner

*: These intervals are combined from several published studies using the highest and lowest limits
**: Option bio-waste addition in kg $person^{-1}$ $year^{-1}$

Suitable alternatives for treatment of faecal matter are anaerobic digestion, composting and vermicomposting depended on the type of faecal matter (Shalabi, 2006). From different models of toilets, faecal matter can be collected from the household wastewater mainly in two ways: separate products (faeces and urine), or as one combined material (faeces plus urine or black water). However, many previous studies report that the most beneficial way to recover excretal nutrients is to collect urine and faeces separately (Otterpohl *et al.*, 1997; Yahav *et al.*, 2009). According to Otterpohl *et al.* (1997), separated urine and faeces can be treated more efficiently than the combined material.

Non-urine faecal matter (Fm) has a complex and variable composition that is derived from blackwater (Otterpohl *et al.*, 1997). The material mainly consists of undigested organic matter such as fibres made up of carbon, which seem to be a great substrate for earthworms (Yadav *et al.*, 2010). Approximately one-third of the Fm consists of food remains, one-third is intestinal bacteria and one-third is from the intestine itself (enterocytes and liquids) (Shalabi, 2006). Although Fm contains fewer nutrients than urine, the humus produced from the solid material actually contains high contents of P_2O_5 and K_2O (3.0-5.4% and 1.0-2.5%, respectively) (Faechem, 1983).

To date, the composting of Fm has been widely used for sanitation of this OSW materials (WHO, 2006; Yadav *et al.*, 2010). During composting, the Fm can be treated efficiently because pathogens are killed as a result of elevated temperatures and their lower competitiveness as opposed to thermophilic microbes (Faechem *et al.*, 1983). However, Yadav *et al.* (2010) reported that a sufficiently high temperature for pathogen destruction in Fm is difficult to achieve because the temperature during composting normally only increases by 10-15^{o}C above the ambient temperature. On the other hand, vermicomposting was focused on as another biological treatment for Fm instead of a classical method (composting). According to Shalabi (2006), vermicomposting might be an appropriate alternative to classical composting to convert Fm into valuable vermicompost (Shalabi, 2006). Several previous studies have reported that Fm could be treated by vermicomposting, but with different pre-treatment and experimental setups (Table 2.11).

Table 2.11: Vermicomposting of faecal matter (Fm) and mixtures of Fm and chemicals or other amendment materials, calculations of DM

Substrate	Pre-treatment	Earthworms		Period/ weeks	Reference
		Species	Density/ g kg^{-1}		
Fresh Fm	na	*E. fetida & D. veneta*	~170	13	Shalabi (2006)
Fresh Fm	120 g $CaCO_3$ $kg\ substrate^{-1}$	*E. fetida, L. rubellus & D. veneta*	~170	16	Shalabi (2006)
Fresh Fm	1-week pre-composting	*E. fetida*	200-300	16	Yadav *et al.* (2010)
Mixtures of Fm, soil & Fm vermicompost	1-week pre-composting	*E. fetida*	200-300	16	Yadav *et al.* (2010)

na: data not available

2.6. ANAEROBIC DIGESTION AND VERMICOMPOSTING OF SEPARATED SOLIDS OF DIGESTED BIOGAS SLURRY

Anaerobic digestion (AD) is one of the sustainable methods for OSW treatment under the concept of OSW management (Christensen, 2010). During the AD process, organic materials are degraded and produce biogas (CH_4 and CO_2) (Lastella *et al*., 2002) (Fig. 2.7). In agriculture-based countries, biogas is produced from household reactors to produce energy for lighting and cooking (Lastella *et al*., 2002). For commercial biogas production in developed countries such as Germany, animal slurries, maize plants and urban wastes are the prime in-put sources for the biogas plants.

Figure 2.7. A biogas plant system in Bonn, Germany

The effluent of biogas plants (digested wastes) is used as an organic fertiliser for agriculture. In some cases, the effluent is separated into a solid and liquid phase (Fig. 2.8). The liquid phase (leachate) can be used directly as a fertiliser (Suthar, 2010).

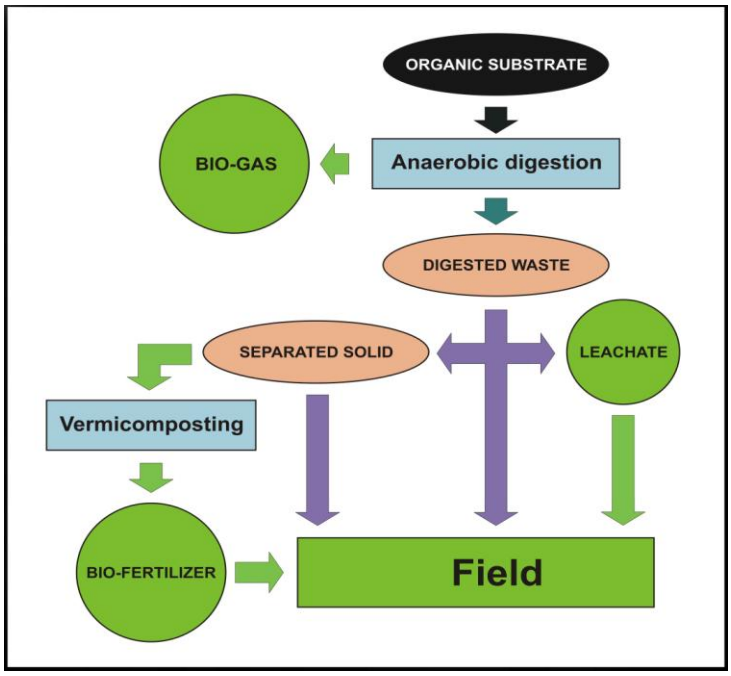

Figure 2.8. Sanitary treatment of organic solid waste materials by anaerobic digestion treatment combined with vermicomposting (*own creation*)

The purpose of separating is to reduce the nutrient load in the liquid phase. The liquid phase contains more than 90% of water and its economic use is limited. For example, a 500 KW_{el} biogas plant has to manage ~20 m^3 daily of effluent. With less nutrients in the effluent, more effluent can be applied per unit area of field. This results in lower costs of effluent management. The separated solids of digested biogas slurry (Bs) (Fig. 2.9) should be treated further for efficient OSW management. The Bs can be added directly to fields to improve soil structure. The Bs is also considered as a good input substrate of composting and vermicomposting processes to achieve maturity before use for agriculture.

Figure 2.9. Separated solids of digested biogas slurry (Bs) from a biogas plant at Schöpingen, Germany

In developing countries such as India, Bs material is often deposited in open places near the biogas unit and these deposits become an active breeding site for disease vector insects (e.g. houseflies) (Suthar, 2010). Because of sanitation requirements, the Bs material should be treated with several sanitisation alternatives such as composting or vermicomposting (Garg *et al*., 2006).

With a high pH value (>8.0), fresh Bs material can be utilised effectively as a C_{org} source in arable soils (Ernst *et al.*, 2008). Furthermore, this substrate contains easily digestible and assimilated carbohydrates and proteins (Jeyabal & Kuppuswamy, 2001; Suthar, 2010). However, in comparison with the digested biogas leachate, the lower nutrient concentration in the Bs material can be a limit for direct use as an effective fertiliser in cropping systems (Lastella *et al.*, 2002). With further treatments such as the alternative of vermicomposting, Bs material can be stabilised and converted to mature compost. Later on, the product can be applied as a hygienic bio-fertiliser source for plants as well as a soil structure improver to the soil.

Like Fm, fresh Bs should be pre-treated initially and mixed with other amendment materials before being fed to earthworms for the vermicomposting process (Garg *et al*., 2006; Sanwan *et al.*, 2008; Suthar, 2010) (Table 2.12).

Table 2.12: Vermicomposting of the separated solids of digested biogas slurry (Bs) and mixtures of Bs with other amendment materials, calculations of DM

Substrate	Pre-treatment	Earthworms		Period/ weeks	Reference
		Species	Density/ g kg^{-1}		
Bs, cow dung, sugarcane, coirpith & weeds	na	*E. eugeniae*	~3.8	17	Jeyabal & Kuppuswamy (2001)
Bs & solid textile mill sludge	Daily 2-week mixing	*E. fetida*	3.3-8.3	15	Garg *et al*. (2006)
Bs, cow dung & sugar mill sludge	2-week pre-composting	*E. fetida*	11.7-13.3	13	Sangwan *et al.* (2008a)
Bs, fermented slurry & soil	na	*L. terrestris, A. longa & A. calininosa*	3.7-4.1	6	Ernst *et al.* (2008)
Bs & vegetable-market solid waste	3-week pre-composting	*E. fetida*	6.9-7.4	15	Suthar (2009)
Bs & crop residues	3-week pre-composting	*E. fetida*	~ 5.0	15	Suthar (2010)

na: data not available

2.7. OTHER TREATMENTS OF ORGANIC SOLID WASTES FOR SANITATION

The OSW materials can be defined by four typical substrates depending on their sources. (*i*) Municipal organic solid waste, collected mainly from urban areas

including household, supermarket, restaurant, street and garden wastes. (*ii*) All kinds of industrial waste. A large amount of this waste is produced by many industrial activities (Christensen, 2010b). (*iii*) Hazardous wastes, which are produced from hospitals or specific institutions and can be considered a source of OSW. (*iv*) Agriculture residues from livestock activities, including both crop by-products and animal manures.

Despite the fact that all the OSW materials have a high potential to recycle nutrients, they are also considered a source of pathogen transfer in cases of low sanitation conditions. Besides the vermicomposting alternative, under the EcoSan concept and sanitisation requirements, four other systems can be considered suitable for this purpose. They are sanitary landfill, incineration, bio-digestion and composting. Depending on the properties of various OSW sources, a suitable system must be chosen for sanitation treatment.

2.7.1. Sanitary landfill

A sanitary landfill is often established in abandoned or unused quarries, bore pits or mine shafts. A properly designed and well managed landfill can be a hygienic means of disposing of organic solid waste while requiring relatively low financial input (Ettala, 1988). This method has been in use for a long time, not only in agricultural activities but also in urban areas, because of its easy implementation (Eshet *et al*., 2005).

The sanitary landfill is the simplest way to hygienically remove pathogen sources in OSW from animals and humans. Besides, this method remains common practice in almost all countries, especially developing countries (Belevi & Baccini, 1989; El-Fadel *et al*., 1997). Disposing of the OSW materials in a sanitary landfill involves burying a mixture of all wastes (Mata-Alvarez *et al.*, 2000). In the developing countries where there are less financial resources available for the administration of OSW management, the mixture of various wastes is not sorted to specific parts to provide for further efficient treatment (Eshet *et al*., 2005). As an emergency method for sanitation, sanitary landfills are established in controlled areas which are located far from urban areas of dense population. Deposited waste is normally compacted to increase its density and stability, and covered to prevent attracting mice or rats. However, a disadvantage of this method is the generation of output wastes such as landfill gas (LFG) and landfill leachate. LFG is considered to be a serious agent in global warming and leaching sludge is mentioned as a major factor of groundwater pollution (Rashid *et al*., 2010).

2.7.2. Incineration

Disposal through incineration involves combustion of OSWs which have a high dry matter content. These OSWs is especially suited to this method as they contain a high potential of heat energy as well as disease pathogens (Viétez & Ghosh, 1999). Incineration and other high temperature treatment systems for sanitisation are sometimes described as burning methods (Dempsey & Oppelt, 1993). Incinerators

convert the OSWs into energy (heat, electricity) and destroy contaminating disease sources.

The incineration is carried out on both a small scale by personal individuals (hospitals, farms, etc.) and on a larger scale by local authorities or industrial zones (Linak & Wendt, 1993). It is recognised as a practical method for disposing of certain hazardous wastes (Ghosh *et al.*, 2000). Moreover, incineration can be common in some countries where land is scarce, as these facilities generally do not require as much area as the sanitary landfill method (Ikeguchi, 1994). Beneficially, this method produces heat that can be used as an energy resource (Eshet *et al.*, 2005; Finnveden *et al.*, 2007). However, incineration is a controversial method of waste disposal, due to issues such as emission of gaseous pollutants.

2.7.3. Anaerobic digestion (bio-digestion)

Among several alternatives for OSW treatment, anaerobic digestion is considered a beneficial method for achieving sanitisation. The energy content of the OSW can be used directly as a combustion fuel or indirectly by processing into electricity (Abraham *et al*., 2007; Lastella *et al*., 2003). Since the Kyoto Protocol on greenhouse gas (GHG) emissions was signed in 1997, in order to meet the GHG emissions requirement of the EU, to reduce GHG emission in the period 2008-2012 by 8.0% (to below the 1990 level), many large-scale biogas plants have been established (Amon *et al*., 2007). For example, in Germany more than 3400 biogas plants were built between 1997 and 2009 (Maciejczyk, 2010) (Fig. 2.10).

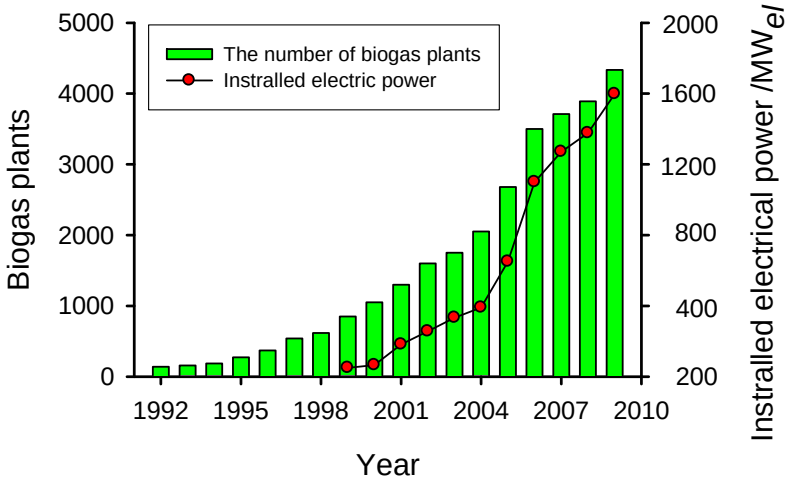

Figure 2.10. Biogas plant expansion and installed electrical capacity in Germany (Maciejczyk, 2010)

Simple construction and the relatively cheap cost of small-scale biogas plants seem to be the reasons for their wide application in developing countries where a huge resource of agricultural waste is available (Monteny *et al*., 2006). With the anaerobic bio-digestion mechanism, biogas plants of a farm-scale size can be used directly as a fuel for cooking or heating (Ayalon *et al*., 2001). Furthermore, a larger-scale system not only solves the hygiene problems but can also produce biogas with a high CH_4

yield. The biogas from biogas plants can be used as fuel for boilers to generate steam and electricity (Mata-Alvaez *et al.*, 2000a; Rashid *et al.*, 2010). In addition, sanitary biogas systems have been developed to ensure that no pathogens are present in the digested output material from the anaerobic digestion process. Vermicomposting is considered an alternative in combination with bio-digestion for a more efficient and hygienic treatment of OSW materials.

2.7.4. Composting

Several OSW materials which contain high organic dry matter (oDM) but low DM such as household/garden wastes and food scraps/by-products from agriculture, can be recycled hygienically by aerobic treatments (vermi-/composting) (Senesi, 1989). OSW materials are universally recognised as important contributors to the maintenance of global fertility and productivity of soil. However, without sanitation treatment, they are also considered pathogen-spreading sources in the nutrient cycle. The sanitary systems with aerobic treatments have been investigated to ensure that products from OSW materials are acceptable for agriculture (Mata-Alvarez *et al.*, 2000b; Senesi, 1989).

The intention of aerobic treatments is to accelerate the decomposition of organic matter under controlled conditions during a period of time (Bernal *et al*., 2009; Ndegwa & Thomson, 2001). There are many technologies for treatments varying in complexity from a simple home-compost pile to an industrial scale enclosed-vessel digestor (Bernal *et al*., 2009; Neklyudov *et al.*, 2008). Besides vermicomposting, another aerobic treatment is the aerobic process by heating, namely composting (Garg *et al*., 2008; Maboeta & Rensburg, 2003).

Like vermicomposting, composting is a biological method for OSW management (Edwards *et al.*, 1998). Expansion of composting facilities has increased in recent years. For example, ~400 composting plants were established in 2009 and treated ~7.0 million tons of OSW materials in Germany (BGK, 2009) (Fig. 2.11).

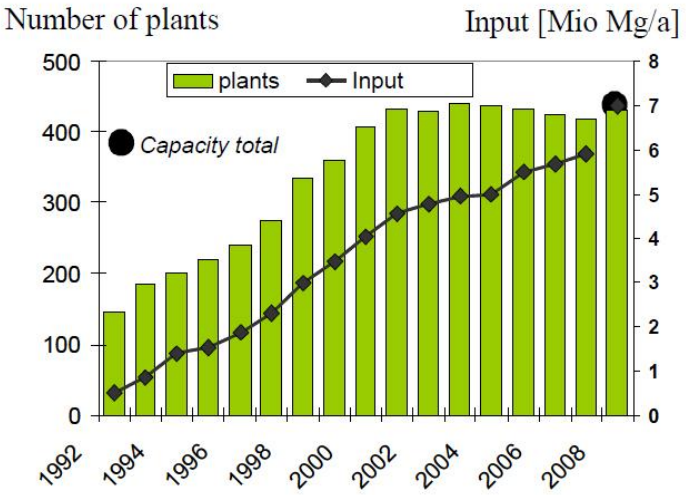

Figure 2.11. Expansion of composting facilities and input-substrate capacity in Germany (BGK, 2009)

2.8. DIFFERENCES BETWEEN VERMICOMPOSTING AND COMPOSTING

In comparison with compost produced from the same parent material, vermicompost is richer in content of available nutrients after the feeding substrate passes through the worm gut (Zhenjun, 2004). Furthermore, vermicompost is more effective for soil improvement than compost after the same period of maturity. A comparison of vermicomposting and composting is presented in Table 2.13 (Somani 2008).

Table 2.13: Comparison of composting and vermicomposting with some advantages of process, product and application, adapted from Frederickson *et al*., (2007); Lazcano *et al.* (2008) and Somani (2008)

Parameter	Composting	Vermicomposting
Heating requirement	Remained & controlled	Avoiding excess heat
By-product	No by-product	Worm biomass
Decomposition time	Long (≥ 6 months)	Less
Decomposition degree of OSW material	Incomplete in large & heterogeneous particles	Complete, resulting in small & uniform particles
Smell of product	Still bad odour	Less bad odour
Available salt content in product	Increased	More increased
pH level of product	Less reduced, pH>7	Reduced, near neutral
C/N ratio of product	Decreased	More decreased
Available N-P-K in product	Increased	More increased
Humification & stabilisation of product	Increased	More increased
Pathogenic microbes in product	Reduced	More reduced
Texture & structure of soil with product	Improved	More improved
Yield of plant crops with product amendment	Increased	More increased

2.9. THE NEED FOR VERMITECHNOLOGY IN DEVELOPING COUNTRIES

Vermicomposting can be considered an innovative type of biotechnology that does not call for expensive laboratories or sophisticated industrial equipment. Moreover, vermicomposting is a biological and environmentally friendly treatment that meets the sustainability goal of MDG7 and ties in with the EcoSan concept (Arancon *et al*., 2005; Gunadi *et al*., 2003). There are several practical and financial reasons to invest in vermicomposting applications in low-income areas, especially in tropical countries (Edwards, 2007; Maboeta & Rensburg, 2003; Somani, 2008) (Table 2.14).

Table 2.14: Reasons to invest in vermicomposting applications in developing countries adapted from Edwards (2007); Maboeta & Rensburg (2003) and Somani (2008)

Factor	Reason to invest in developing countries
Investment & finance	The application requires less capital and simple technologies than other alternatives.
Source of OSW material and earthworms	Almost all developing countries are located in tropical areas and have various species of earthworms and more available & unused OSW materials produced by livestock and agricultural activities than developed countries.
Establishment	The application is established easily of any scale and anywhere in developing countries, which more seldom have centralised OSW treatment facilities as found in developed countries.
Maintenance and operation	Civil construction and labour costs are lower in developing countries.
Hygiene and environmental protection	Numerous human health risks relating to sanitation still exist in developing countries.
Sustainable development	Developing countries suffer from population pressure and factors associated with the degradation of the natural resource base more than developed countries

3. MATERIALS AND METHODS

To fulfill the research objectives, three main groups of experiments were conducted. Firstly, the vermicomposting suitability of OSW materials, including biowaste (Bw), non-urine faecal matter (Fm) and the separated solids of digested biogas slurry (Bs) were tested in the presence of *E. fetida*. Secondly, the influence of three essential physico-chemical parameters (pH, salt content and TAN) on *E. fetida* was tested in an aqueous solution. Single- and multi-factor effects of these parameters were investigated to find the optimum conditions for worm survival. Finally, vermicomposting experiments were carried out on three test OSW materials to evaluate the efficiency of *E. fetida* in monoculture treatment and *E. fetida* with *D. veneta* in polyculture treatment.

3.1. ORGANIC SOLID WASTE SUITABILITY TEST FOR VERMICOMPOSTING OF *E. FETIDA*

E. fetida does not accept all kinds of fresh organic material. Hence preliminary vermicomposting tests were conducted to investigate the acceptability of the proposed substrates. Three different variations were tested, each with distinct physico-chemical characteristics. *Variation A* test was comprised of fresh Bw, Fm and Bs. *Variation B* test was comprised of mixtures of varying proportions of fresh Fm and Bs. *Variation C* test only used 3-day pre-treated Bs (Bs3-HCl), which was also acidified by HCl to reduce the high pH of Bs.

3.1.1. Earthworm (*E. fetida*)

Earthworms were purchased from Regenwurmfarm Tacke GmbH Company, Borken, Germany. Mature *E. fetida* (~9-12 weeks old) with a weight of 0.25-0.4 g worm^{-1} and a length of 2.5-5.0 cm worm^{-1} were randomly picked from a population cultivated in cow dung for use in the experiments.

3.1.2. Feeding materials

3.1.2.1. Bio-waste

Bio-waste (Bw) was collected from Cussanushaus, an international dormitory of Bonn University, Germany. The Bw material was a mixture of vegetables, eggshells, bread, fruits and milk products produced by 15 students for a period of three days. After collection, Bw was cut manually into small pieces (Φ≤ 1.0 cm). Finally, the homogeneous Bw was stored at room temperature for three days before being used for vermicomposting.

3.1.2.2. Non-urine faecal matter

Non-urine black water was collected from a building with six households in Kaiserslautern, Germany. The black water passed through a filter bag to separate the non-urine faecal matter (Fm). The Fm was then homogenised manually in a 100-L plastic container with a mixing rod and the container was covered and stored until the Fm substrate was used for vermicomposting.

3.1.2.3. Separated solids of digested biogas slurry

Separated solids of digested biogas slurry (Bs) were obtained from the storage tank of an on-farm biogas plant in Schöpingen, Germany. The dried Bs was taken from the storage tank and was then piled on the ground for one week. A sample of ~100 kg was taken from the pile and transported to the greenhouse facility of the Plant Nutrition Institute, INRES, Bonn University. Later on, it was stored in two covered 100-L plastic containers. It is worth mentioning that the input material for the biogas plant was a mixture of cattle slurry and maize.

Physico-chemical characteristics of fresh Bw, Fm and Bs are given in Table 3.1.

Table 3.1: Main physico-chemical properties of the three fresh OSW materials in a suitability test for the vermicomposting of *E. fetida*. pH level, EC and TAN content were analysed in fresh substrate, the others were analysed in DM, but all data given of DM

Parameter	Fresh Bw	Fresh Fm	Fresh Bs
DM/ %	27.6	11.4	26.0
pH	5.3	5.8	8.4
EC/ mS cm^{-1}	9.1	3.2	3.7
TAN/ g kg^{-1}	*<3.8	*<9.3	11.4
**NH_3/ mg kg^{-1}	<0.4	<3.3	1406.2
C_{tot}/ %	45.6	45.8	43.8
N_{tot}/ %	3.8	2.1	1.4
C/N ratio	14.0	21.6	31.3
P_{tot}/ %	2.9	0.8	1.1
K_{tot}/ %	0.6	0.1	1.1

*:''<'' Detection limit of the modified Quantofix-N-Volumeter method (*see 3.4.4.2*)
**: Calculated from TAN content and pH level (*see 3.4.4.4*)

3.1.3. Experimental design for the the suitability test of OSW materials

The whole experimental setup and more details of the three tests (*Variations A-C*) are given in Table 3.2.

Table 3.2: Experimental design for the acceptance of feeding material of *E. fetida* at 22 ± 5°C including ***variation A***: fresh substrate (Bw, Fm and Bs); ***variation B***: mixture of fresh Bs and fresh Fm, and ***variation C***: Bs3 pre-treated with HCl; Bs3 is the TAN pre-treated Bs for three days; '**Bk**' is control without earthworm

Test exp.		Feeding material			*E. fetida*		Test period/ days
Variation	Code	Substrate	Ratio/ w:w	Weight/ kg box^{-1}	Mass/ g box^{-1}	No./ box^{-1}	
A	Bw	Fresh Bw	-	2.0	100	~400	20
	Bw**Bk**	Fresh Bw	-	2.0	0	0	20
	Fm	Fresh Fm	-	2.0	100	~400	20
	Fm**Bk**	Fresh Fm	-	2.0	0	0	20
	Bs	Fresh Bs	-	2.0	100	~ 400	20
	Bs**Bk**	Fresh Bs	-	2.0	0	0	20
B	Mix1	Bs + Fm	1:2	0.8 + 1.6	100	398	30
	Mix2	Bs + Fm	1:4	0.6 + 1.8	100	393	30
	Mix3	Bs + Fm	1:6	0.4 + 2.0	100	286	30
	Mix4	Bs + Fm	1:8	0.3 + 2.1	100	361	30
C	Bs3-A1	Bs3 + HCl 0.5%	2:1	0.5 + 0.25	50	229	25
	Bs3-A2	Bs3 + HCl 0.7%	2:1	0.5 + 0.25	50	359	25
	Bs3-A3	Bs3 + HCl 1.0%	2:1	0.5 + 0.25	50	309	25
	Bs3-A4	Bs3 + HCl 1.2%	2:1	0.5 + 0.25	50	317	25

Suitability vermicomposting experiments were conducted in square wooden boxes (0.4 x 0.5 x 0.3 m). Each box was divided into two parts separated by a wooden wall (Fig. 3.1A). This separating wall had four small round windows (Φ = 10 cm), covered with a plastic mesh (Φ = 0.3 mm). Earthworms could move from one side to the other while the plastic mesh prevented substrate transfer. The bottom of the box was covered with metal mesh (Φ = 10 mm) for aeration.

Figure 3.1. (**A**) Design of an experimental box for suitability vermicomposting and (**B**) the experimental setup with the tested OSW material in one side and *E. fetida* with bedding in the other.

At the beginning, the test feeding materials (0.5-2.4 kg box^{-1}) were added to one side of the box (Fig. 3.1B). Approximately 200 g of reference bedding material (worm medium from the supplier) with 100 g *E. fetida* were added to the other side. In parallel, the same test substrate without *E. fetida* was used as the control.

After being left for a few days in the box and no more feed for *E. fetida* existed in the side with the bedding material (considered a well accepted habitat), the worms were expected to move through the plastic mesh to the other side of the box with the test substrate in order to survive. If the worms did not accept the test material as a feed, they were expected to escape out and die.

During the 25-30-day process, the experimental boxes were covered by wooden roofs to maintain moisture and darkness. Each box was placed on a wooden tray where dead earthworms were collected daily. All experiments were carried out in the cellar of the greenhouse at the Plant Nutrition Institute, INRES, Bonn University, at room temperature (22 ± 5^{o}C) (Table 3.1). At the end, earthworms were collected manually from the test substrate only and then counted and weighed.

3.1.3.1. Suitability of fresh organic solid wastes

Variation A tested the suitability of individual fresh Bw, Fm and Bs as feed for *E. fetida* (Table 3.2). No new substrate or additional water was fed into the reactors during the 20 days of the process. The pH level, DM and EC of the test substrates were measured only at the beginning and the end of the experiment.

3.1.3.2. Suitability of mixed organic solid wastes

Variation B tested the suitability of mixtures of fresh Bs and Fm as worm feed (Table 3.2). The mixed substrates were made with varying proportions of fresh Bs and Fm from 1:2 to 1:8 by fresh weight. The physico-chemical properties of four mixed substrates are given in Table 3.3.

Table 3.3: Characteristics of fresh Bs and Fm and their mixtures (Mix1-4) in different ratios by fresh weight before use in the suitability test; EC was calculated for DM

Parameter	Fresh Bs	Fresh Fm	Mixtures of fresh Bs and Fm			
			Mix1	Mix2	Mix3	Mix4
Bs : Fm ratio (w:w)	1:0	0:1	1:2	1:4	1:6	1:8
DM/ %	34.4	12.1	17.4	13.8	14.7	14.1
pH	8.8	5.4	8.1	7.3	6.6	6.3
EC/ mS cm^{-1}	4.7	8.5	5.2	6.0	6.9	7.3

During the 30-day experiment, the materials were moistened by adding 100 mL of distilled water on day 6 and day 11 to maintain a suitable DM content for the earthworms. pH level, DM and EC of the tested materials were measured every three days. Like all other test variations, all surviving *E. fetida* were determined by hand-sorting without OSW from the test materials and counted after the process.

3.1.3.3. Suitability of pre-treated organic solid wastes

Variation C tested the suitability of the four Bs pre-treated with HCl as a feed (Table 3.2). Fresh Bs had ~12.0 g TAN kg^{-1} (including ~4.0 g NH_3-N kg^{-1}) and a pH level of ~9.0 (Table 3.4).

Table 3.4: Physico-chemical properties of fresh Bs, 3-day pre-treated Bs (Bs3) and pre-treated Bs-HCl (Bs3-A1-4) before use as a feed in the suitability tests

Parameter	Fresh Bs	Bs3	Mixtures of Bs3 and HCl			
			Bs3-A1	Bs3-A2	Bs3-A3	Bs3-A4
HCl concentration/ %	-	-	0.5	0.7	1.0	1.2
Adjusted HCl dosage/ g kg^{-1}	-	-	11.8	15.5	23.6	26.4
DM/ %	26.3	37.5	22.5	21.9	22.3	21.9
pH	8.9	9.1	8.2	7.5	7.0	6.5
EC/ mS cm^{-1}	5.1	4.6	5.3	6.5	8.0	9.1
*TAN/ g kg^{-1}	12.1	3.4	3.3	3.3	3.3	3.3
**NH_3/ mg kg^{-1}	2120	1530	266.7	58.5	36.5	9.4

*: Measured by the modified Quantofix-N-Volumeter method (*see 3.4.4.2*)
**: Calculated from pH level and TAN content (*see 3.4.4.4*)

Prior to acidification, fresh Bs was dried on an aluminium tray (40 x 60 cm) for TAN reduction at room temperature (~20°C) for three days. TAN and the pH level of the Bs material were measured daily in this period. The 3-day pre-treated Bs (Bs3) (containing 3.4 g TAN kg^{-1}) was acidified to pH levels between 8.2 and 6.5 by different HCl dosages (Table 3.4). At these HCl adjustments, the NH_3 concentration decreased from >1530 mg kg^{-1} to <300 mg kg^{-1}.

The pH level, DM content and EC value of the four test materials were determined every three days. During the 25 days of the experiment, all Bs3-HCl materials were moistened by distilled water on day 1 (100 mL); day 4 (200 mL); day 15 (50 mL) and day 23 (100 mL).

3.2. SINGLE- AND MULTI-FACTOR TESTS OF INFLUENCES ON THE SURVIVAL RATE OF *E. FETIDA*

Little is known about the physico-chemical parameters of favourable organic substrates for sustained vermicomposting as indicated by worm survival rate (Edwards, 2008; Kaplan *et al.*, 1980). While it is not an easy task to adjust the chemical parameters in a solid organic medium properly in order to test their effect, chemical parameters can be easily controlled in an aqueous solution. Hence, the aqueous medium was chosen to test the effects of the selected chemical parameters on the earthworms. The pH level, salt content and TAN were tested to investigate the factors with the greatest influence on earthworm survival rate.

3.2.1. Single-factor experimental design

Single-factor experiments were carried out to test the effect of one chemical parameter at a time on earthworms (*E. fetida*) while the other two were kept constant in an aqueous solution (Table 3.5).

Table 3.5: Overview design of single-factor tests for the survival rate of *E. fetida* (*n* = 3)

Single-factor test		Fixed factor (EC/ mS cm^{-1})	Chemical
Factor	Test range		
pH	2.0	EC = 3 & TAN = 0	Citric acid
	3.0-7.0	EC = 3 & TAN = 0	Citric acid/Na_2HPO_4
	8.0	EC = 3 & TAN = 0	KH_2PO_4/Na_2HPO_4
	9.0	EC = 3 & TAN = 0	Glycine/NaOH
	5.0-8.7	EC = 8 & TAN = 0	KH_2PO_4/Na_2HPO_4
EC	0-32.2 mS cm^{-1}	pH = 5.4	KCl
TAN	30-480 mmol L^{-1}	pH = 8 & EC= 3-40	NH_4HCO_3

For pH, the range 2.0-9.0 was tested with one pH unit representing one treatment, i.e. 2.0, 3.0, 4.0, etc. Four different buffer systems, each covering a narrow pH range, were used to produce the desired pH range. In order to test the effect of the different buffers on earthworms in another experiment, the buffer system (KH_2PO_4/Na_2HPO_4) was used to cover the pH level between 5.0 and 8.7. This pH range is known as the acceptable range for vermicomposting.

For salt content, an amount of up to 40.0 g KCl L^{-1} (EC 32.2 mS cm^{-1}) was used to investigate the influence of salt content. For the TAN content, test solutions were prepared from 30 mmol L^{-1} to 480 mmol L^{-1} by NH_4HCO_3.

3.2.2. Multi-factor experimental design

Multi-factor experiments were designed to test the simultaneous effect of two chemical parameters on the *E. fetida* survival rate while the third was kept constant in an aqueous solution (Table 3.3). Chemical parameters levels were adjusted in four steps: (*i*) The pH was roughly adjusted by KH_2PO_4/Na_2HPO_4 buffer; (*ii*) NH_4HCO_3 was added to adjust the TAN; (*iii*) KH_2PO_4 or Na_2HPO_4 salt was used to adjust the pH level precisely; (*iv*) KCl was used to adjust the EC.

Mature *E. fetida* (~9-12 weeks old) with a weight ~0.3-0.4 g $worm^{-1}$ (~2.5-4.5 cm $worm^{-1}$) were purchased from Regenwurmfarm Tacke GmbH, Borken, Germany. For all single- and multi-factor experiments, distilled water was used as a control at 20°C. One worm was placed in a 0.1 L $bottle^{-1}$ and its survival rate was recorded in hours (Fig. 3.2). *E. fetida* was considered to be dead when it showed no reaction after being touched with a small spoon.

Table 3.6: Overview design of multi-factor tests for the survival rate of *E. fetida* (*n* = 3)

Multi-factor test		Fixed factor (EC/ mS cm^{-1})	Chemical
Factor	Test range (EC/ mS cm^{-1} & TAN/ mmol L^{-1})		
pH & EC	pH: 5.0-8.7 & EC: 8.0-20.0	TAN = 0	KH_2PO_4/Na_2HPO_4 & KCl
pH & TAN	pH: 6.0-8.0 & TAN: 0-20.0	EC = 8	KH_2PO_4/Na_2HPO_4; NH_4HCO_3 & KCl
		EC = 14	KH_2PO_4/Na_2HPO_4; NH_4HCO_3 & KCl
EC & TAN	EC: 8.0-20.0 & TAN: 0-45.0	pH = 6	KH_2PO_4/Na_2HPO_4; KCl & NH_4HCO_3
		pH = 7	KH_2PO_4/Na_2HPO_4; KCl & NH_4HCO_3
		pH = 8	KH_2PO_4/Na_2HPO_4; KCl & NH_4HCO_3

Figure 3.2. Single- and multi-factor tests of pH, EC and TAN for the survival rate of *E. fetida* (*n* = 3)

3.2.3. Statistics and regression equation construction

All treatments were replicated three times (*n* = 3). An ANOVA Test with Duncan mean separation (SPSS 17.0) was used to evaluate significant differences between treatments. Based on data from the multi-factor tests (*see 3.2.1*), a linear multi-factorial regression equation was constructed to predict earthworm survival rate from pH, EC and TAN content (*N* = 201) (Expert-Design 7.1.5).

$$f_t = B_0 + \sum B_i X_i \ (i = 1\text{-}3) \qquad [1]$$

3.3. MONOCULTURE AND POLYCULTURE VERMICOMPOSTING OF *E. FETIDA* AND *D. VENETA*

This experiment examined vermicomposting of three OSW materials from different sources. The feeding substrates were Bs, Fm and Bw. Three important physico-chemical parameters for earthworms (pH, EC and TAN) were identified in the previous experiment (*see 3.2*). Moreover, the testing of the multi-factor influence of these factors on worm survival rates indentified suitable methods for pre-treatment of the OSM materials. Pre-composting of fresh Bs and Bw material produced suitable feeding materials for the earthworms. Thus, two pre-treated single substrates (single Bs and single Bw) and non-pre-treated Fm (single Fm) as well as three mixtures of these substrates (combined BsBw, combined FmBw and combined BsFmBw) were used as feed for vermicomposting.

In vermicomposting experiments, mixtures of *E. fetida* and *D. veneta* were incubated for polyculture treatment in comparison with monoculture treatment (*E. fetida*).

3.3.1. Earthworms (*E. fetida* and *D. veneta*)

Mature *E. fetida* (~250-400 mg worm^{-1} and ~2.5-4.0 cm worm^{-1}) were randomly picked, counted and balanced for the vermicomposting experiments (Fig. 3.3 and Table 3.7). The species was purchased from Global-Wormen Company, Enschede, the Netherlands.

Similarly, mature *D. veneta* (~1.0-1.2 g worm^{-1} and ~8-10 cm worm^{-1}), which were introduced by Superwürm e.K. Farm, Düren, Germany, were separated from the bedding material (Fig. 3.3 & Table 3.7). After counting and weighing, they were mixed with *E. fetida* for polyculture incubations in different substrates.

Figure 3.3. The performance of *E. fetida* (small) in comparison to *D.veneta* (bigger)

Table 3.7: Comparison of biological properties of *E. fetida* and *D. veneta* for vermicomposting

Properties	*E. fetida*	*D. veneta*
Distribution	Worldwide	Europe
Individual size (length/ cm & mass/ g)	Small (2.5-4.0 & 0.2-0.4)	Big (8-10 & 0.8-1.1)
Growth rate (life cycle/ days)	Rapid (45-51)	Slow (97-214)
Hand-sorting	Normal	Easy

3.3.2. Feeding materials

3.3.2.1. Separated solids of digested biogas slurry

The fresh Bs was obtained from the same source mentioned in the suitability test (*see 3.1.2.3*). After TAN pre-treatment (*see 3.3.3.2*), the pre-treated Bs was used directly as a single feeding substrate for vermicomposting. It was also mixed with other materials (Bw and Fm) to produce combined substrates (BsBw and BsFmBw).

3.3.2.2. Non-urine faecal matter

The fresh Fm was collected from the black water container of a urine-separated toilet of the Lambertsmühle building, in Burscheid, Germany (Fig. 3.4). The sanitation system of this building belongs to a project of the OtterWasser GmbH, Germany. After transporting the waste to the greenhouse at the Plant Nutrition Institute, INRES, Bonn University, the material was homogenised by mixing manually in a 100-L plastic container. It was then stored in the covered plastic container without any further pre-treatment before being use for vermicomposting.

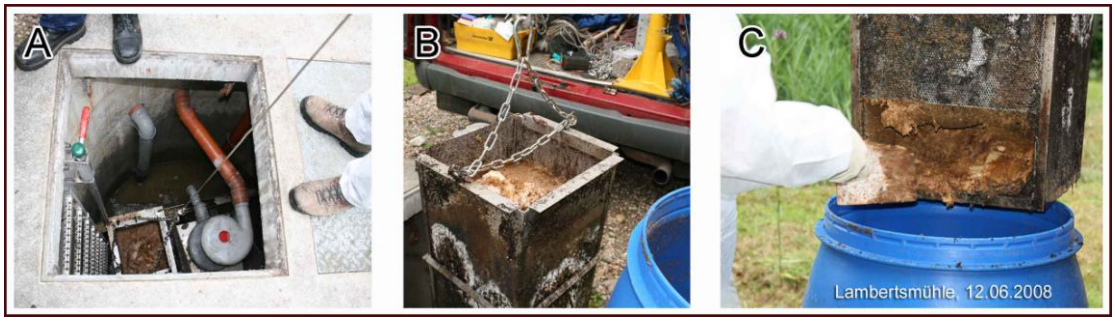

Figure 3.4. (**A**) Black water container of separated toilets, (**B**) the sieve collector of solid part from black water and (**C**) non-urine faecal matter (Fm) in Lambertsmühle building of OtterWasser GmbH, Germany

3.3.2.3. Bio-waste

The fresh Bw (or BwMix) was a mixture of two OSW materials, namely Bw1 and Bw2 (Fig. 3.5A).

Figure 3.5. Cutting (**A**) garden waste (Bw1) and (**B**) fruit & vegetable waste (Bw2) to smaller fragments (Φ≤ 1.0 cm) before mixing and pre-composting

The first type (Bw1) was collected from the Remondis Waste Treatment Centre of Bonn, Germany. The substrate contained mainly garden wastes including leaves, small tree branches and grass. Despite the fact that Bw1 was low in nutrients, it was a good bedding material for vermicultures.

The second type (Bw2) was mainly fruit (apple, lemon, orange, grape, melon, etc.) and vegetable waste (carrot, salad, cabbage, cucumber, etc.) (Fig. 3.5B). The Bw2 was collected from Bergfeld's Bioladen Supermarket, Bonn, Germany. The Bw1 and Bw2 materials were cut into small pieces (Φ ≤1.0 cm) manually and then mixed together. The mixture of Bw1 and Bw2 (BwMix) was then pre-composted for 20 days (*see 3.3.3.2*).

After pre-composting, the Bw was considered an input feed for earthworms. It could be used directly (single Bw) or mixed with others (combined BsBw, combined FmBw and combined BsFmBw). The physico-chemical characteristics of fresh Bs, Fm, and Bw are given in Table 3.8.

Table 3.8: Physico-chemical properties and nutrient content in fresh feeding materials including Bs; Fm, and Bw (Bw1; Bw2 and their mixture-BwMix), all data of DM

Parameter	Fresh Bs	Fresh Fm	Fresh Bw (BwMix)		
			Fresh Bw1	Fresh Bw2	BwMix
DM/ %	25.3	20.0	31.5	16.1	27.3
pH	8.8	5.9	5.9	4.0	4.4
EC/ mS cm^{-1}	6.3	2.8	5.4	4.8	5.2
*TAN/ g kg^{-1}	9.8	<0.4	<0.2	<0.5	<0.3
**NH_3/ mg kg^{-1}	2568.2	<0.2	<0.1	<0.1	<0.1
oDM/ %	89.3	94.3	53.1	88.7	65.7
C_{tot}/ %	45.3	54.7	27.8	45.9	31.7
N_{tot}/ %	1.6	2.9	2.0	2.0	1.6
C/N	28.8	19.0	14.2	23.5	22.3
P_{tot}/ %	1.0	0.6	0.4	0.4	0.3
K_{tot}/ %	0.7	0.1	0.5	1.4	0.9

*: '<' Detection limit of the Scheibler method (*see 3.4.4.3*)
**: Calculated from TAN content and pH level (*see 3.4.4.4*)

3.3.3. Pre-treatment of fresh OSW materials for vermicomposting

3.3.3.1. Pre-treatment of bio-waste

The fresh Bw (or BwMix) was pre-composted in a small pile (~80 kg and 60 cm height) for 20 days (Fig. 3.6A). At day 6, ~20 kg of additional fresh Bw2 was added to this pile. During the 20-day pre-composting period, Bw was turned on days 1, 6, 7, 12, 16 and 20 when the pile temperature (30 cm from the top) was measured.

Figure 3.6. Pre-treatment of (**A**) bio-waste by 20-day thermophilic composting on a plastic sheet and (**B**) separated solids of digested biogas slurry by 15-day barrel-rolling in garden conditions

3.3.3.2. Pre-treatment of separated solids of digested biogas slurry

The fresh Bs (~30 kg barrel^{-1}) were pre-composted in two metal barrels in a garden for 15 days (Fig. 3.6B). The material was mixed by rolling the barrels two or three times per day.

3.3.3.3. Pre-treatment of non-urine faecal matter

The fresh Fm was stored in a 100-L plastic container at normal conditions during a three-week period when other fresh substrates (Bs and Bw) were in the processing stage of pre-treatments.

3.3.3.4. Feeding materials for vermicomposting

After the pre-treatment of fresh OSW materials, three single substrates (Bs, Fm and Bw) were used directly as a feed for earthworms (Fig. 3.7). In addition, the substrates were combined to produce three combined substrates, namely BsBw, FmBw and BsFmBw.

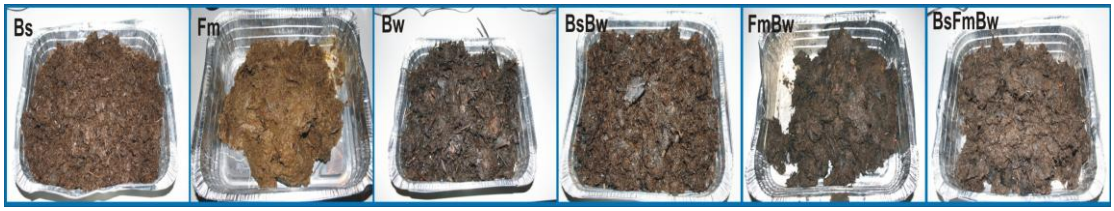

Figure 3.7. Six feeding materials including single substrates (Bs, Fm and Bw) and combined substrates (BsBw, FmBw and BsFmBw)) for vermicomposting with *E. fetida* and *D. veneta*

The physico-chemical properties and nutrient concentration of the six feed materials are given in Table 3.9.

Table 3.9: Physico-chemical properties and nutrient concentration of single feeding substrates (Bs, Fm and Bw) and combined feeding substrates (BsBw, FmBw and BsFmBw) used for vermicomposting, all data of DM

Parameter	Bs	Fm	Bw	BsBw	FmBw	BsFmBw
DM/ %	14.4	20.1	31.3	19.6	25.0	22.2
pH	8.1	7.0	8.0	7.8	7.4	7.8
EC/ mS cm^{-1}	5.7	2.9	4.6	5.1	4.6	4.5
*TAN/ g kg^{-1}	0.5	7.4	<0.4	<0.4	<0.4	<0.3
**NH_3/ mg kg^{-1}	35.5	41.2	<12.3	<8.0	<5.0	<11.2
oDM/ %	87.6	94.3	66.4	65.4	71.0	72.8
C_{tot}/ %	43.8	36.4	33.6	37.4	37.2	37.7
N_{tot}/ %	1.9	2.1	1.6	1.7	1.8	1.8
C/N ratio	23.1	17.3	21.0	22.0	20.7	20.9
P_{tot}/ %	0.9	0.6	0.3	0.6	0.6	0.7
K_{tot}/ %	0.7	0.1	0.9	0.7	0.5	0.6

*: Measured by Scheibler method, '<' means the limit of method detection (*see 3.4.4.3*)
**: Calculated from TAN content and pH level (*see 3.4.4.4*)

3.3.4. Experimental design for vermicomposting

PVC pipes (Φ = 20 cm) were cut into tubes with a length of 0.4 m (Fig. 3.8A). One end was covered by a metal mesh (Φ = 1.0 cm) for gravitational dewatering. The upper end was covered with a plastic lid to maintain dark conditions at 22 ± 5°C and to prevent the escape of earthworms. The box was placed on two wooden supports (~5 cm height) on an aluminum tray (30 x 40 cm) for aeration. All reactors were put on three-story wooden shelves in a 50 m^2 cellar (Fig. 3.8B).

Figure 3.8. (**A**) Batch-scale boxes for vermicomposting (*n* = 3) and (**B**) monoculture and polyculture treatments of six vermicomposting substrates in a cellar of the Plant Nutrition Institute, INRES, Bonn University

An overview of the experimental design for six feeding materials with monoculture and polyculture treatments with *E. fetida* and *D. veneta* is given in Table 3.10.

Table 3.10: Experimental design (*n* = 3) for monoculture (mono), polyculture (poly) and the control of three single substrates (Bs, Fm and Bw) and three combined substrates (BsBw, FmBw and BsFmBw)

Exp. code	Feeding material			*E. fetida*/ Mean± SD		*D. veneta*/ M±SD	
	Sub.	Wt/kg	ratio	Mass/ g	No.	Mass/ g	No.
Bs-mono	Bs	3.0	-	100± 0.2	442 ± 127	0	0
Bs-poly	Bs	3.0	-	50 ± 0.1	203 ± 19	50 ± 0.1	71 ± 11
Bs-control	Bs	2.0	-	0	0	0	0
Fm-mono	Fm	3.0	-	100± 0.1	425 ± 67	0	0
Fm-poly	Fm	3.0	-	50 ± 0.1	284 ± 47	50 ± 0.4	61 ± 7
Fm-control	Fm	2.0	-	0	0	0	0
Bw-mono	Bw	3.0	-	101± 0.0	481 ± 24	0	0
Bw-poly	Bw	3.0	-	50 ± 0.0	217 ± 48	50 ± 0.3	65 ± 3
Bw-control	Bw	2.0	-	0	0	0	0
BsBw-mono	Bs+Bw	3.0	1:1	100± 0.1	490 ± 35	0	0
BsBw-poly	Bs+Bw	3.0	1:1	50 ± 0.0	257 ± 20	50 ± 0.1	54 ± 3
BsBw-control	Bs+Bw	2.0	1:1	0	0	0	0
FmBw-mono	Fm+Bw	3.0	1:1	100± 0.1	571 ± 6	0	0
FmBw-poly	Fm+Bw	3.0	1:1	50 ± 0.0	328 ± 88	50 ± 0.0	52 ± 2
FmBw-control	Fm+Bw	2.0	1:1	0	0	0	0
BsFmBw-mono	Bs+Fm+Bw	3.0	1:1:1	100± 0.1	540 ± 8	0	0
BsFmBw-poly	Bs+Fm+Bw	3.0	1:1:1	50 ± 0.0	348 ± 56	50 ± 5.3	66 ± 8
BsFmBw-control	Bs+Fm+Bw	2.0	1:1:1	0	0	0	0

Only *E. fetida* was incubated in monoculture treatment, whereas *D. veneta* was mixed with *E. fetida* (1:1/ w:w) in polyculture treatment. At the beginning, 3.0 kg of each input material were fed into the experimental box. Then, 100 g of counted earthworms were added onto the substrate surface in each vermicomposting box. In parallel, 2.0 kg of the same material without earthworms were used as a control.

During the 12 weeks of the process, ~30 g of fresh material box^{-1} were sampled once every two weeks to analyse physico-chemical parameters (DM, pH, EC, TAN, NH_3 and organic content (oDM). The vermicomposting substrates and controls were moistened by distilled water when the DM content in any substrate was recorded to be >30%.

At the end of the process, surviving earthworms were assessed by hand-sorting and counting, and then the biomass was weighed without vermicomposted substrate. In addition, the five materials (Bs, Bw, BsBw, FmBw and BsFmBw) were sieved into two sizes: Φ <4 mm and Φ ≥4 mm (Fig. 3.9).

Figure 3.9. Materials sieved into two sizes: the big fraction (Φ ≥4 mm) was named incompletely digested substrate (IDS) and the smaller fraction (Φ <4 mm) vermicompost (VC)

The larger size fraction was regarded as incompletely digested substrate (IDS), while the smaller size fraction was considered as vermicompost (VC). The two fractions of all vermicomposted substrates were stored separately in aluminium trays at room temperature for the 12 weeks of maturation.

Physico-chemical properties and nutrient concentration in both 12-week vermicomposting fractions (IDS and VC) of all OSW materials were determined after 6 weeks (IDS18 and VC18) and 12 weeks (IDS24 and VC24) of maturation. The final product (VC24) of all OSW materials was tested for respiration activity for four days (AT_4) and for hygiene status.

3.4. PHYSICO-CHEMICAL ANALYSIS

To determine the physico-chemical properties, samples were analysed according to (BGK, 1994) (Table 3.11). TAN was measured by the modifying Quantofix-N-Volumeter for solid samples and the Scheibler method (*see 3.4.4.2*). The C/N ratio and nutrient concentration were analysed according to the soil analysis methods of the PE-INRES Institute, Bonn University. The maturity of vermicompost was measured by the AT_4. Finally, the hygiene parameters were tested by the Hygienic & Public Health Institute, Bonn University.

Table 3.11: Parameters analysed in vermicomposting of OSW materials, all data as % of DM

Parameter	Abbreviation	Unit	Methods/tools
Earthworm biomass & numbers	W_{wo} & Nr.	g & worm	Hand-sorting, weighing & counting
Temperature	T	^oC	Thermometer, Germany
Dry matter	DM	%	Heating, 105^oC, 24 hours, Germany
pH level	pH	-	Sample: H_2O = 1:10/ w:v, electrode glass sensor, SevenEasy, Germany
Electric conductivity	EC	mS cm^{-1}	Sample:H_2O = 1:10/ w:v, sensor, LP 340, Germany
Total ammonium nitrogen	TAN	g kg^{-1}	Modified Quantofix-N-Volumeter & Scheibler method, Germany
Ammonia	NH_3	mg kg^{-1}	Calculated from TAN content & pH by the equation [8]
Organic dry matter	oDM	%	Muffle furnace/ 550^oC & 6 hours, Germany
Total carbon	C_{tot}	%	Atomic elemental Analyser, EC3000, Italy
Total nitrogen	N_{tot}	%	Atomic elemental Analyser, EU3000, Italy
Total phosphorus	P_{tot}	%	Molybdänblau method, IR sensor, ECOM 6122, Germany
Total potassium	K_{tot}	%	Elemental Photometer, ELEX 6361, Germany
Respiration activity for four days	AT_4	mg O_2 g^{-1}	AT_4, incubation, 4 days, OxiTop® sensor, Germany
Number of *E. coli* & *Enterococcus* spp.	-	CFU g^{-1}	Incubation in Chromocult®Coliform Agar & Enterococus®Selective Agar
Occurrence of *Salmonella* spp.	Positive/ negative	+/ -	Incubation in Rappaport-Vassiliadis
Number of coliphages	-	CPU g^{-1}	Incubation in single-agar layer, ISO 10707-1 & ISO 10705-2

3.4.1. Temperature measurement and maintenance

During pre-composting of the fresh Bw (*see 3.3.3.1*), the Bw pile temperature was measured by a thermometer at a depth of 0.3 m from the top of the pile before the material was mixed manually. During the vermicomposting process, from April 2007 to November 2007 (*see 3.1*) and from September 2008 to March 2009 (*see 3.3*), the experiment temperature remained in the range 18-27^oC.

3.4.2. Dry matter (DM) and moisture

Approximately 15 g of sample (fresh OSW material, vermicomposted substrate or vermicompost) without earthworms were weighed on an aluminium tray (tray weight was recorded previously). The tray with the sample was then dried at 105^{o}C for 24 hours. The dried sample was weighed again after cooling to room temperature for 12 hours. Dry matter (DM) content in the material was calculated by equation [2]:

$$\mathbf{DM/\ \% = (W_{S\text{-}AF\text{-}T} - W_T)/\ W_{S\text{-}BE} \times 100\%} \quad [2]$$

where:

$\mathbf{W_{S\text{-}AF\text{-}T}}$: Weight of sample and aluminium tray after drying (g)

$\mathbf{W_T}$: Weight of aluminium tray (g)

$\mathbf{W_{S\text{-}BE}}$: Weight of sample before drying (~15 g)

3.4.3. pH level and electric conductivity (EC)

A 10.0 g portion of fresh substrate was weighed and placed in a 250-mL plastic bottle. After adding 100 mL of distilled water, the bottle was shaken for two hours. The pH levels were measured directly in the suspended solution by a pH meter (SevenEasy Metler Toledo, Germany).

With the same suspended solution, the electric conductivity (EC) was measured by an EC meter (LP 340, Germany) at room temperature. The EC value of each sample was adjusted to its DM (already known) using the equation [3]:

$$\mathbf{EC/\ mS\ cm^{-1} = EC_{FS} \times DM} \quad [3]$$

where:

$\mathbf{EC_{FS}}$: EC value of fresh sample recorded by the EC meter (mS cm^{-1})

DM: Dry matter (%)

3.4.4. Total ammonium nitrogen (TAN) and ammonia (NH_3) concentration calculation

3.4.4.1. Principle of Quantofix-N-Volumeter method for TAN measurement

Based on a volumetric method, the Quantofix-N-Volumeter device (Fig. 3.10) was used to determine TAN content (TAN = sum of NH_4^+-N and NH_3-N) in a liquid sample (e.g. biogas slurry) (Klasse & Werner, 1987; Klasse, 1998). In the bio-digestion process, the TAN content in digested biogas slurry can be tested quickly by this device (Van Kessel & Reeves III, 2000). The mechanism of this method is volumetric determination of nitrogen gas (N_2) which is produced from redox reactions between NH_4^+/NH_3-containing substrate and the Quantofix reagent (NaOCl/OH^-):

$$2\ NH_4^+ + 3\ NaOCl + 2\ OH^- = N_2^{\uparrow} + 3\ NaCl + 5\ H_2O \quad (a)$$

$$2\ NH_3 + 3\ NaOCl = N_2^{\uparrow} + 3\ NaCl + 3\ H_2O \quad (b)$$

3.4.4.2. Modified Quantofix-N-Volumeter method for solid samples

Quantofix is a volumetric method and was designed for 0.1 L of slurry sample. This method can be used for solids as well, although the liquid reactant needs to come into contact with the solid sample. To overcome this problem, the solid sample was mixed with water (10%) before using the Quantofix device (Fig. 3.10).

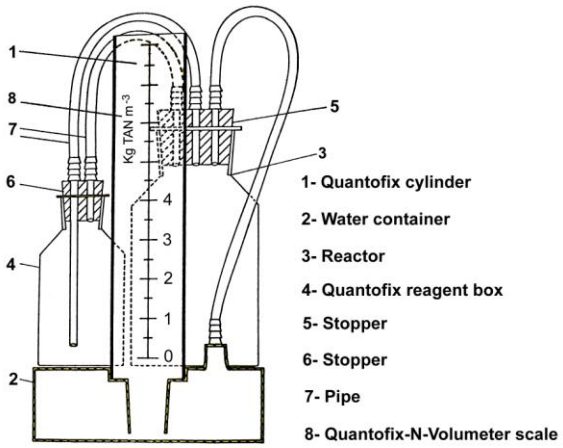

Figure 3.10. The Quantofix-N-Volumeter device (*own creation*)

Procedure: Tap water is poured into the water container (2) via the Quantofix cylinder (1) until the water surface level reaches the starting point (point zero). Then, 10.0 g solid sample of known DM are mixed with 100 mL distilled water. The mixture is added to the reactor (3). In parallel, the Quantofix reagent box (4) is filled with 150 mL Quantofix reagent. Plastic stoppers (5 and 6) are fixed on the reactor and the reagent box, which are connected together by the Teflon pipes (7).

Measurement can then be started by turning over the Quantofix reagent box to pour the reagent into the reactor (3), where the reactions (a) and (b) happen. The reactor is shaken for 5 minutes and then allowed to sit still for 5 minutes. N_2 gas emitted from the reactions presses the water from the water container up to the Quantofix cylinder. The water level in the Quantofix cylinder is then recorded on the Quantofix scale on the Quantofix cylinder, which is accurate to 0.1 units. Care must be taken to ensure that a tight seal is obtained and that the water level does not fall. If this occurs, the determination procedure must be repeated.

TAN calculation: Instead of the value of TAN content which is recorded by the water level in the Quantofix cylinder in units of kg TAN m^{-3}, the TAN content in solid samples is calculated in units of g TAN kg^{-1} using the equation [4]:

$$\textbf{TAN content/ g kg}^{-1} = \textbf{Mw}_{N2} \textbf{ x P x (H}_{Qf} \textbf{ x Qf)/ (R x T x W}_S\textbf{)} \quad [4]$$

where:

Mw_{N2}: Molecular weight of N_2 = 28.0 g mol^{-1}

P: Air pressure (atm)

H_{Qf}: Water level height in the Quantofix cylinder (Quantofix-N-Volumeter scale unit)

Qf: Cylinder volume of one Quantofix-N-Volumeter scale unit (L)= π x 0.31dm x $(0.61dm/2)^2$= 0.09 L

R: Universal gas constant = 0.082 L atm mol^{-1} K^{-1}

T: Measured temperature (K)

W_S: Weight of dry solid sample (kg) = W_{FS} x DM/100

W_{FS}: Weight of fresh solid sample = 0.01 kg

DM: Dry matter (%)

At standard pressure and temperature (P = 1.0 atm and T = 25°C = 293K), the TAN content in solid material can be calculated by the equation [5]:

$$\textbf{TAN content/ g kg}^{-1}\textbf{= 1 055 x H}_{Qf}\textbf{/ DM} \quad [5]$$

Detection limit of the method: 0.1 units is the minimum volume that can be recorded by the Quantofix-N-Volumeter scale ($H_{Qf\text{-}min}$). Therefore, 1.1 g TAN kg^{-1} is the smallest amount which can be detected in solid sample (assuming DM = 100%) by the adjusted Quantofix-N-Volumeter method.

3.4.4.3. Scheibler method for small solid samples

Based on the mechanism of the adjusted Quantofix-N-Volumeter method, the Scheibler device was modified to accurately measure TAN content in smaller solid samples (≤10.0 g fresh substrate) (Fig. 3.11).

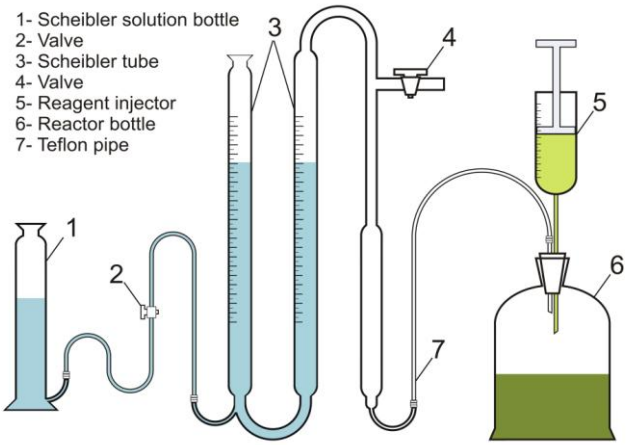

Figure 3.11. Scheibler method for TAN measurement for small solid samples (*own creation*)

Procedure: The liquid from the Scheibler solution bottle (1) is pumped into the Scheibler tube (3) by opening the valve (2). The valve (2) is then closed to keep the liquid in the Scheibler tube (3). The valve (2) is also used to control the liquid level in the U-tube (3) at the starting point.

10.0 g fresh sample (with known DM) and 100 mL distilled water are added to the 250-mL reactor bottle (6). In parallel, 150 mL Quantofix reagent ($NaOCl/OH^-$) are added to the reagent injector (5). The reactor bottle (6) is fixed tightly to the injector

(5) by a stopper. The reactor bottle (6) is also connected to the Scheibler U-tube (3) by a pipe (7).

At the beginning of the measurement process, the Quantofix reagent in the injector (5) is injected into the reactor (6). The valve (4) is opened to leave the liquid in two tubes (3) at the same level at the starting point (V_{Start}) (air pressure balancing). After that, the valve (4) is closed, the reactor bottle (6) is shaken for two minutes and allowed to react completely within eight minutes. During the measurement period, the N_2 gas that forms in the reactor (6) goes through a pipe (7) and presses the liquid in the two Scheibler tubes (3) to new different levels. The valve (4) is opened to control the liquid in the two Scheibler U-tubes (3) to the same level (V_{End}) (air pressure balancing). Finally, the difference between the initial level (V_{Start}) and the final level V_{End} is recorded as the volume of N_2 gas (V_{Sb}). Care must be taken to ensure that a tight seal is obtained and that the water level does not fall. If this occurrs, the determination procedure must be repeated.

TAN calculation: The TAN content in solid samples is calculated by the equation [6]:

$$\textbf{TAN content/ g kg}^{-1} = [\textbf{Mw}_{N2} \times \textbf{P} \times \textbf{V}_{Sb}] / [\textbf{R} \times \textbf{T} \times \textbf{W}_S] \quad [6]$$

where:

$\textbf{Mw}_{N2}$: Molecular weight of N_2 = 28.0 g mol^{-1}

P: Air pressure (atm)

$\textbf{V}_{Sb}$: Measured volume of N_2 gas by the Scheibler device = V_{End} - V_{Start} (L)

R: Universal gas constant = 0.082 L atm mol^{-1} K^{-1}

T: Room temperature during measuring (K)

$\textbf{W}_S$: Weight of dry solid sample (kg) = W_{FS} x DM/ 100

$\textbf{W}_{FS}$: Weight of fresh solid sample = 0.01 kg

DM: Dry matter content (%)

At standard conditions (P = 1.0 atm, T = 25°C = 293 K), the TAN content in the sample can be calculated by the equation [7]:

$$\textbf{TAN content/ g kg}^{-1} = 11\,654 \times \textbf{V}_{Sb} / \textbf{DM} \quad [7]$$

Detection limit of the method: With the miniaturised system, 0.5 x 10^{-3} L is the minimum volume ($V_{Sb\text{-}min}$) which can be recorded by the Scheibler device. Consequently, 0.06 g TAN kg^{-1} is the smallest amount which can be detected in solid sample (assuming DM = 100%) by the Scheibler method.

Within the same size of solid sample (10.0 g), the Scheibler method can detect TAN >180 times more accurately than the adjusted Quantofix-N-Volumeter method.

3.4.4.4. NH_3 calculation

From TAN content and the pH value of each sample, NH_3 concentration was calculated by the equation [8]:

$$[NH_3]/\ g\ kg^{-1} = 10^{(pH\text{-}pK)} \times TAN\ content\ (g\ kg^{-1}) / [1+10^{(pH\text{-}pK)}] \quad [8]$$

where:

p*K*: Acid coefficient of NH_4^+/NH_3 = 9.25 (20°C)

3.4.5. Total carbon (C_{tot}) and total nitrogen (N_{tot})

The vermicomposted substrates or vermicomposts were dried and then ground homogeneously. Approximately 5.0 mg of the dried sample was weighed, packed and pressed into a condensed pellet shape in aluminium foil. The pellets were kept in a vacuum chamber until they were analysed by a GC elemental analyser (EuroEA 3000, Italy).

3.4.6. Total phosphorus (P_{tot}) and total potassium (K_{tot})

The total phosphorus (P_{tot}) concentration was analysed by the Molybdänblau method (Benz & Kelly, 1969). Approx. 0.2 g dried substrate/vermicompost was ground and placed in a Teflon chamber. Then 4.0 mL HNO_3 (63%) was added and the Teflon chamber containing the sample was heated in a muffle furnace at 550°C for six hours. When the Teflon chamber had cooled to room temperature, the digested solution was poured into a 25-mL graduated flask. The Teflon chamber was washed with deionised water several times and then, the graduated flask was filled to the 25-mL marker with deionised water. The digested solution was filtered through a funnel consisting of white ribbon filter paper (MN640m, Φ = 125 mm) into 100-mL plastic vessels. The filtered leachate was used as the final solution for P_{tot} and K_{tot} analyses.

P_{tot} analysis: Leachate (0.1 mL) was transferred with a micropipette into a 50-mL graduated flask. Then 8.0 mL colour indicator solution (a mixture of Ammonium Heptamolybdate-tetrahydrate, Potassium Antimony (III) oxitatrate, Ascorbic acid and sulphur acid) was added to the flask. The graduated flask was filled to the 50-mL marker with deionised water. After reaction time (~15 minutes), the P_{tot} solution was measured by an UV-VIS photometer (Eppendorf ECOM 6122, Germany) at a wavelength of 578 nm.

K_{tot} analysis: The same leachate as above (0.5 mL) was transferred to a 5.0-mL graduated flask. The flask was filled to the 5.0-mL marker with deionised water and then the K_{tot}-solution was measured by a flame photometer (Eppendorf ELEX 6361, Germany).

3.4.7. Organic dry matter (oDM)

Approximately 1.0 g of dried substrate/vermicompost was weighed and placed in a porcelain boat. The boat containing the sample was then heated in a muffle furnace at 550°C for six hours, cooled to room temperature and weighed again. The oDM content was calculated using the equation [9]:

$$oDM/\ \% = (W_{S\text{-}AF\text{-}B} - W_B) \times 100 / W_{S\text{-}BE} \quad [9]$$

where:

$\mathbf{W_{S\text{-}AF\text{-}B}}$: Weight of substrate plus porcelain boat after heating (g)

$\mathbf{W_B}$: Weight of porcelain boat (g)

$\mathbf{W_{S\text{-}BE}}$: Weight of substrate before heating (~1.0 g)

3.4.8. Maturity test using the static respiration method "Atmungsaktivität" (AT_4)

Respiration activity for four days (AT_4) was used to determine the microbial activity in the vermicompost based on the O_2 consumption (Fig. 3.12).

Figure 3.12. Respiration activity for four days (AT_4) of measurements on maturity in the incubation cabinet

The 24-week vermicomposts of all substrates with a particle size Φ <4 mm (VC24) were used for the AT_4 measurements (n = 3). Distilled water was added to the sample (DM~80%) to adjust the moisture content to 40-60% of water-holding capacity. Then 40.0 g of the sample was placed in a 2.0-L glass bottle. A 10-mL rubber tube containing 12 pellets of solid potassium hydroxide (KOH) (~3.5 g bottle^{-1}) for CO_2 trapping was inserted vertically into the glass bottle. The bottles were then covered with the rubber septa, capped, closed airtight and placed in a climate cabinet at constant temperature (25^{o}C). The samples were incubated for six days. The internal pressure change was recorded daily by OxiTop® sensors. The AT_4 was calculated from the pressure change within four days through the following the equation [10]:

$$\mathbf{AT_4 / mg\ O_2\ g^{-1} = (Mw_{O2} \times V_B \times \Delta\rho) / (R \times T \times W_S)} \quad [10]$$

where:

$\mathbf{Mw_{O2}}$: Molecular weight of O_2 = 32 000 mg mol^{-1}

$\mathbf{V_B}$: Volume of bottle = 2.0 L

$\mathbf{\Delta\rho}$: Decrease of pressure in the bottle/ hPa

R: Universal gas constant = 83.144 L hPa mol^{-1} K^{-1}

T: Temperature of the incubated cabinet = 298 K

W_S: Weight of dry sample= W_{FS} x DM/ 100

W_{FS}: Weight of fresh sample = 40.0 g

DM: Dry matter (%)

3.4.9. Hygiene parameters

Fresh organic wastes, two-week pre-treated materials and 24-week vermicomposts (Φ <4 mm) were tested for hygiene parameters. All samples were collected and stored in plastic bags to avoid contamination. The parameters were analysed in the laboratory of the Hygiene and Public Health Institute, University of Bonn. The samples were processed within 24 hours after sampling.

Escherichia coli was counted in a Chromocult®Coliform Agar (Merck) after 24 hours incubation at 36°C.

Enterococcus spp. was enumerated on Enterococcus Selective Agar according to Slanetz & Bartley (Merck) after 48 hours of incubation at 36°C.

Salmonella spp. was determined by using the presence/ absence (negative/ positive) test in which 1.0 or 10.0 g of fresh samples were placed in sterile flasks and incubated with 100 mL Rappaport-Vassiliadis broth for 24 hours at 36 ± 1 °C. Then 1.0 mL from each flask was used to further incubate with another 100 mL Rappaport-Vassiliadis broth for 24 hours at 44 ± 1°C. Next, the liquid was transferred to a Hektoen agar plate using an inoculating loop and the agar plate was incubated for 24 hours at 36 ± 1°C.

Somatic coliphages were counted by the single-agar-layer technique as described in ISO 10707-1 and ISO 10705-2.

3.5. STATISTICS

All treatments were replicated three times (*n*= 3). The one way ANOVA, multiple regression and standard post-hoc Duncan mean separation tests were used to evaluate differences between treatments at a significance level of 95% (P<0.05) using the SPSS 17.0 software package. The data were processed with Microsoft Excel 2007. Figures and graphs were drawn by Sigma Plot 10.0.

4. RESULTS

4.1. SUITABILITY TEST OF ORGANIC SOLID WASTES FOR VERMICOMPOSTING OF *E. FETIDA*

In this part, the results of physico-chemical properties of the three OSW materials and the biomass of the surviving earthworms are given after a quick-test to determine suitability of the substrates for vermicomposting.

4.1.1. Properties of fresh OSW materials and suitability for vermicomposting

The DM content in fresh Fm was quite low (11.4%), whereas fresh Bw and fresh Bs had acceptable DM content in the range 20-30% (Table 3.1). This range is known to be a suitable DM content for *E. fetida*. pH levels of fresh Bw and fresh Fm were acidic (5.3 and 5.8, respectively) compared with the alkaline pH of fresh Bs (8.4). The TAN content was low in the fresh Bw and Fm (<3.8 and <9.3 g kg^{-1}, respectively). In contrast, fresh Bs contained a high TAN content of 11.4 g kg^{-1}, which included ~1.4 g NH_3 kg^{-1}. C_{tot} had a similar content in all fresh materials (43.8-45.5%), but N_{tot} concentration varied from 1.4% to 3.8%. Consequently, the C/N ratio had a high variability from 14.0 to 31.3. For other nutrients, P_{tot} concentration in fresh Bw was quite high (2.9%) in comparison with that in fresh Fm and Bs (0.8 and 1.1%, respectively). K_{tot} had the highest content in fresh Bs (1.1%), whereas there was less of this element in the fresh Bw and Fm (0.6 and 0.1%, respectively).

After 20 days of suitability tests of all the three OSW materials for vermicomposting, the biomass of *E. fetida* decreased to <5% compared with the initial biomass (100%) (Fig. 4.1A). Fresh Fm, which had the highest acceptance by earthworms (5%) of the three substrates, seemed to be the most suitable feeding substrate among the three OSW materials. The fresh Bw and Bs were not accepted directly by the earthworms.

All three test OSW materials had higher DM content (~3-5%) than the controls at day 20 of the process (Fig. 4.1B), whereas the EC increased in all substrates during the incubation, but less than in the controls (Fig. 4.1C). The EC of the 20-day Bw was the highest (9.0 mS cm^{-1}) of the OSW materials (<6.0 mS cm^{-1}). From <6.0 in the fresh Bw and Fm, the pH increased to 6.5-7.0 after 20 days (Fig. 4.1D). However, the pH of fresh Bs (8.5) decreased to 7.5 at day 20.

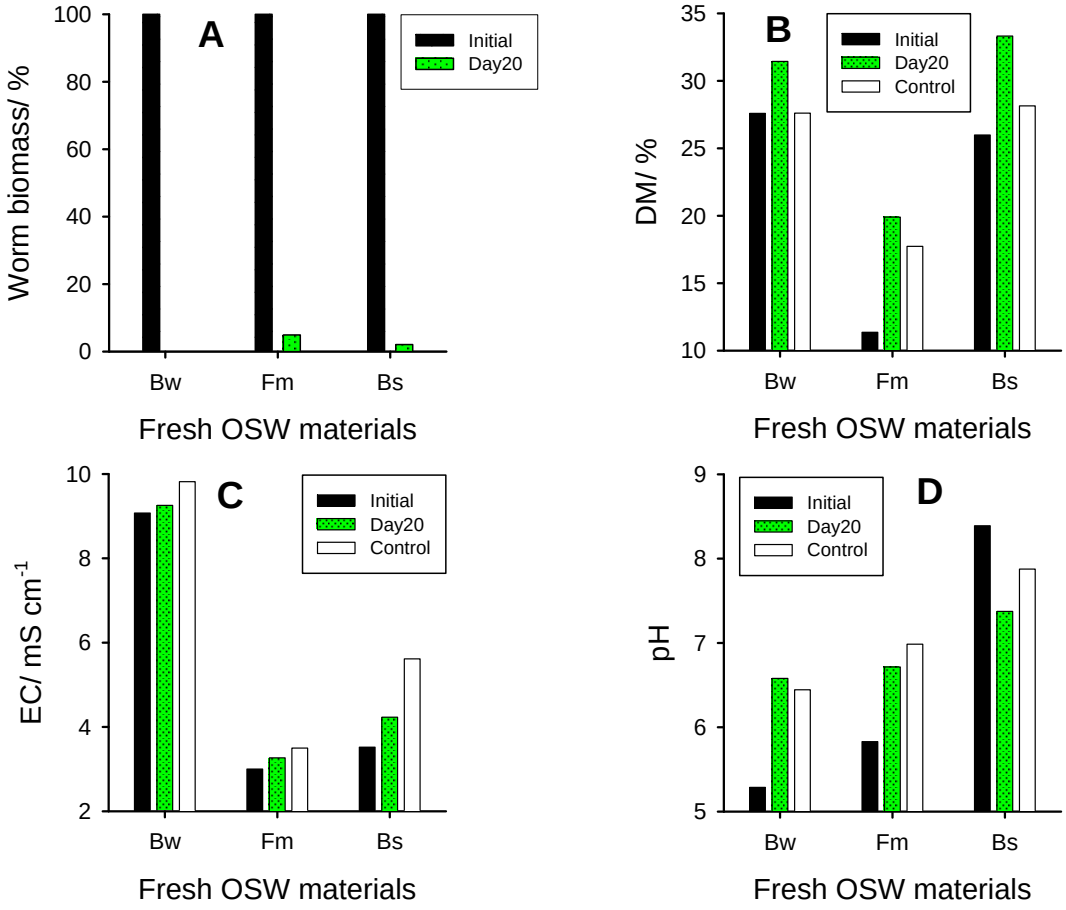

Figure 4.1. Changes in (**A**) earthworm biomass, (**B**) DM, (**C**) EC and (**D**) pH level of fresh feeding substrates at the beginning (initial), after 20 days (Day20) and in the control for vermicomposting suitability

4.1.2. Suitability of mixture of fresh organic solid wastes for vermicomposting

To adjust the pH level of the OSW materials for the suitability for vermicomposting, fresh Bs (with a high pH of 8.8) was mixed with fresh Fm (a lower pH of 5.4) in different ratios (1:2 to 1:8 by fresh weight) to produce more suitable pH levels (6.3-8.1) of the combined substrates than in the fresh substrates (Table 3.3). Depending on the Fm proportions in Fm-Bs mixtures, the EC in the four mixed substrates showed a slight increase from 5.2 to 7.3 mS cm^{-1}, while the DM content varied in the range 14.1-17.4%.

During the first 10 days of the suitability test, almost all surviving *E. fetida* (80.5%; 97.7%; 87.2% and 91.6% of the introduced earthworm numbers) were found in both bedding and tested substrates of the Mix1; Mix2; Mix3 and Mix4, respectively (Fig. 4.2A). During the period from day 10 to day 15 of the suitability test, no surviving *E. fetida* were found in the 200g-reference material (perhaps, due to lack of feed) and all earthworms were observed in the test substrates. However, the number of *E. fetida* decreased rapidly in the Mix1 substrate after 15 days (~50%), whereas the

number in the other mixtures, decreased gradually (>72%). At the end of the 30-day process, the Mix4 (pH6.3 at the initial) had more surviving earthworms (65.8%) than the Mix1-3 substrates (31.0%, 58.4% and 58.5%, respectively).

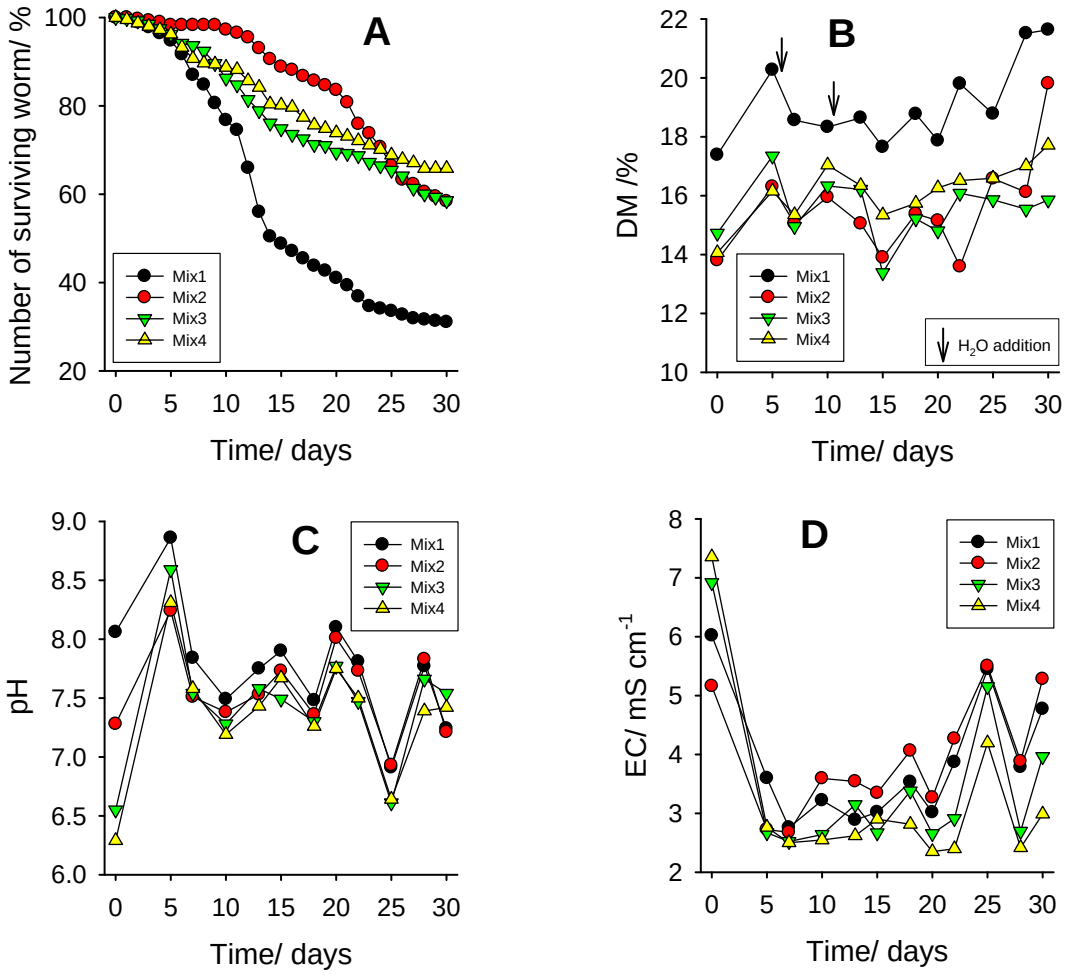

Figure 4.2. Changes in (**A**) the number of the surviving *E. fetida*, (**B**) DM content, (**C**) pH and (**D**) salt content in four mixtures of fresh Bs and Fm in ratios of 1:2 (Mix1); 1:4 (Mix2); 1:6 (Mix3) and 1:8 (Mix4) during 30 days of suitability test for vermicomposting

During 30 days of the process, the DM content in Mix1 was quite high (~20%), whereas the DM in the three other substrates (Mix2-4) was <16% (Fig. 4.2B). The pH level of all four mixtures increased after the first five days of the process, up to >pH 8 (Fig 4.2C). Later, pH levels decreased and remained around 7.5. The EC of all mixed substrates decreased to a point of ~3.0 mS cm^{-1} after five days (Fig. 4.2D). The EC remained at 3.0 mS cm^{-1} until day 20 and then slightly increased until day 30.

4.1.3. Suitability of the pre-treated separated solids of digested biogas slurry for vermicomposting

The TAN content in the fresh Bs decreased quickly during the 5-day pre-treatment (Fig 4.3). The large TAN content in fresh Bs (12.1 g kg^{-1}) decreased significantly

(>60%) after only the first three days (p<0.05). The NH_3 concentration in the fresh Bs (3.9 ± 0.2 g NH_3 kg^{-1}) was reduced by >75% within five days. However, the pH level was stable around 9.0.

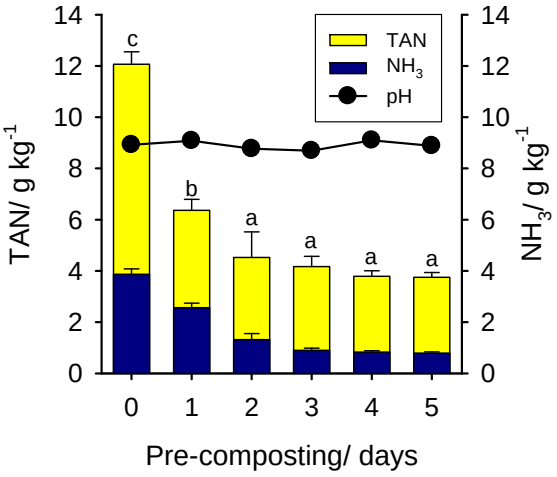

Figure 4.3. Changes in pH, TAN and NH_3 concentration in Bs during five days of TAN pre-composting, data was as DM, values are means ± SD (n= 3). For the TAN parameter, significant differences between plots are indicated by different suffixes a-c (p<0.05)

After acidification by HCl (*see 3.1.2*), the DM content in the four 3-day pre-treated materials (Bs3-HCl) was <22.0%, which is regarded as an acceptable moisture content for the development of *E. fetida*. With the addition of HCl acid, the pH levels of Bs3-HCl substrates decreased from 9.1 to 8.2, to 7.5, to 7.0 and to 6.5 in Bs3-A1, Bs3-A2 Bs3-A3 and Bs3-A4, respectively (Table 3.4). The EC values of Bs3-HCl increased from 5.3 mS cm^{-1} to 9.1 mS cm^{-1} depending on HCl dosage (Table 3.4). All the Bs3-HCl substrates still had the same TAN (~3.3 g kg^{-1}) independent of the HCl dosage, but NH_3 varied in different concentrations, decreasing from 267 to 9.4 mg kg^{-1} along with the decrease in the pH levels (Table 3.4).

In the suitability tests of the Bs3-HCl substrates, the number of surviving *E. fetida* in all substrates decreased followed the decrease in HCl dosage during the first four days (Fig. 4.4A). The earthworms died quickly in Bs3-A1 (>80%) and Bs3-A2 (~40%) after the first four days. However, both Bs3-A3 and Bs3-A4 seemed to be accepted by *E. fetida* (only <5% dead worms were found after that period). Later, a rapid decrease in *E. fetida* numbers in both Bs3-A2 and Bs3-A3 was observed, especially at day 15.

Both Bs3-A3 and Bs3-A4 may be considered suitable for *E. fetida* after 25 days, with >44.8% survival of worms, whereas Bs3-A1 and Bs3-A2 were not (only <27.3% survival of worms). Of the four Bs3-HCl substrates, Bs3-A4 might be the best material, with 66.7% surviving earthworms after the 25-day test. On the other hand, Bs3-A1 had only 14.5% survival of earthworms after 25 days.

The DM content in Bs3-A3 and Bs3-A4 increased to >22% at day 5, whereas that in Bs3-A1 and Bs3-A2 were >23% at day 15 and day 23 (Bs3-A4) (Fig. 4.4B). The pH level in Bs3-A1 and Bs3-A2 was high at day 4 (>8.3), whereas that of the other two

substrates (Bs3-A3 and Bs3-A4) increased to ~7.5 after four days (Fig. 4.4C). Decreases in pH levels to ~7.5 were observed in both Bs3-A1 and Bs3-A2 after HCl addition at day 4. Later on, the level in all four Bs3-HCl substrates decreased gradually from day 5 to pH 7.0 on day 25. The EC of the four initial Bs3-HCl substrates varied in the range 5.3-9.1 mS cm^{-1} depending on the HCl dosage (Fig. 4.4D). An increase in the EC was observed in Bs3-A1 and Bs3-A2 to ~8.0 mS cm^{-1} when the HCl addition was adjusted at day4. Later, the EC of the four substrates remained at ~8.0 mS cm^{-1} from day 5 to day 25.

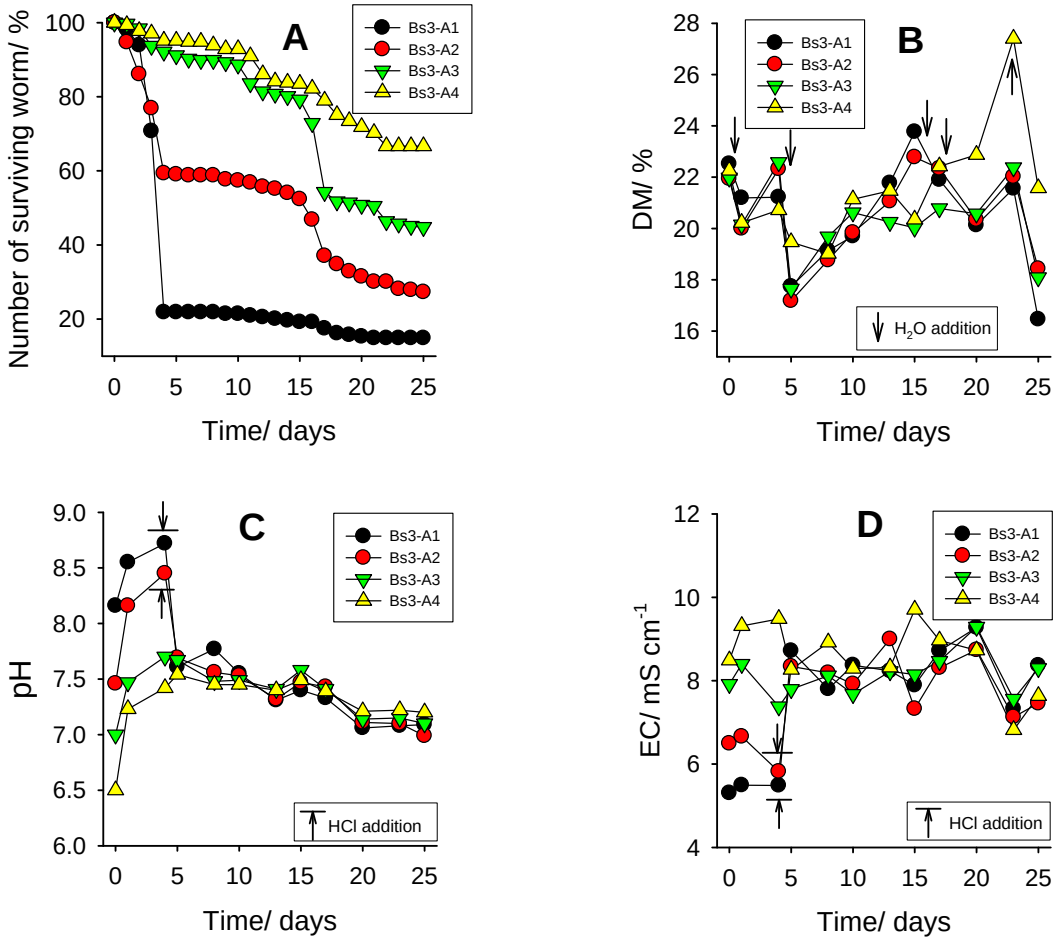

Figure 4.4. Changes in (**A**) the number of the survived *E. fetida*, (**B**) DM content, (**C**) pH level and (**D**) EC in four 3-day TAN pre-treated Bs (Bs3) by different HCl dosages: 11.8 g kg^{-1} (Bs3-A1); 15.5 g kg^{-1} (Bs3-A2); 23.6 g kg^{-1} (Bs3-A3) and 26.4 g kg^{-1} (Bs3-A4) during 25 days of the suitability test for vermicomposting

4.2. SINGLE- AND MULTI-FACTOR TEST OF INFLUENCES ON THE SURVIVAL RATE OF *E. FETIDA*

The results of the suitability test for vermicomposting with *E. fetida* (*see 4.1*) can be used to investigate the correlations between the survival rate of *E. fetida* and the three main factors (pH, EC and TAN) in single- and multi-variable analysis.

4.2.1. Single-factor influences on *E. fetida* survival rate of pH level, salt content and total ammonium nitrogen

4.2.1.1. Single-factor influence on E. fetida survival rate of pH level and buffer system

In general, the worm survival rate increased with increasing pH in the range 2.0-8.0 (Fig. 4.5A).

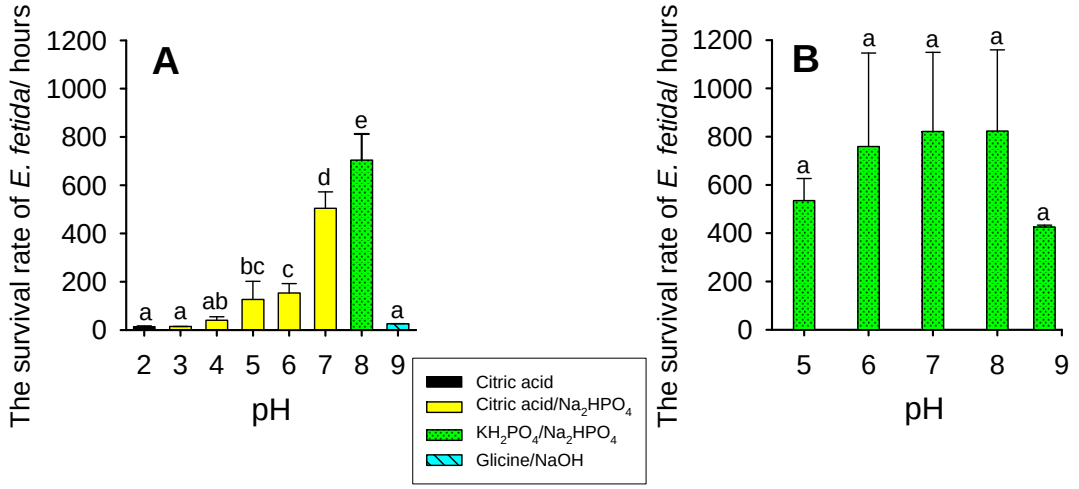

Figure 4.5. (A) Influence of pH level on the survival rate of *E. fetida* and (B) influence of pH adjusted by only KH_2PO_4/Na_2HPO_4 buffer. Bars indicate Mean ± Standard deviation (SD) (n = 3); significant differences between plots are indicated by different suffixes a-e (p<0.05)

At pH ≤4.0, the earthworm survival rate was limited, <41 ± 11 hours. When the pH level was in the range of 5.0-6.0, the survival rate was between 127 ± 75 and 153 ± 39 hours. At pH 7.0 and 8.0, the earthworm's survival rates were highest, at 460 ± 164 hours and 708 ± 153 hours, respectively. At pH 9.0 the earthworms died rapidly (<11 ± 7 hours). However, the buffer for pH9 (Glicine/NaOH) was different in comparison with the other buffers for either pH3-7 (Citric acid/Na_2HPO_4) or pH8 (KH_2PO_4/Na_2HPO_4).

When the pH was adjusted only by a KH_2PO_4/Na_2HPO_4 buffer (Fig. 4.5B), the earthworms showed a high survival rate at all pH values tested (>400 hours). The Duncan test showed no significant difference in survival rates between pH levels in the range 5.0-8.7 (p<0.05).

4.2.1.2. Single-factor influence on E. fetida survival rate of salt content

In distilled water (EC<0.1 µS cm^{-1}), the earthworm survival rate was 808 ± 194 hours (Fig. 4.6). The earthworm survival rate reached a peak of 1380 ± 255 hours at an EC of 4.5 mS cm^{-1}. A rapid decrease was observed thereafter with increasing salt content. At high salt levels of 20.0 g KCl L^{-1} (EC = 32.0 mS cm^{-1}), the earthworms died quickly (<0.27 ± 0.01 hours).

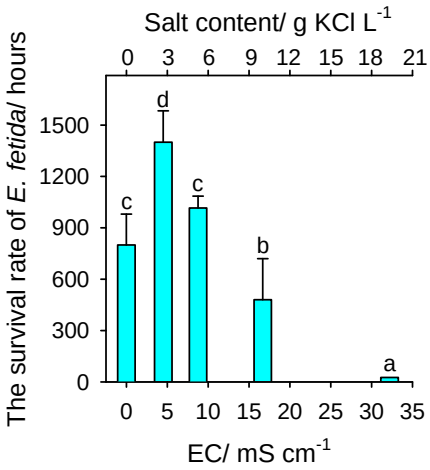

Figure 4.6. Influence of EC adjusted by KCl on the survival rate of *E. fetida* at pH 5.4. Values are means ± SD (*n* = 3); significant differences between columns are indicated by different suffixes a-d ($p<0.05$)

4.2.1.3. Single-factor influence on E. fetida survival rate of total ammonium nitrogen

TAN had a negative effect on the activity of *E. fetida* at pH 8.1 (Fig. 4.7). At TAN>120 mmol L^{-1} with 8.0 mmol NH_3 L^{-1}, the survival rates of *E. fetida* were low (<0.17 ± 0.01 hours). Even in the case of 30 mmol TAN L^{-1} (containing 2.0 mmolNH_3 L^{-1}), the earthworm survival rate was only 0.87 ± 0.06 hours.

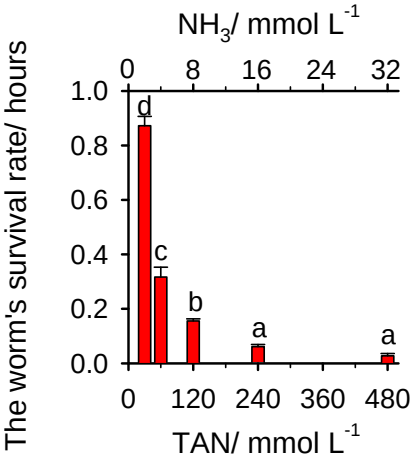

Figure 4.7. Effect of TAN and NH_3 on the survival rate of *E. fetida* at pH 8.1 and EC 3-40 mS cm^{-1}. Values are means ± SD (*n* = 3); significant differences between columns are indicated by suffixes a-d ($p<0.05$)

4.2.2. Multi-factor influences on *E. fetida* survival rate of pH level, salt content and total ammonium nitrogen

4.2.2.1. Multi-factor influence on E. fetida survival rate of pH level and salt content

The pH and EC optimum for *E. fetida* were ~pH 6.0 and EC<10 mS cm^{-1} with a earthworm survival rate of ~1200 hours (Fig. 4.8).

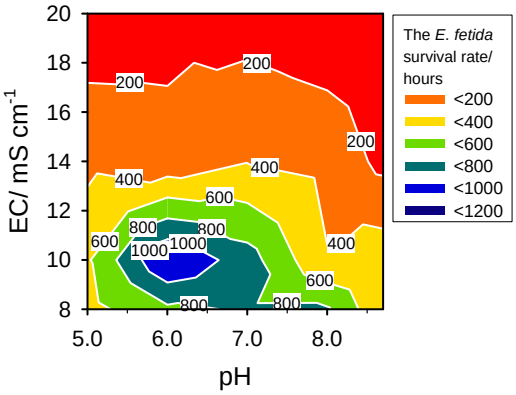

Figure 4.8. Multi-factor influence of pH and EC on the survival rate of *E. fetida*. The 3D-contour graph is composed of pH level (horizontal), EC value (vertical) and isolines of worm survival rate (*n* = 3)

At EC levels of 10-12 mS cm^{-1}, the worms were observed to be tolerant to the test solution, with a survival rate of >600 hours at all pH levels in the range 5.0-7.5. However, the survival rate of *E. fetida* was less (<400 hours) at pH>7.5 when compared with that at acidic and neutral pH values.

At higher EC levels (12-14 mS cm^{-1}), the earthworm survival rate decreased along with the increase of EC. However, a high survival rate (>400 hours) was observed in all cases when the pH increased from 5.0 to 8.0. At the same EC levels, the *E. fetida* survival rate decreased strongly at pH>8.0.

At the EC range 14-18 mS cm^{-1}, *E. fetida* was still quite resistant, with a survival rate >200 hours, independent of pH in the range of 5.0-8.0. At EC>18 mS cm^{-1}, the survival rate of *E. fetida* was <200 hours at any pH value.

4.2.2.2. Multi-factor influence on E. fetida survival rate of pH level and total ammonium nitrogen

At the same EC, the survival rate of *E. fetida* decreased with increasing pH level and TAN content (Fig. 4.9A-B).

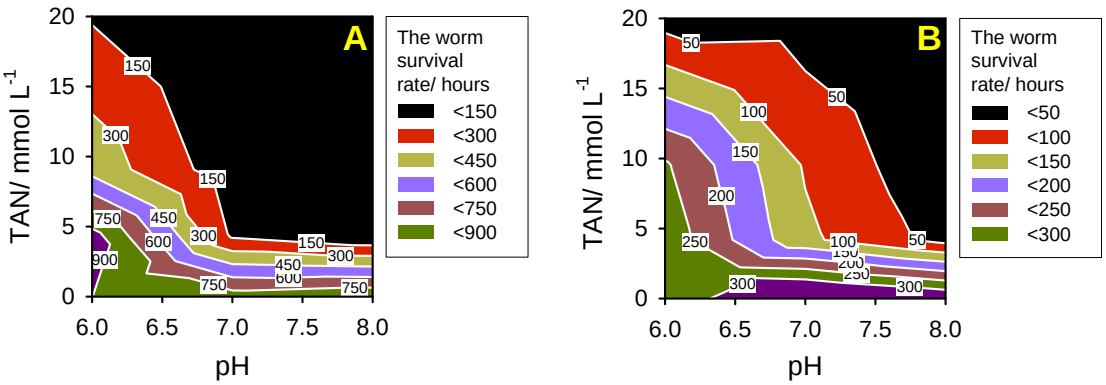

Figure 4.9. Multi-factor influence of pH and TAN on the survival rate of *E. fetida* at (**A**) a low salt content (EC 8 mS cm^{-1}) and (**B**) a high salt content (EC 14 mS cm^{-1}). The 3D-contour graphs are composed of pH level (horizontal), TAN (vertical) and iso-lines of worm survival rate (*n* = 3)

In the presence of TAN, in the test solution at different controlled pH levels, the survival rate of *E. fetida* changed dramatically when the solution changed from acid (pH<7) to alkaline (pH>7).

In the case of a lower salt content (EC 8 mS cm^{-1}) at pH<7.0, *E. fetida* seemed to be tolerant to TAN<20 mmol L^{-1}, with a survival rate >100 hours (Fig. 4.9A). The highest survival rates of *E. fetida* were around pH 6.0 and TAN<5.0 mmol L^{-1} (>900 hours). On the other hand, at pH>7.0 and TAN<5.0 mmol L^{-1}, the survival rate decreased sharply (from 800 hours to 100 hours) along with an increase in TAN, independent of the pH increase. At pH>7.0 and TAN>5.0 mmol L^{-1}, the survival rate of *E. fetida* was less (<100 hours).

In the case of a higher salt content (14 mS cm^{-1}), at pH<7.0 and TAN<17 mmol L^{-1}, the survival rate of *E. fetida* decreased from 300 hours to 100 hours as TAN increased (Fig. 4.9B). Otherwise, earthworm activity was limited (survival rate <100 hours) when TAN>17 mmol L^{-1}. At pH>7.0 and TAN>5.0 mmol L^{-1}, the earthworm' survival rate was only <100 hours. At TAN<5.0 mmol L^{-1}, the earthworm survival rate increased as TAN decreased, but independent of pH increase in the range of pH6-8.

4.2.2.3. Multi-factor influence on E. fetida survival rate of salt content and total ammonium nitrogen

In general, earthworm activity decreased as TAN increased at all pH levels (Fig. 4.10A-C). However, the influence of salt content on earthworm survival rate differed, depending on pH level.

At pH 8.0, earthworm activity was limited in the presence of TAN, but independent of an increase in EC (Fig. 4.10A). The worm survival rate was only 5.0 hours at TAN content <10 mmol L^{-1} (including ~6% NH_3).

At pH 7.0, TAN still strongly influenced earthworm activity, even at a low EC level, 8.0 mS cm^{-1} (Fig. 4.10B). However, at this neutral pH, the survival rate of *E. fetida* was ~8-10 times higher in comparison with that at pH 8.0 at the same EC and TAN levels. At low TAN content (<20 mmol L^{-1}), influences of both TAN and EC on *E. fetida* were observed when the salt content increased. Nevertheless, there was no different influence of EC on earthworm survival rate at the same TAN content at TAN>20 mmol L^{-1}.

At pH 6.0, the earthworms were quite tolerant, with survival rates of 100-1200 hours (Fig. 4.10C). Depending on TAN content, worm activity decreased strongly when EC increased. In these acidic conditions, earthworm survival rate was >150 hours when EC was <12.0 mS cm^{-1}, even though TAN was up to 30 mmol L^{-1} (but containing only 0.06% NH_3).

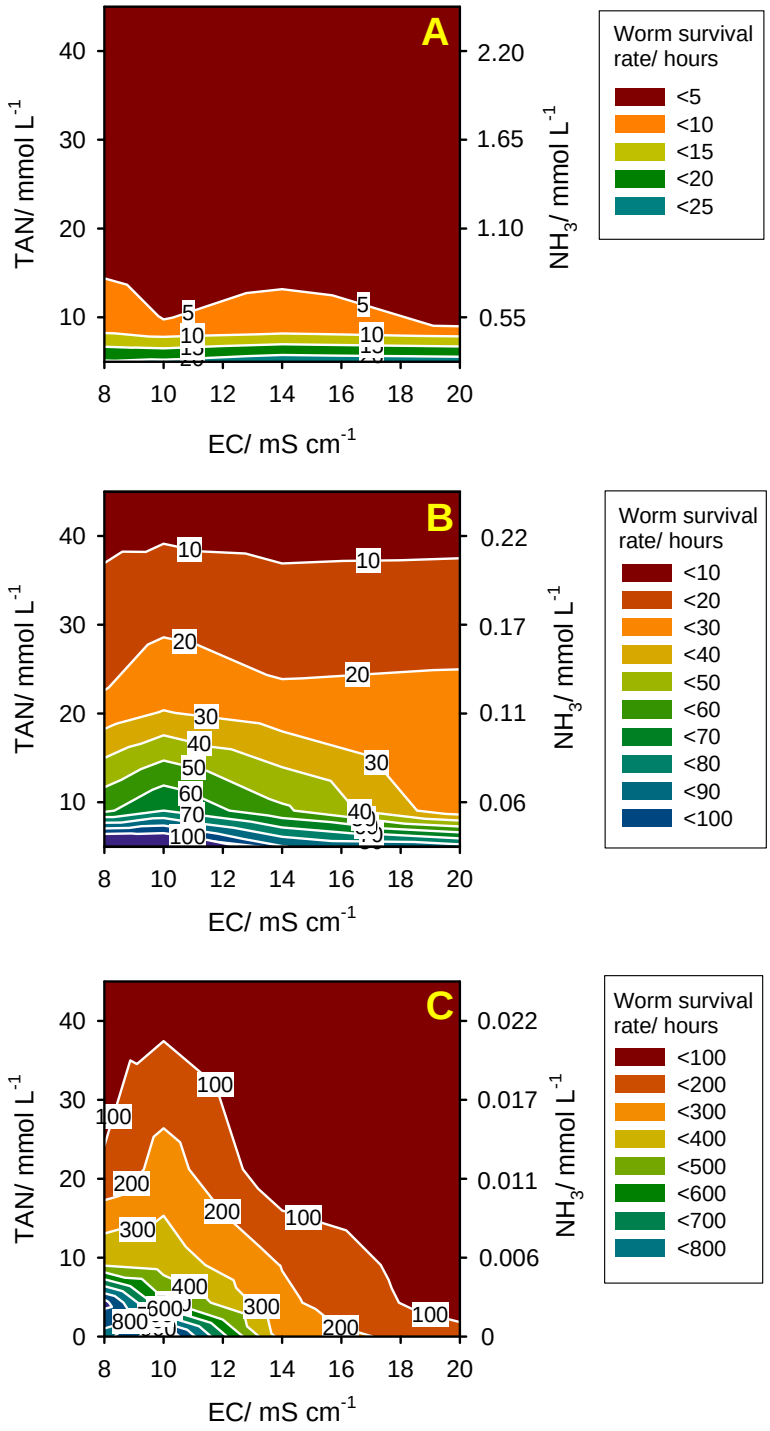

Figure 4.10. Multi-factor influence of EC and TAN on the survival rate of *E. fetida* at (**A**) pH 8.0; (**B**) pH 7.0 and (**C**) pH 6.0. The 3D-contour graphs are composed of EC (horizon), TAN and NH_3 (vertical) and isolines of earthworm survival rate (n = 3)

The correlation between the survival rate of *E. fetida* and NH_3 concentration could be observed clearly when TAN was lower than 50 mmol L^{-1} and EC was 8.0 mS cm^{-1} (Fig. 4.11). The survival rate of *E. fetida* decreased dramatically when NH_3 concentration increased.

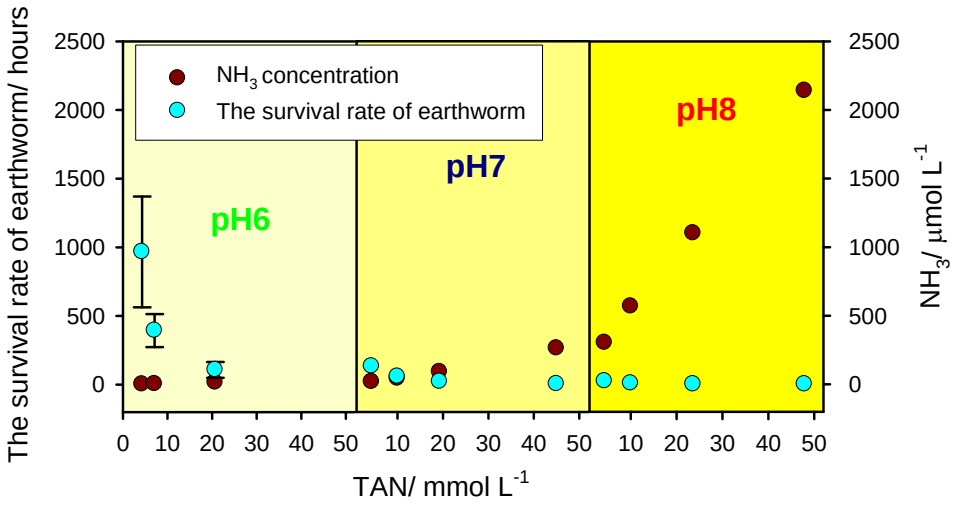

Figure 4.11. Correlation between NH_3 concentration and survival rate of *E. fetida* at different pH levels (6.0, 7.0 and 8.0) at TAN = 0-50 mmol L^{-1} and EC = 8 mS cm^{-1}. Values are means ± SD (*n* = 3)

4.2.3. Regression equation analysis

Using the ANOVA for response surface linear model, worm survival rate was estimated by three independent variables (X_i, *i* =1-3) for pH, EC and TAN (Table 4.1).

Table 4.1: Model and unstandardised coefficients of the regression equation of pH, EC and TAN content for the survival rate of *E. fetida* (*N* = 201)

*__Model summary__: ANOVA for response surface linear model (R^2 = 0.4, R_{Adj}^2 = 0.4); Dependent variable: $f_t = B_0 + B_1*X_1 + B_2*X_2 + B_3*X_3$

Independent variables				**Unstandardised
X_i	Factor	Test range	Unit	coefficient (B_i ± SD)
Constant coefficient	-	-	hours	+1109 ± 124
X_1	pH	5-9	-	-74 ± 16
X_2	EC	0-22	mS cm^{-1}	-23 ± 3
X_3	TAN	0-50	mmol L^{-1}	-7.7 ± 1.0

*: The model is significant (*P*<0.0001)
**: All values of unstandardised coefficients significant (*P*<0.001)

The regression equation for the three factors (pH, EC and TAN content) achieved from the multi-factor test was:

Worm survival rate/ hours = 1109 – 74***pH** – 23***EC** – 7.7***TAN** [11]

Equation [11] showed that all pH, EC and TAN had negative influences on the earthworm survival rate. In the absence of EC & TAN (e.g. pure water with pH = 7.0, EC and TAN = 0), *E. fetida* can survive ~500 hours. On the other hand, in the presence of either TAN or EC, the earthworm survival rate decreased in an increase of the two parameters. The worm survival rate as calculated by equation [11] was an indicator of the potential of different OSW materials for vermicomposting.

4.3. MONOCULTURE AND POLYCULTURE VERMICOMPOSTING OF *E. FETIDA* AND *D. VENETA*

This section is divided into three main sub-sections, including: (*i*) initial characteristics of the three fresh OSW materials and pre-treatments for vermicomposting suitability (*ii*) the development of earthworms and changes in characteristics of vermicomposted substrates during the vermicomposting of monoculture treatment (only *E. fetida*) and polyculture treatment (*E. fetida* and *D. veneta*); and (*iii*) the qualities of the vermicomposted products and the reductions in pathogens.

4.3.1. Initial characteristics of fresh OSW materials and pre-treatment for vermicomposting suitability

4.3.1.1. Physico-chemical properties of fresh OSW materials

In its original form, fresh Bs had a high pH level (8.8), a low salt content (EC 6.3 mS cm^{-1}) and a high TAN content (9.8 g kg^{-1}), which included ~2.6 g NH_3 kg^{-1} (Table 3.8). Nevertheless, it had a suitable C/N ratio (~30) for vermicomposting and the concentrations of N_{tot}-P_{tot}-K_{tot} were 1.6%, 1.0% and 0.7%, respectively.

Fresh Fm had acceptable parameters of DM (20%) and pH level (~6.0), but a high oDM content was observed (94.3%) (Table 3.8). This fresh substrate contained less TAN (<0.4 g kg^{-1}). C_{tot} concentration in the fresh Fm was high (54.7%) in comparison with that in the fresh Bs and Bw (~30-45%). Consequently, even with high N_{tot} concentration (2.9%), the C/N ratio was quite low (19). Both P_{tot} and K_{tot} concentrations were low (0.6% and 0.1%, respectively).

Fresh Bw1 had a high DM (31.5%), a low oDM content (53.1%) and a low C_{tot} concentration (27.8%), whereas N_{tot} concentration was 2.0%, resulting in a low C/N ratio (14.2). Furthermore, other nutrient concentrations in the Bw1 were quite low (0.4% P_{tot} and 0.5% K_{tot}). On the other hand, fresh Bw2 had a low DM (16.1%) and a low pH level (4.0), but its oDM content was high (88.7%). Consequently, a mixture of Bw1 and Bw2 (BwMix) seemed to have suitable properties for vermicomposting (pH 4.4, TAN <0.3 g kg^{-1}, oDM 65.7% and C/N ratio 22.3) (Table 3.8).

4.3.1.2. Changes in physico-chemical properties in Bs during pre-composting

From the results of the suitability tests of fresh Bs, Fm, and Bw for vermicomposting (*see 4.1*) and the multi-factor influences on *E. fetida* (*see 4.2*), pre-treatment can be considered as the first step of the vermicompost process to avoid earthworm toxicity.

During the TAN pre-treatment, the amount of TAN in Bs was reduced from 9.8 g kg^{-1} to 2.3 g kg^{-1} after 15 days (Fig. 4.12A). NH_3 concentration in fresh Bs was >2.6 g kg^{-1} which was reduced significantly to ~0.8 g NH_3 kg^{-1} after 15 days of the pre-treatment. DM content in Bs increased, to ~45% after the process (Fig. 4.12B). Furthermore, EC decreased with a reduction from 5.7 to 3.7 mS cm^{-1} after 15 days (Fig. 4.12C).

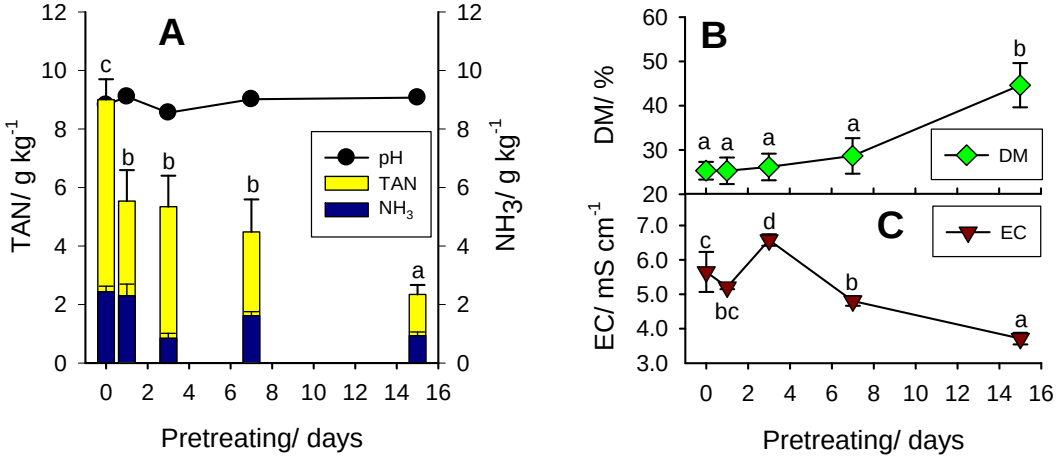

Figure 4.12. Changes in (**A**) pH, TAN, NH_3 concentration; (**B**) DM and (**C**) EC of fresh Bs substrate during the 15-day pre-treatment. Values are means ± SD (n = 3). For TAN, DM and EC parameters, significant differences between plots are indicated by different suffixes a-d (p<0.05)

4.3.1.3. Changes in physico-chemical properties in Bw during pre-composting

Temperature of the Bw heap (20 cm from the top) increased from 24°C to >50°C within 6 days of the pre-composting process (Fig. 4.13A). At day 6, new Bw2 material was added to the compost heap and then the temperature decreased steadily to 34°C at day 20. The pH of Bw increased rapidly from 4.9 at the start to 8.1 at day 6 and then decreased from ~8.0 to 5.8 when the new Bw2 (pH 4.4) was introduced at that day, but it increased again, to pH 7.7 at day 20 (Fig. 4.13B).

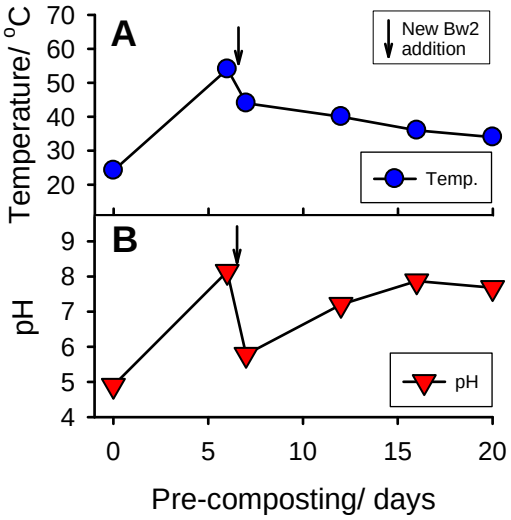

Figure 4.13. Changes in (**A**) temperature of Bw and (**B**) pH of Bw during the 20-day pre-composting. New Bw2 (DM 11.6%, pH 4.4, EC 8.9 mS cm^{-1}, TAN <0.5 g kg^{-1}) was added at day 6 of the process

4.3.1.4. Physico-chemical properties of pre-treated substrates

The physico-chemical properties of 6 feeding materials for vermicomposting (three single substrates and three combined substrates) did not differ greatly after pre-

treatment (Table 3.9). These materials contained 14-31% DM and pH levels were in the range 7.0-8.1. Single Bs had the highest salt content (EC 5.7 mS cm^{-1}). The EC of the other five substrates was <5.1 mS cm^{-1}, with that of single Fm being only 2.9 mS cm^{-1}. TAN content in single Fm was high (7.4 g kg^{-1} including 41.2 mg NH_3 kg^{-1}), whereas that in the other five substrates was lower (<0.5 g TAN kg^{-1}). Both single Bs and single Fm were rich in organic matter, with oDM >87%, whereas single Bw contained only 66.4% oDM. The highest C_{tot} concentration was in the single Bs (43.8%), and the lowest in single Bw (33.6%), whereas single Fm component included 36.4% C_{tot}. There was ~2.0% N_{tot} concentration in components of all six materials. C/N ratio of all the six feeding substrates was in the range 18.0-24.3 at the beginning of vermicomposting.

4.3.2. Physico-chemical changes in feeding materials during monoculture and polyculture vermicomposting

4.3.2.1. Changes in dry matter content during vermicomposting

Dry matter (DM) content in all feeding materials varied in the range 20-30% during 12 weeks of vermicomposting. The DM content in the controls (the same substrate without worms) increased rapidly, to ~80% after 12 weeks (Fig. 4.14). Although the controls were also moistened frequently at the same time as the vermicomposted substrates, the materials dried and had fragmented forms. There were significant differences in DM content between worm treatments and control for feeding materials (p<0.05), but no significant difference was observed between DM content in feeding substrates of monoculture and polyculture treatment (p<0.05).

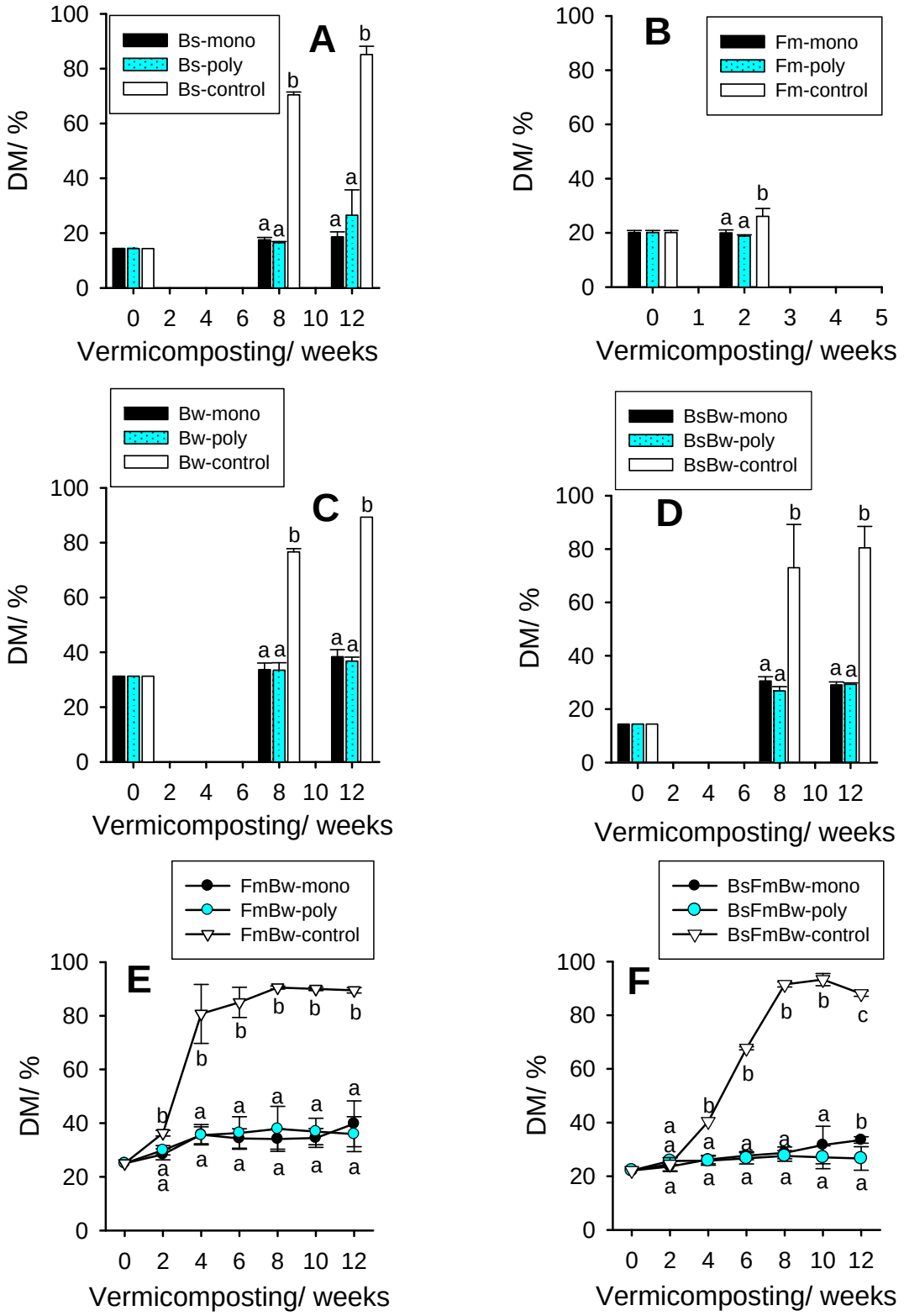

Figure 4.14. Changes in DM content in (**A**) single Bs, (**B**) single Fm, (**C**) single Bw, (**D**) combined BsBw, (**E**) combined FmBw, and (**F**) combined BsFmBw after 12 weeks of monoculture and polyculture vermicomposting. Values are mean ± SD (n = 3), significant differences between treatments at any given time are indicated by different suffixes a-c (p<0.05)

4.3.2.2. Changes in organic dry matter during vermicomposting

Varying in a range from ~65 up to 95% of DM at the beginning, organic dry matter (oDM) in all feeding substrates decreased with time of vermicomposting (Fig. 4.15).

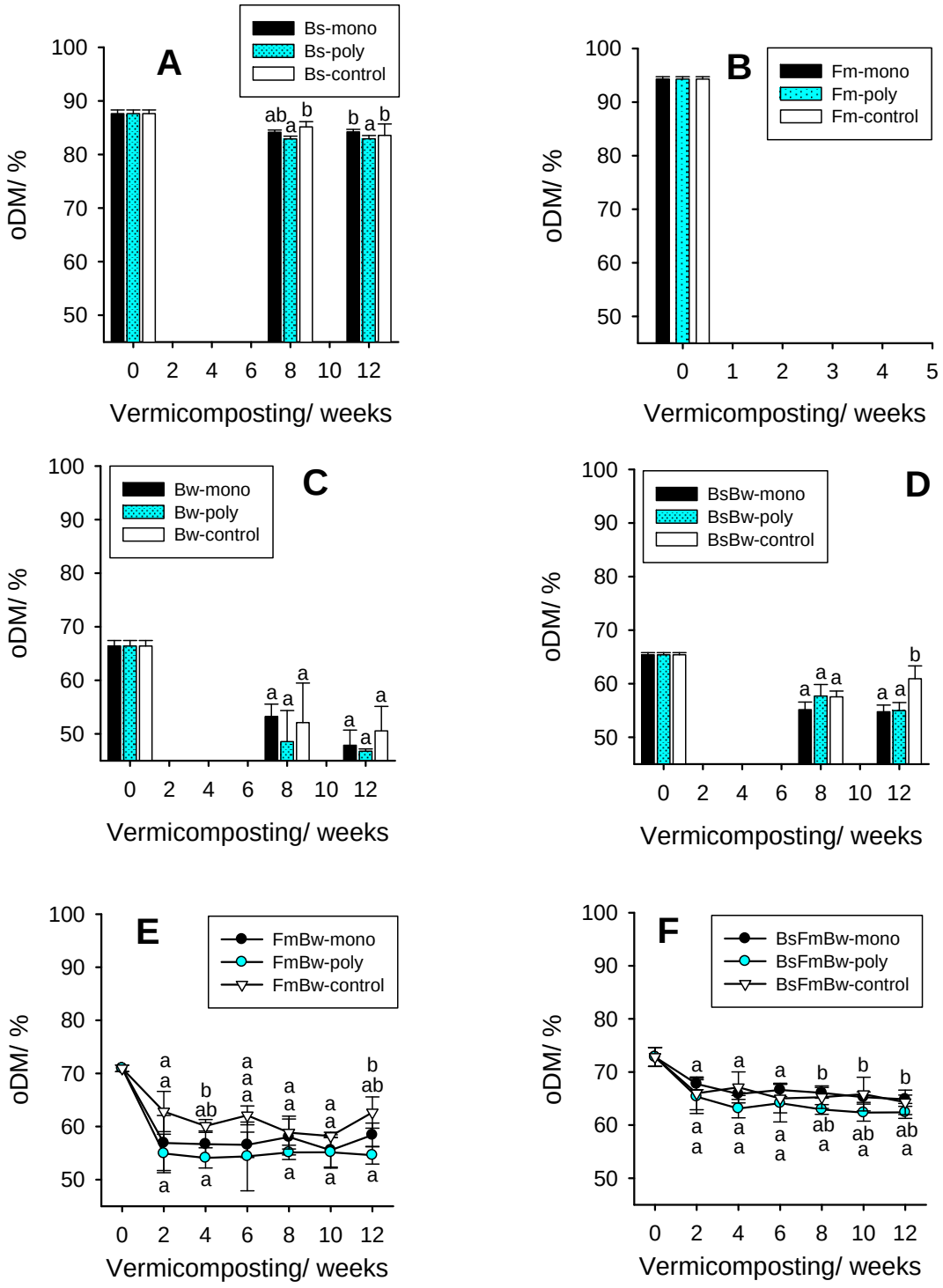

Figure 4.15. Changes in oDM content (of DM) in (**A**) single Bs, (**B**) single Fm, (**C**) single Bw, (**D**) combined BsBw, (**E**) combined FmBw, and (**F**) combined BsFmBw after 12 weeks of monoculture and polyculture vermicomposting. Values are mean ± SD (n = 3), significant differences between treatments at any given time are indicated by different suffixes a-b (p<0.05)

With a high amount of oDM at the beginning of the vermicomposting process (87.6% of DM), the oDM content in single Bs quickly decreased in both treatments and more quickly than in the control (Fig. 4.15A). There was no significant difference between oDM content in Bs-mono and Bs-control at week 8 and week 12 (P<0.05). Nevertheless, oDM content in Bs-poly was significantly lower than in Bs-control (p<0.05).

The oDM content in single Fm was ~95% of DM at the beginning of the vermicomposting process, and was the highest value when compared with the other substrates at the beginning (Fig. 4. 15B).

The oDM content in the single Bw reduced to ~50% of DM after 8 weeks (Fig. 4.15C). However, there was no significant difference between the oDM in single Bw of both worm treatments and the control at any given time (p<0.05).

The oDM content in combined BsBw decreased from 65.5% to <57.8% of DM after 12 weeks of vermicomposting, whereas that in BsBw-control was reduced to 60.9 ± 2.4% of DM (Fig. 4.15D). There was no significant difference between oDM content in BsBw-mono, BsBw-poly and BsBw-control at week 8 (p<0.05). However, the oDM in BsBw of both treatments had a lower content than the control at week 12 (p<0.05). Otherwise, the oDM in 12-week BsBw-poly had no significant difference to that in 12-week BsBw-mono (p<0.05).

Containing 71.0 ± 0.6 % of DM at the beginning, the oDM content in combined FmBw decreased to ~10% after the 12-week vermicomposting process (Fig. 4.15E). In the control, the oDM was reduced to less than that in both monoculture and polyculture treatment at any given time, although the Duncan test did not show significant differences (p<0.05). However, there was a significantly lower oDM amount in FmBw-poly than in FmBw-control at week 12 (p<0.05).

The oDM content in combined BsFmBw (72.8 ± 1.8% of DM at the beginning) decreased to ~65% of DM in both worm treatments and in control after 12 weeks (Fig. 4.15F). There was no significant difference between the oDM content in BsFmBw-mono and BsFmBw-control at any given time, whereas the oDM content in BsFmBw-poly was significantly lower when compared with the control (p<0.05).

4.3.2.3. Changes in pH level during vermicomposting

Apart from the pH increase in single Bw at week 12 of vermicomposting, the other pH levels decreased significantly over 12 weeks of the process, whereas those of controls had a lower reduction (Fig. 4.16). There were significant differences between the pH level of both vermicomposted substrates and the control (p<0.05). Nevertheless, only the pH level of single Bs-mono was significantly lower than that of single Bs-poly at any given time (p<0.05), whereas no significant difference between the pH of other substrates between monoculture and polyculture treatment was found (p<0.05).

The pH level of single Bs increased slightly from 8.1 to 8.2-8.3 in both worm treatments and the control during the first 2 weeks (Fig. 4.16A). The pH decreased toward the acidic level (6.3-6.8) in both worm treatments from week 2 to week 12,

whereas in Bs-control, a reduction in pH was observed until week 8, then it increased to pH 7.8 at week 12. During 12 weeks of the process, the pH of single Bs-poly reduced significantly faster than the level in single Bs-mono (p<0.05).

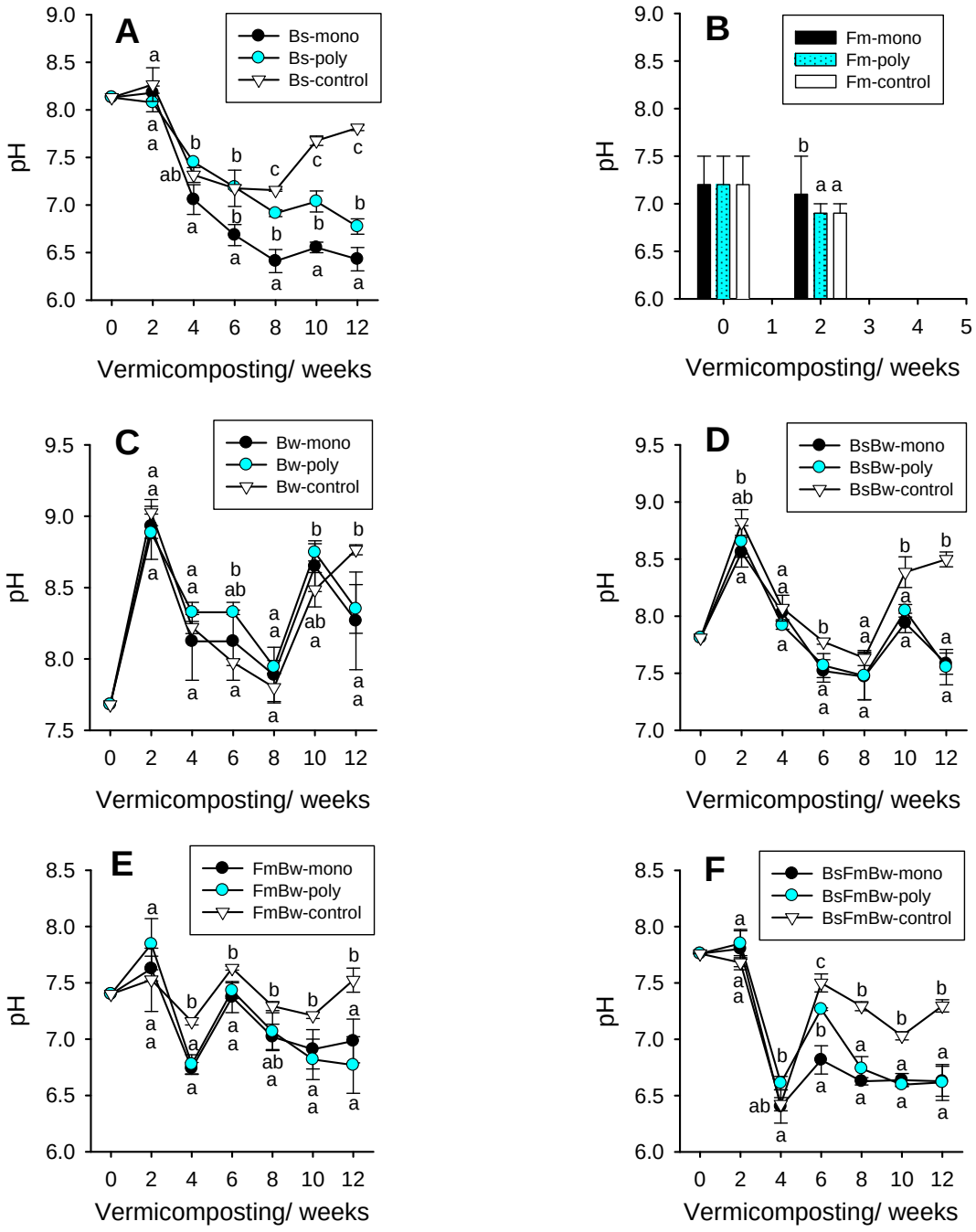

Figure 4.16. Changes in pH level in (**A**) single Bs, (**B**) single Fm, (**C**) single Bw, (**D**) combined BsBw, (**E**) combined FmBw and (**F**) combined BsFmBw after 12 weeks of monoculture and poly-culture vermicomposting. Values are mean ± SD (n = 3), differences between treatments at the same time are indicated by different suffixes a-c (p<0.05)

The pH level of single Fm of both worm treatments and control did not change much during the first two weeks (Fig. 4.16B).

The pH level of single Bw increased quickly, up to ~9.0 after the first 2 weeks of vermicomposting (Fig. 4.16C). The level in both worm treatments and control decreased to pH 7.8 from week 2 to week 8. Later, the pH of Bw-mono and Bw-poly increased again to ~8.5 at week 10 and dropped to ~8.3 at week 12. There was no significant difference in pH level of single Bw between worm treatments and control at any given time from week 2 to week 10 (p<0.05). However, the pH of Bw-mono and Bw-poly was significantly lower than in Bw-control at week 12 (p<0.05).

In both worm treatments of combined BsBw, pH level increased from 7.7 to 8.5 at the first 2 weeks of the process, whereas the control increased up to ~pH 9.0 (Fig. 4.16D). In all substrates, the pH decreased to ~7.5 from week 2 to week 8. Later, there was another increase in the pH level of the vermicomposted substrates. At the end of the process, the pH level in BsBw-mono and BsBw-poly was ~7.5, while that in BsBw-control was higher (~8.5) (p<0.05).

The pH level in combined FmBw increased slightly during the first 2 weeks as well as from week 4 to week 6 of the process (~8.0). However, it decreased to ~7.0 after 12 weeks (Fig. 4.16E). On the other hand, the pH in FmBw-control remained significantly higher compared with the worm treatments for any given time (p<0.05). However, the pH of FmBw-poly was similar to the level of FmBw-mono (p<0.05). The pH level of FmBw in both worm treatments was ~7.0 at the end of process, while the control had a higher level of pH 7.5 (p<0.05).

The pH level of combined BsFmBw in both worm treatments and control remained stable during the first 2 weeks, but it decreased quickly from ~7.7 in week 2 to ~6.5 at week 4 (Fig. 4.16F). Later, the pH level increased again to ~7.0 in both BsFmBw-mono and BsFmBw-poly, whereas the pH was 7.5 in BsFmBw-control. The pH of BsFmBw-mono was significantly higher than that of BsFmBw-poly and BsFmBw-control at week 6 (p<0.05). The pH level in both worm treatments decreased slightly to <7.0 from week 6 to week 12, whereas it remained >7.0 in the control at that time. During this period, a significantly lower pH level in BsFmBw-mono and BsFmBw-poly in comparison with BsFmBw-control was observed (p<0.05).

4.3.2.4. Changes in the electric conductivity during vermicomposting

In both monoculture and polyculture treatment, EC level of all feeding substrates showed an increase, whereas that in the control decreased slightly or was stable during the process (Fig. 4.17). There was a significant difference between the EC in both worm treatments and control at any given time (p<0.05). However, no significant difference was found between the EC in monoculture and polyculture treatment (p<0.05).

At the beginning, EC of single Bs was the highest (~5.5 mS cm^{-1}) (Fig. 4.17A), whereas that of the single Fm was lowest, ~1.0 mS cm^{-1} (Fig. 4.17B) in comparison with those of other substrates (Fig. 4.17C-F). The EC level in single Bw and three combined substrates (BsBw, FmBw and BsFmBw) was ~4.5 mS cm^{-1}. For single Bs, a strong increase in EC was observed, but only in monoculture treatment (Fig. 4.17A). Other OSW substrates (Bw, BsBw and FmBw) in both worm treatments and

the control had a slight increase in EC levels (Fig. 4.17C-E). The EC increase in combined BsFmBw differed most in comparison with the initial value. It increased quickly from ~4.5 mS cm^{-1} to ~7.5 mS cm^{-1} after 4 weeks the process (Fig. 4.17F).

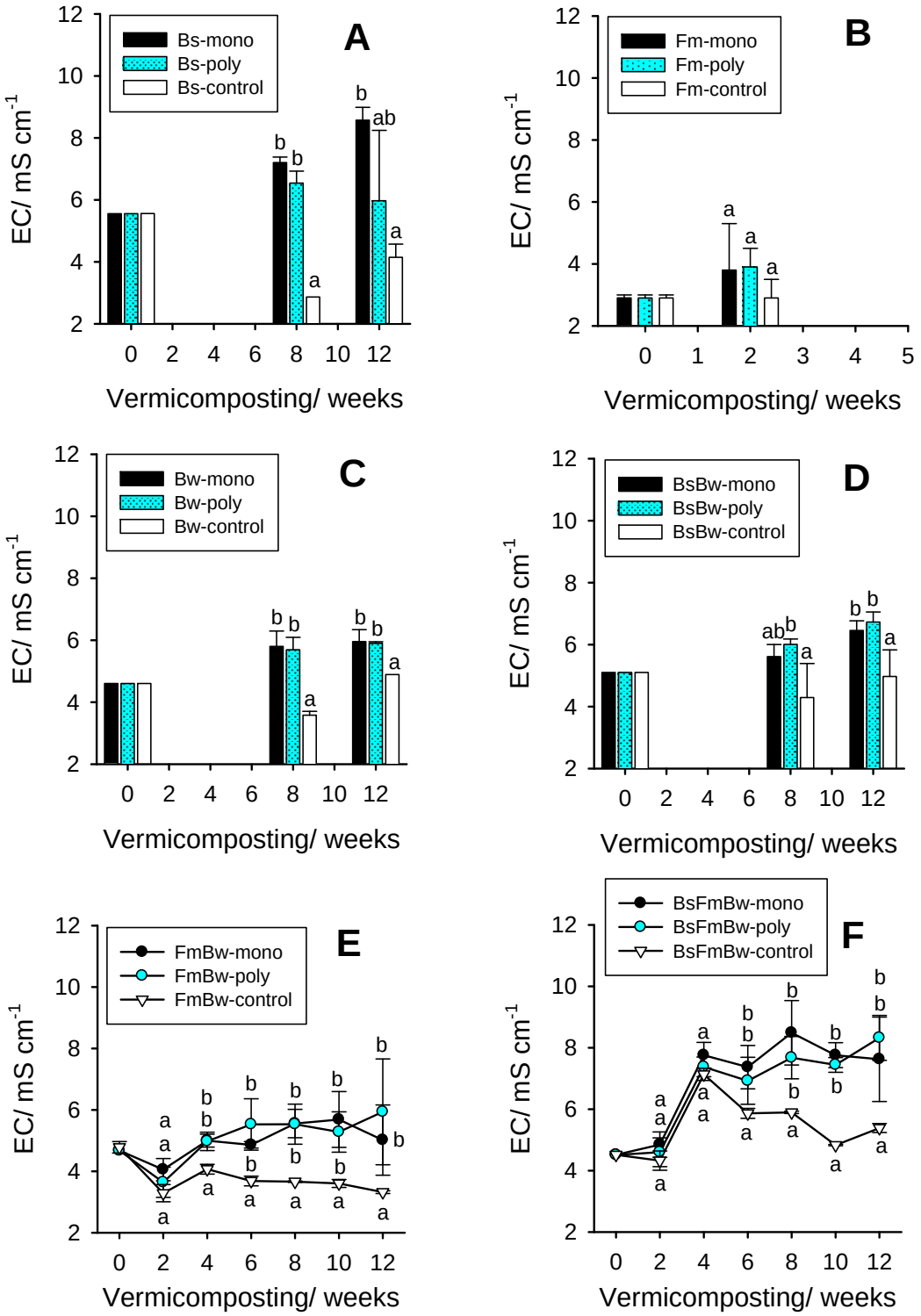

Figure 4.17. Changes in EC in (**A**) single Bs, (**B**) single Fm, (**C**) single Bw, (**D**) combined BsBw, (**E**) combined FmBw and (**F**) combined BsFmBw after 12 weeks of monoculture and polyculture vermicomposting. Values are mean ± SD (*n* = 3), significant differences between treatments at any given time are indicated by different suffixes a-b ($p<0.05$)

4.3.2.5. Changes in TAN and NH_3 concentration during vermicomposting

At the beginning, there was a low TAN content in all feeding substrates (<0.5 g kg^{-1}) except single Fm (~6.0 g kg^{-1}) (Fig. 4.18).

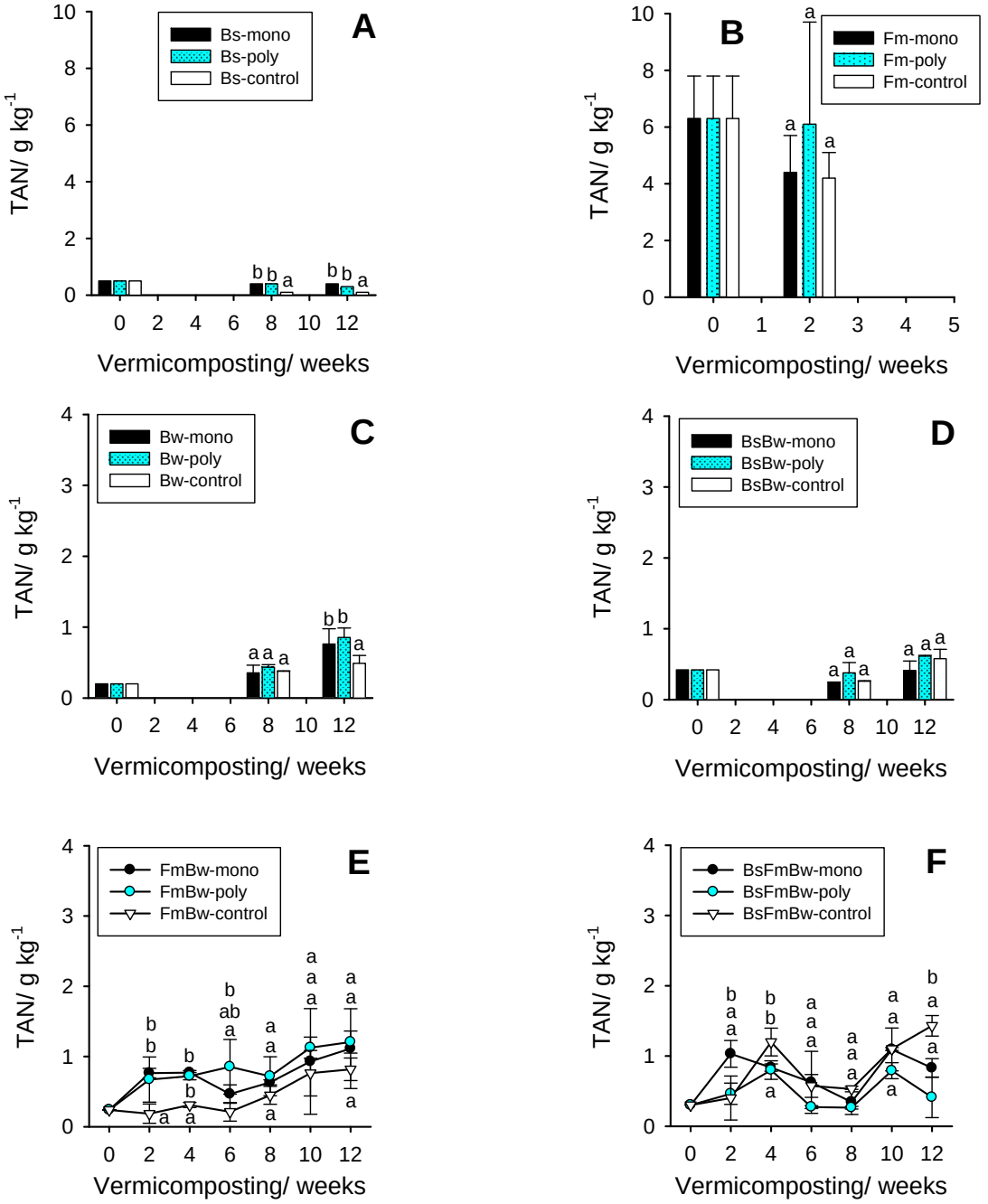

Figure 4.18. Changes in TAN content in (**A**) single Bs, (**B**) single Fm, (**C**) single Bw, (**D**) combined BsBw, (**E**) combined FmBw and (**F**) combined BsFmBw after 12 weeks of monoculture and polyculture vermicomposting. Values are mean ± SD (n = 3), significant differences between treatments at any given time are indicated by different suffixes a-b (p<0.05)

TAN content was stable in the single Bs during 12 weeks of the process (Fig. 4.18A), whereas that in the other vermicomposted substrates (Bw, BsBw, FmBw and

BsFmBw) showed slight increases (Fig. 4.18C-F). However, there was no significant difference between the TAN content in the substrates of monoculture and polyculture treatments at any given time (p<0.05).

Closely correlated to pH level, NH_3 concentration in all feeding substrates varied in a large range from 4.0 to 40 mg kg^{-1} (Fig. 4. 19).

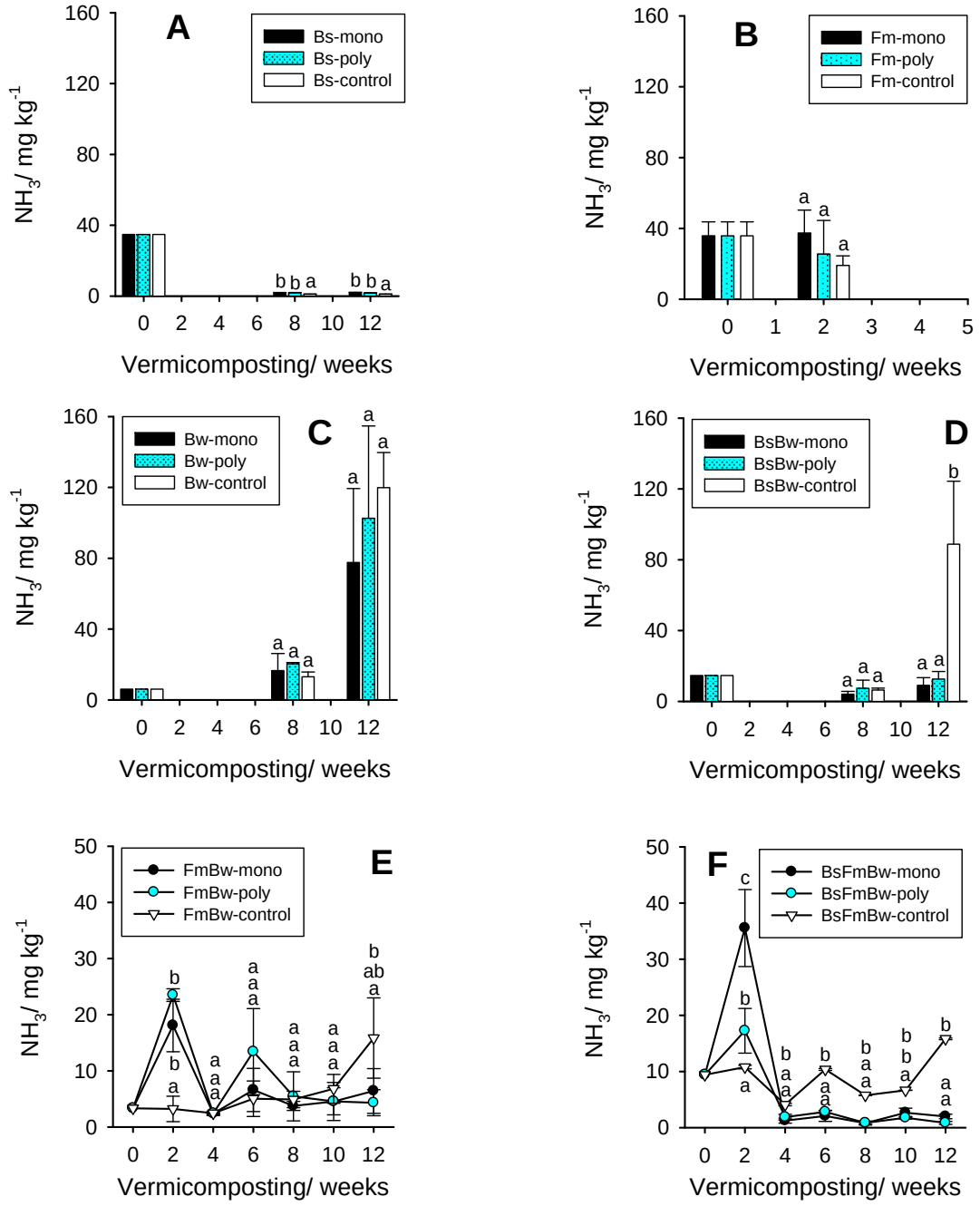

Figure 4.19. Changes in NH_3 concentration in (**A**) single Bs, (**B**) single Fm, (**C**) single Bw, (**D**) combined BsBw, (**E**) combined FmBw and (**F**) combined BsFmBw after 12 weeks of monoculture and polyculture vermicomposting. Values are mean ± SD (n = 3), significant differences between treatments at any given time are indicated by different suffixes a-b (p<0.05)

In single Bs, NH_3 concentration decreased from ~40 mg kg^{-1} at the beginning (pH 8.1) to <1.4 mg kg^{-1} (pH<6.8) after 12 weeks of treatment, whereas the concentration in Bs-control was 4.5 mg kg^{-1} (pH 7.8) at week12 (Fig. 4.19A). There was a significant difference between NH_3 concentration in both worm treatments and the control, but no significant difference between the amount in Bs-mono and Bs-poly (p<0.05).

In single Fm, NH_3 concentration was quite high at the begining (35.8 ± 7.9 mg kg^{-1}) (pH 7.2) (Fig. 4.19B). The NH_3 concentration in the substrates remained at ~36 mg kg^{-1} (pH~7.0) after 2 weeks and at that time no worms were alive in the treatments.

In contrast to single Bs and Fm, NH_3 concentration in single Bw increased, from 6.1 mg kg^{-1} at the beginning (pH 7.7) up to >100 mg kg^{-1} after 12 weeks (pH~8.5) (Fig. 4.19C). However, there was no significant difference between worm treatments and the control at any given time (p<0.05).

NH_3 concentration was low in combined BsBw, in the range 5-15 mg kg^{-1} (pH~7.5) during 12 weeks of vermicomposting, except that in BsBw-control (90 ± 40 mg kg^{-1}) at week 12 (pH 8.5) (Fig. 4.18D). There were no significant differences between NH_3 concentration in both worm treatments and the control at any given time (p<0.05).

NH_3 concentration in combined FmBw varied in the range 4.0-25 mg kg^{-1} (pH~7.2) after 12 weeks (Fig. 4.19E). At week 2 and week 6, the worm treatment showed higher NH_3 concentration (>10 mg kg^{-1}). At week 12, the NH_3 concentration was highest in the control.

NH_3 concentration in combined BsFmBw was 9.4 mg kg^{-1} at the beginning of the 12-week vermicomposting (pH 7.7) (Fig. 4.19F). In BsFmBw-mono, NH_3 concentration increased quickly, up to 35.5 ± 6.9 mg kg^{-1} at week 2 (pH 7.9), which was significantly higher than that in BsFmBw-poly and BsFmBw-control (<18 mg kg^{-1}) (pH~7.8). NH_3 concentration in BsFmBw of both worm treatments was <3.0 mg kg^{-1} from week 4 to week 12 (pH~6.7), whereas BsFmBw-control contained a significantly higher amount (>5.0 mg kg^{-1}) at any given time of the period (pH~7.4) (p<0.05).

4.3.3. Development of earthworms after vermicomposting

The development of earthworms was considered as the change in the numbers and biomass of surviving worms after 12 weeks of vermicomposting. In general, both the number of worms and the biomass decreased during the 12 weeks (Fig. 4.20). The worm numbers in both treatments of single Bs decreased by ~82% after 12 weeks of vermicomposting (Fig. 4.20A), whereas all earthworms (100%) died in single Fm or escaped from the feeding substrate after the first few days of the process (Fig. 4.20B). The single Fm seemed not to be completely accepted by *E. fetida* and *D. veneta*, either in monoculture or in polyculture treatments. The worm numbers and biomass in single Bw in both worm treatments decreased by ~96% after 12 weeks (Fig. 4.20C). There was no significant difference in worm numbers and biomass in monoculture and polyculture treatments for all three single vermicomposted substrates (Bs, Fm and Bw) after 12 weeks of the process (p<0.05) (Fig. 4.20A-C).

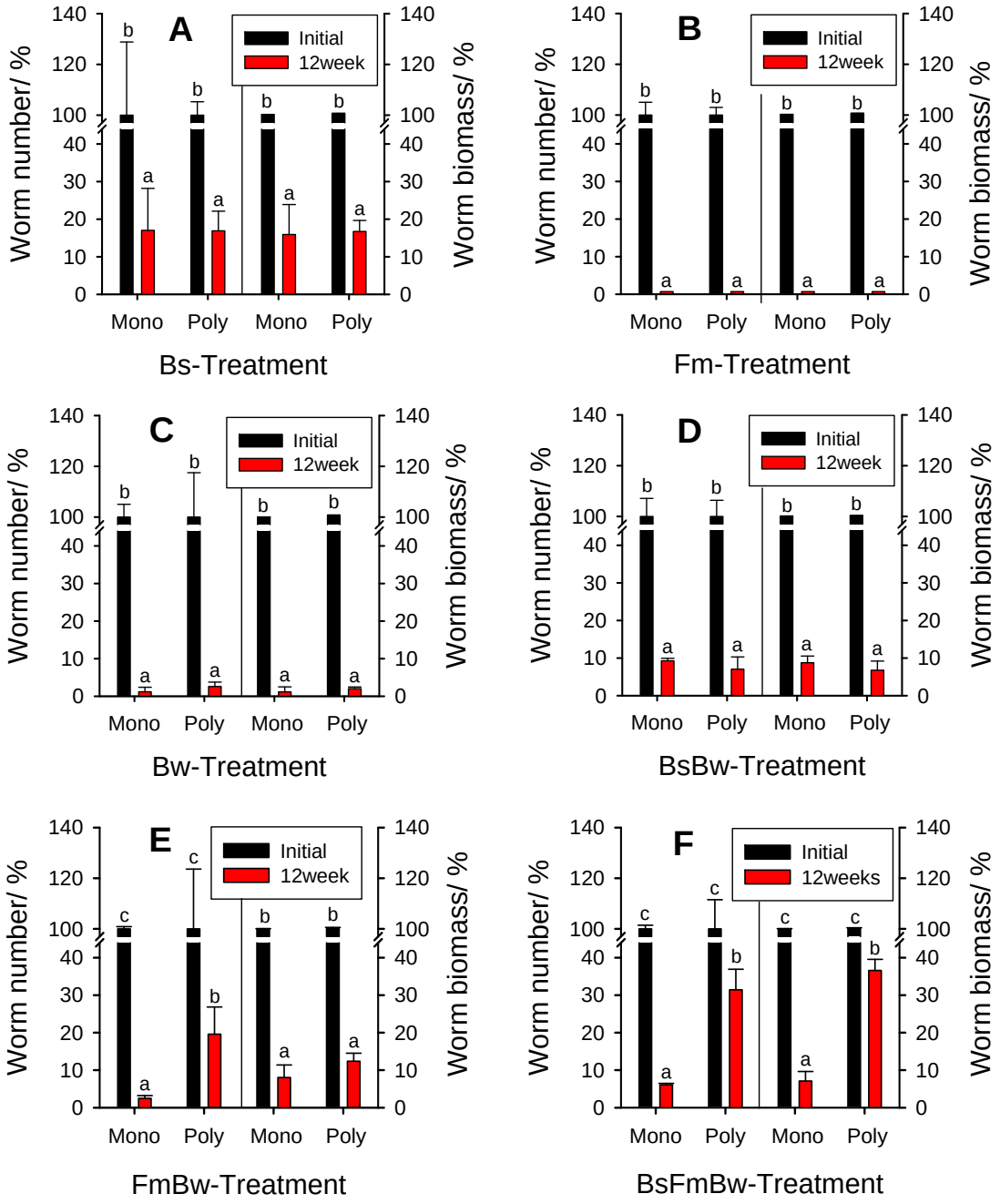

Figure 4.20. Changes in worm numbers and biomass in (**A**) single Bs, (**B**) single Fm, (**C**) single Bw, (**D**) combined BsBw, (**E**) combined FmBw and (**F**) combined BsFmBw after 12 weeks of monoculture and polyculture vermicomposting. Values are mean ± SD (n = 3), significant differences between columns for each parameter are indicated by different suffixes a-c (p<0.05)

In both monoculture and polyculture treatments of combined BsBw, the number of surviving earthworms was reduced by ~90% after 12 weeks (Fig. 4.20D). Only 8.8 ± 1.8 % *E. fetida* survived in BsBw-mono, whereas the biomass of *E. fetida* and *D. veneta* in BsBw-poly was 6.7 ± 2.5 %. No significant difference in the number of surviving worms or worm biomass was found between monoculture and polyculture treatments (p<0.05).

A reduction in worm numbers was observed in both worm treatments of combined FmBw after 12 weeks, but there was a significantly higher number in polyculture treatment (19.6 ± 7.3%) compared with monoculture treatment (2.5 ± 0.7%) (Fig. 4.20E). In FmBw-mono, the biomass of *E. fetida* was smallest (8.1 ± 3.3%), whereas in FmBw-poly, the amount of *E. fetida* and *D. veneta* was greater (12.4 ± 2.1%).

In monoculture treatment of combined BsFmBw, the number of *E. fetida* decreased to 6% whereas in polyculture treatment, 31.5% surviving worms were found in the substrate after 12 weeks of vermicomposting (Fig. 4.20F). The worm biomass in BsFmBw-poly was 36.6 ± 3.0%, which was significantly higher than that in BsFmBw-mono (7.1 ± 2.6%) after 12 weeks (p<0.05).

4.3.4. Changes in element concentrations in the products after treatment of OSW materials

In general, a reduction in C_{tot} concentration in five feeding materials was observed in both worm treatments and control in comparison with that at the beginning (Fig. 4.21). In contrast, the total nutrient concentrations (N_{tot}-P_{tot}-K_{tot}) in these OSW materials increased significantly after the vermicomposting treatment (p<0.05) (Fig. 4.22-4.24).

4.3.4.1. Changes in total carbon in the vermicomposted products

In both the monoculture and polyculture treatments of the OSW materials, C_{tot} concentration decreased during 12 weeks of storage (Fig. 4.21A-B).

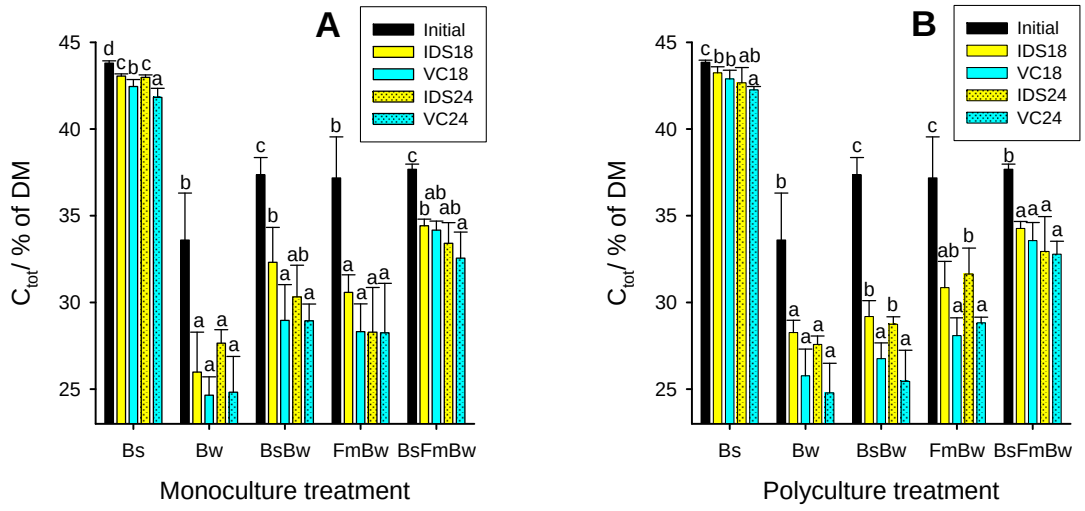

Figure 4.21. Comparisons of C_{tot} concentration (% of DM) in five OSW materials of (**A**) monoculture and (**B**) polyculture treatments at the beginning (Initial), incomplete digested substrate after 18 weeks (IDS18), vermicompost after 18weeks (VC18) and those after 24 weeks (IDS24 and VC24). Values are mean ± SD (n = 3), significant differences between the products of each substrate are indicated by different suffixes a-d (p<0.05)

In monoculture treatments of the five OSW materials, the C_{tot} concentration in Bs-VC18 (42.5 ± 0.4% of DM) and Bs-VC24 (41.8 ± 0.5% of DM) was significantly lower than the amount in Bs-IDS18 and Bs-IDS24 (43.1 ± 0.1% and 43.0 ± 0.1% of DM, respectively) (p<0.05) (Fig. 4.21A). The same lower tendency of C_{tot} concentration was observed in BsBw-VC18 (29.0 ± 2.1% of DM) and BsBw-VC24 (28.9 ± 1.0% of DM) in comparison with BsBw-IDS18 and BsBw-IDS24 (32.3 ± 2.0% and 30.1 ± 1.8% of DM, respectively). However, there was no significant difference between C_{tot} concentration in Bs-VC18 and Bs-VC24 (p<0.05) as well as that in BsBw-VC18 and BsBw-VC24 (p<0.05). There was no significant difference in C_{tot} concentration between IDS and VC products from monoculture treatments of Bw, FmBw and BsFmBw at week 18 (p<0.05) as well as at week 24 (p<0.05) (Fig. 4.21A).

In polyculture treatments of the five OSW materials with the same tendency for a C_{tot} reduction as in the monoculture treatments, lower amounts of C_{tot} were found in Bs-VC, BsBw-VC and FmBw-VC24 compared with Bs-IDS, BsBw-IDS and FmBw-IDS24, respectively (P<0.05) (Fig. 4.21B). Otherwise, the C_{tot} concentration in Bw-VC, FmBw-VC18 and BsFmBw-VC was not significantly lower than that in the IDS products of the same OSW materials at any given time (p<0.05) (Fig. 4.21B).

4.3.4.2. Changes in total nitrogen in the vermicomposted products

Total nitrogen (N_{tot}) concentration in the five OSW materials increased by ~20-50% from the initial amounts, varying from 2.2% to 3.5% of DM after the vermicomposting in both monoculture and polyculture treatments (Fig. 4.22A-B).

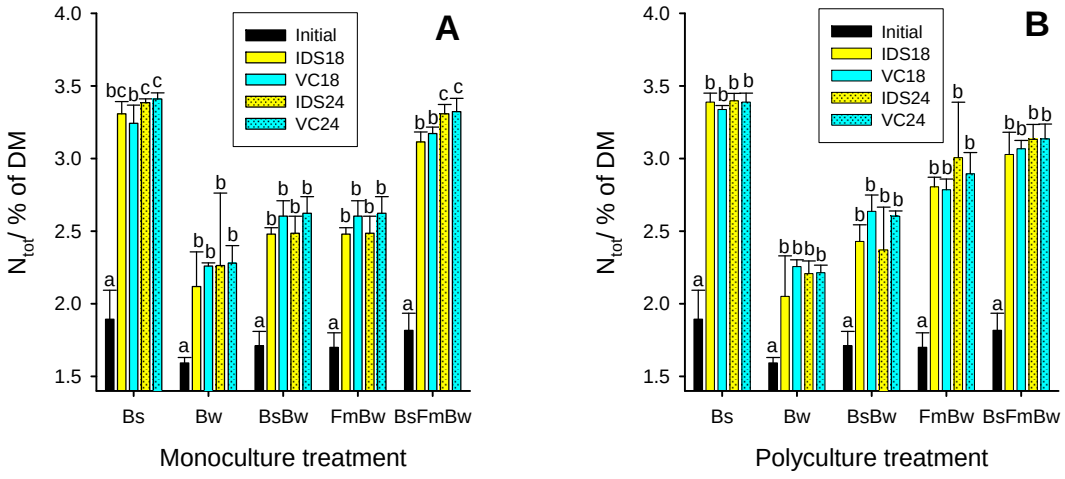

Figure 4.22. Comparisons of N_{tot} concentration (% of DM) in five OSW materials of (**A**) monoculture and (**B**) polyculture treatments at the beginning (Initial), incomplete digested substrate after 18 weeks (IDS18), vermicompost after 18 weeks (VC18) and those after 24 weeks (IDS24 and VC24). Values are mean ± SD (n = 3), significant differences between the products of each substrate are indicated by different suffixes a-c (p<0.05)

In monoculture treatments of the five OSW materials, only single Bs and combined BsFmBw had significantly higher N_{tot} concentration in the 24-week products than in

the 18-week products (p<0.05) (Fig. 4.22A). Nevertheless, the N_{tot} concentrations in Bs-VC and BsFmBw-VC were the same as those in Bs-IDS and BsFmBw-IDS, respectively (p<0.05). In the other OSW materials, no significant difference among the N_{tot} concentration in the four products of single Bw, combined BsBw and combined FmBw was found (p<0.05).

In polyculture treatments of five OSW materials, there was no significant difference in N_{tot} concentration in all products (Bs-IDS18, Bs-VC18, Bs-IDS24 and Bs-IDS24) (p<0.05) (Fig. 4.22B). Similar results were obtained for the change in N_{tot} concentration in the products of the four other OSW materials (Bw, BsBw, FmBw and BsFmBw) (p<0.05).

4.3.4.3. Changes in total phosphorus in the vermicomposted products

Total phosphorus (P_{tot}) concentration in the products of five OSW materials in both the monoculture and polyculture treatments increased between 20% and 50%, when compared with the initial amount (p<0.05) (Fig. 4.23A-B). However, there were significant differences in P_{tot} concentration in four products (IDS18, IDS24, VC18 and VC24) of the five OSW materials (Bs, Bw, BsBw, FmBw and BsFmBw) (p<0.05).

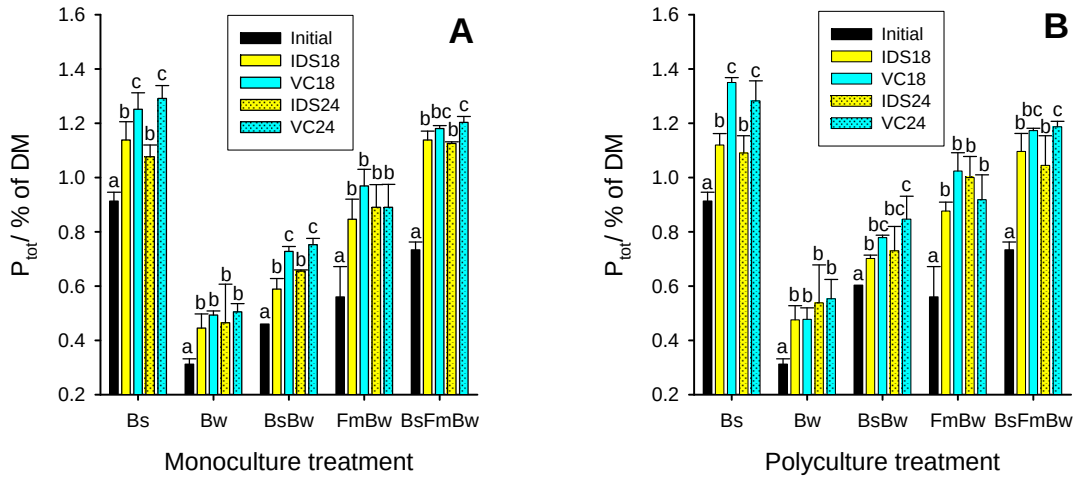

Figure 4.23. Comparisons of P_{tot} concentration (% of DM) in five OSW materials of (**A**) monoculture and (**B**) polyculture treatments at the beginning (Initial), after 18 weeks (IDS18 and VC18) and after 24 weeks (IDS24 and VC24). Values are mean ± SD (n = 3), significant differences between the products of each substrate are indicated by different suffixes a-c (p<0.05)

In the monoculture treatments of OSW materials (Fig. 4.23A), P_{tot} concentration in Bs-VC, BsBw-VC, and BsFmBw-VC was significantly higher than the amount in Bs-IDS, BsBw-IDS, and BsFmBw-IDS at any given time (p<0.05). There was no significant difference in P_{tot} concentration between IDS and VC products of the other substrates (Bw and FmBw) (p<0.05).

Similarly to the monoculture treatments, P_{tot} concentration was the same in the four Bw-products and FmBw-products in polyculture treatments (Fig. 4.23B). However,

there were significantly lower amounts of P_{tot} in Bs-VC, BsBw-VC and BsFmBw-VC in comparison with those in their IDS products (P<0.05). No significant differences were seen when comparing P_{tot} concentration in IDS18 to that in IDS24 products and that in VC18 to that in VC24 products after polyculture treatments of Bs, BsBw and BsFmBw (p<0.05).

4.3.4.4. Changes in total potassium in the vermicomposted products

There was also an increase in total potassium (K_{tot}) concentration in the vermicomposted products (~20-50%) in comparison with the initial K_{tot} concentration in the same OSW materials (p<0.05) (Fig. 4.24A-B). In both monoculture and polyculture treatments, there was no significant differences in K_{tot} concentration between the four products (IDS18, IDS24, VC18 and VC24) of all five OSW materials (p<0.05).

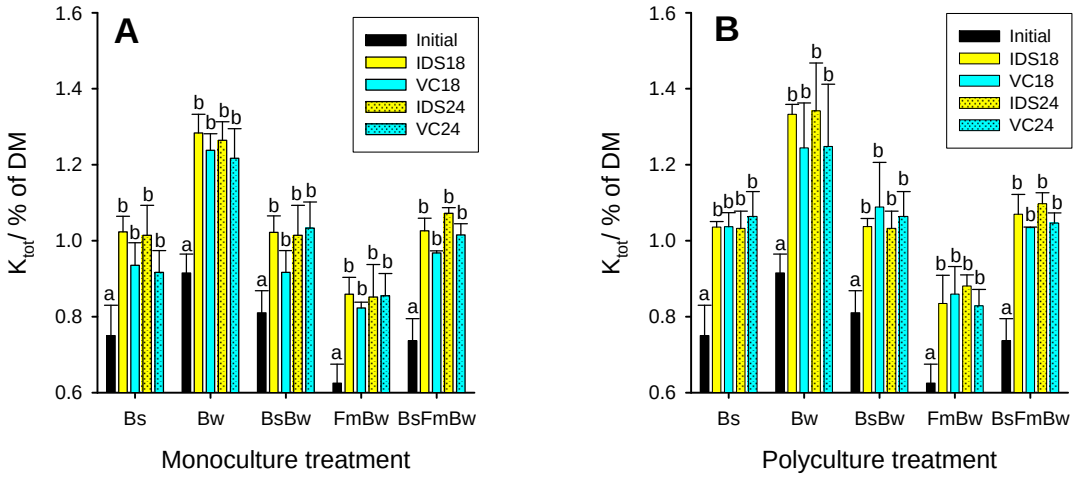

Figure 4.24. Comparisons of K_{tot} concentration (% of DM) in five OSW materials of (**A**) monoculture and (**B**) polyculture treatments at the beginning (Initial), after 18 weeks (IDS18 and VC18) and after 24 weeks (IDS24 and VC24). Values are mean ± SD (n = 3), significant differences between the products of each substrate are indicated by different suffixes a-b (p<0.05)

4.3.5. Maturity of OSW materials after vermicomposting

4.3.5.1. C/N ratio

The C/N ratio of all OSW materials decreased during the 24-week treatment (12-week vermicomposting + 12-week maturity) (Fig. 4.25). The C/N ratio decreased fast during in the first 2-4 weeks. Then, the C/N ratio of all feeding materials decreased gradually during the storage period with maturation (from week 12 to week 24).

C/N ratio of single Bs was reduced from 23.2 to ~12.5 in both worm treatments after 24 weeks, whereas the reduction in the control was less, to 15.0 ± 1.0 at week 24 (Fig. 4.25A). At the beginning, the C/N ratio of single Fm was lowest (~18.0) in comparison with the ratios in the other OSW materials (>20) (Fig. 4.25B).

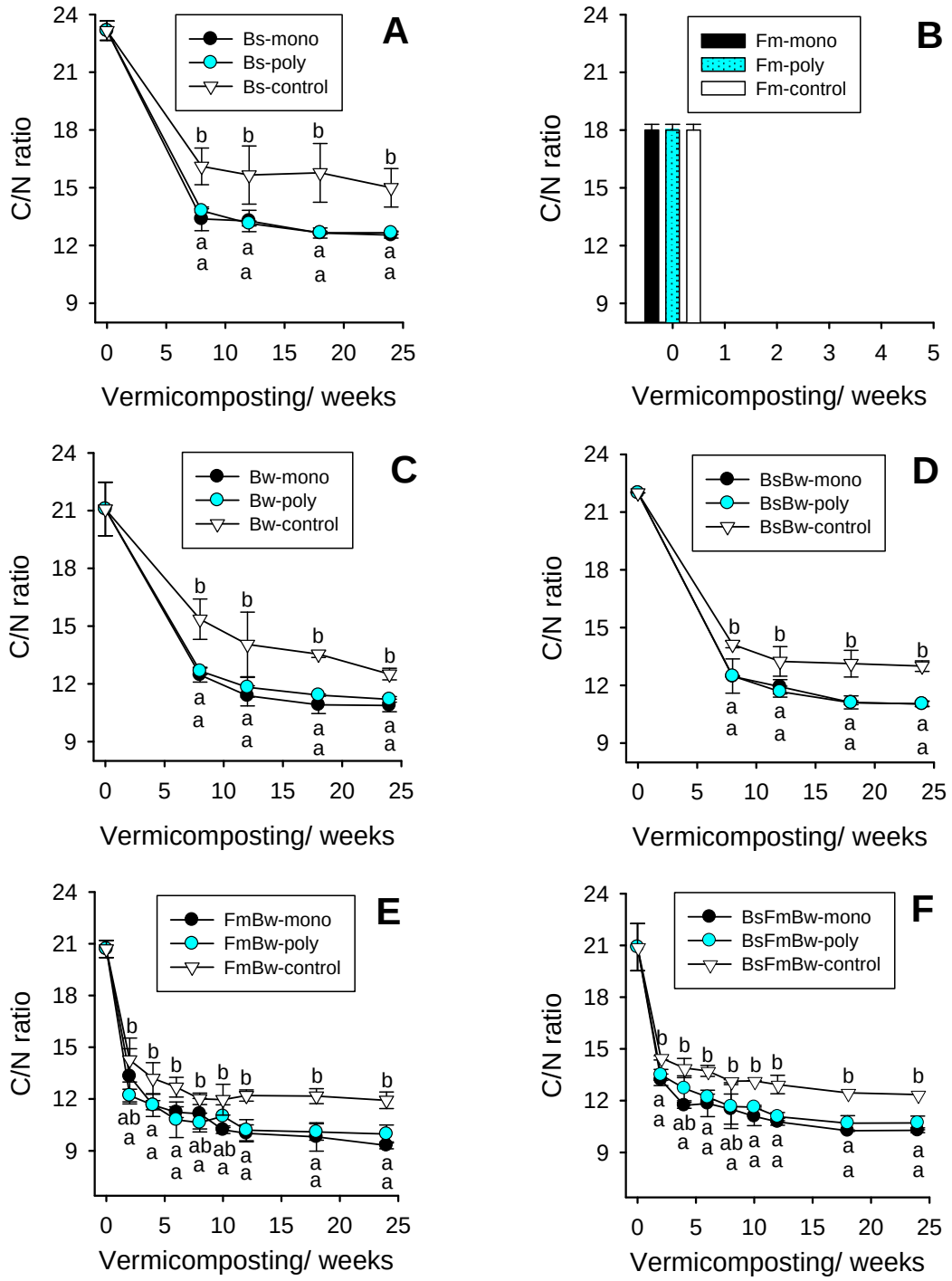

Figure 4.25. Changes in C/N ratio of (**A**) single Bs, (**B**) single Fm, (**C**) single Bw, (**D**) combined BsBw, (**E**) combined FmBw and (**F**) combined BsFmBw during the 24-week process: 12-week vermicomposting + 12-week storage of monoculture treatment (mono), polyculture treatment (poly) and control. Values are mean ± SD (n = 3), significant differences between treatments at any given time are indicated by different suffixes a-b (p<0.05)

C/N ratio of single Bw-mono decreased fastest from 21.1 ± 1.4 at the beginning to 10.9 ± 0.3 after 24 weeks (Fig. 4.25C). The C/N of Bw-poly24 was 11.2 ± 0.2 after 24 weeks, but showed no significant difference compared with Bw-mono24, whereas

that of Bw-control24 was significantly higher, 12.5 ± 0.3 (p<0.05).

As with the treatments of single materials, no significant difference was seen in C/N ratio between monoculture and polyculture treatments of the three combined materials (BsBw, FmBw and BsFmBw) (p<0.05), whereas the C/N ratios of the controls were significantly higher than those of worm treatments at any given time (p<0.05) (Fig. 4.25D-F).

4.3.5.2. Respiration index

The respiration index for four days (AT_4) showed that the final products (VC24) from both monoculture and polyculture treatments (VC24-mono and VC24-poly) of the five OSW materials were significantly lower as compared to the controls (control24) (p<0.05) (Fig. 4.26). However, there was no significant difference between VC24-mono and VC24-poly for each OSW vermicompost (p<0.05).

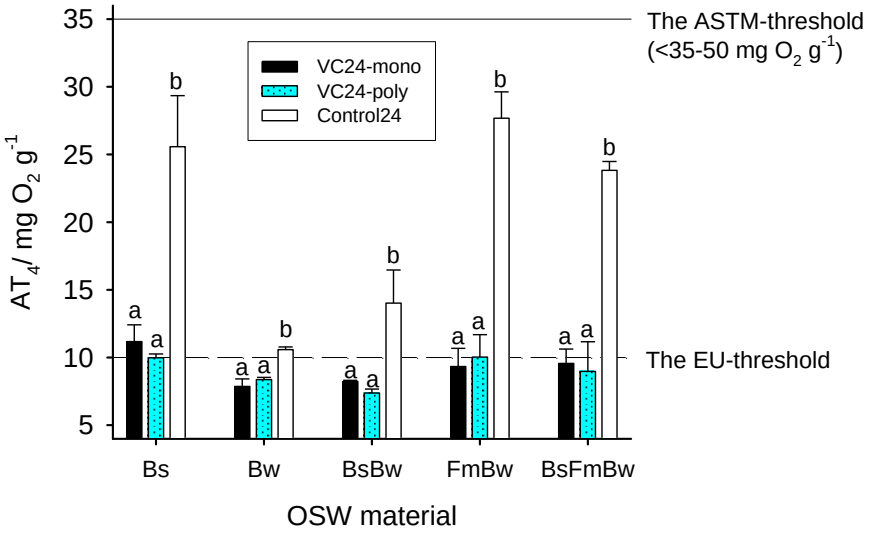

Figure 4.26. Comparisons of the respiration index for four days (AT_4) of vermicomposts after 24 weeks of monoculture treatment (VC24-mono), polyculture treatment (VC24-poly) and the control (control24) from different OSW materials. Values are mean ± SD (n = 3), significant differences between values of each substrate are indicated by different suffixes a-b (p<0.05)

With an exception of Bs, all vermicomposts (VC24) of the five different OSW materials matched the EU regulations for mature composts (<10.0 mg O_2 g^{-1}), whereas all controls had a higher AT_4 as compared to the EU regulation. All substrates were in line with the American standard for testing and materials (ASTM) threshold (<35-50 mg O_2 g^{-1}).

4.3.6. Reductions in hygienie pathogens after vermicomposting

At the beginning, all fresh OSW materials were contaminated by several pathogenic microorganisms including *Salmonella* spp., *Enterococcus* spp., *E. coli* and somatic coliphage (Table 4.2).

Table 4.2: Occurrence of pathogens in the fresh OSW materials used as a feed for vermicomposting, data given for DM except *Salmonella* spp.; '+' means the positive detection in 10.0 g wet sample

Pathogenic indicator	Fresh Bs	Fresh Bw	Fresh Fm
Occurrence of *Salmonella* spp.	+	+	+
Number of *Enterococcus* spp./ CFU g^{-1}	4.2×10^3	1.4×10^7	2.0×10^4
Number of *E. coli*/ CFU g^{-1}	1.9×10^4	2.4×10^7	2.0×10^4
Number of coliphage/ PFU g^{-1}	3.0×10^1	4.5×10^3	1.0×10^2

Analyses of *Salmonella* spp. showed that the bacteria was reduced after the treatments (Table 4.3). No *Salmonella* spp. survived in 10.0 g pre-treated substrate (DM ~30%) after 2 weeks. Later, *Salmonella* spp. bacteria was also not found in the 10.0 g vermicomposts (DM~90%) and the controls (DM~90%).

Table 4.3: Occurrence of *Salmonella spp*. in the fresh substrates, the 2-week pre-treated substrates (2-week pre-sub.), the vermicomposts (VC24) and controls (control24) of the different OSW materials; '+/-' means the positive/negative detection in 10.0 g (wet wt.)

OSW material	Fresh sub.	2-week pre-sub.	24-week products		
			VC24-mono	VC24-poly	Control24
Single Bs	+	-	-	-	-
Single Fm	+	-	na	na	na
Single Bw	+	-	-	-	-
Combined BsBw	+	-	-	-	-
Combined FmBw	+	-	-	-	-
Combined BsFmBw	+	-	-	-	-

na: data not available

The numbers of *Enterococcus* spp. in all OSW materials decreased with time for both worm treatments (Fig. 4.27).

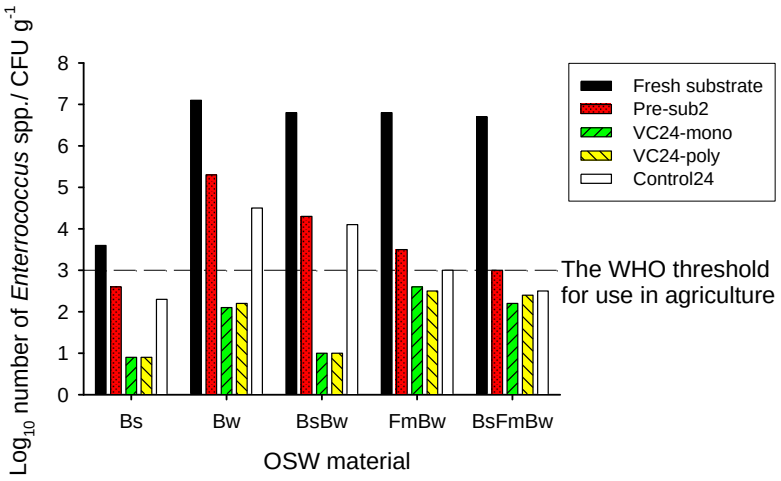

Figure 4.27. Comparison of the $\log_{10}$ number of *Enterococcus* spp. in fresh substrates, 2-week pre-treated substrates (Pre-sub2), 24-week vermicompost (VC24) and the control (control24) among the different OSW materials

The pre-treatment resulted in a decrease of *Enterococcus* spp. in all substrates. In the vermicomposted products, the content of *Enterococcus* spp. was even lower (<3.0 log_{10} units). The overall reduction was the highest for BsBw (>6.0 log_{10} units) and the lowest for Bs (~3.0 log_{10} units). In the controls, *Enterococcus* spp. was always higher as compared to the vermicomposted products. There was no difference in number of *Enterococcus* spp. between the monoculture and polyculture treatments. All vermicomposted products were below the WHO recommendation for substrates to be used in agriculture (<3.0 log_{10} units).

The numbers of *E. coli* in all vermicomposted products decreased below the WHO-threshold for use in agriculture (<10^3 CFU g^{-1}) after the treatments (Fig. 4.28).

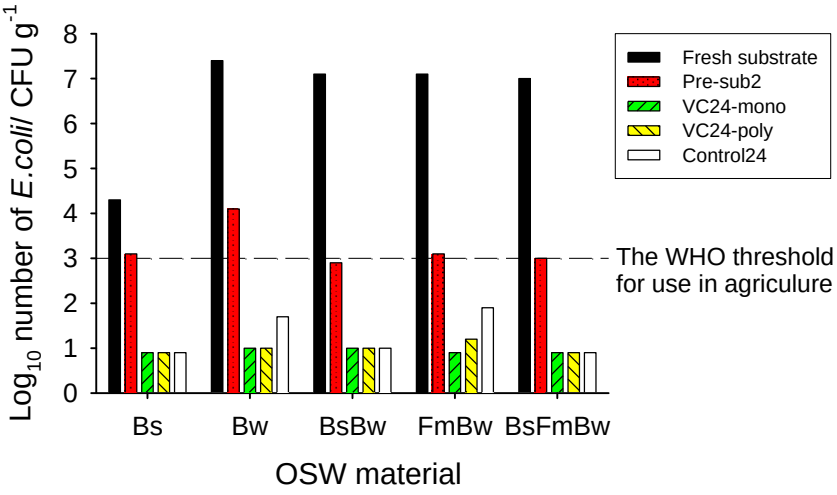

Figure 4.28. Comparison of the log_{10} number of *E. coli* in the fresh substrates, 2-week pre-treated substrates (Pre-sub2), 24-week vermicompost (VC24) and the controls (control24) among the different OSW materials

In single substrates (Bs and Bw), the number of *E. coli* decreased by 1.3 and 3.3 log_{10} units, respectively after the pre-treatment. In the combined substrates (BsBw, FmBw and BsFmBw) which had >10^7 CFU g^{-1} at the beginning, the number of *E. coli* was reduced by around 4.2 log_{10} units. Then, *E. coli* was reduced to less than $10^{0.9}$ CFU g^{-1} in all vermicomposted products after 24 weeks of vermicomposting. There was no difference in the number of *E. coli* between monoculture and polyculture treatments, but the numbers in vermicomposted products were lower than in the controls (≥3.0 log_{10} CFU g^{-1}).

For viruses, the number of somatic coliphage available in all fresh OSW materials was 0.3 x 10^1, 4.5 x 10^3 and 10^1 PFU g^{-1} in Bs, Fm and Bw, respectively (Table 4.3). After only 2 weeks of pre-treatment, these were reduced to zero. Consequently, no further analyses were made on this hygiene parameter after 24 weeks of vermicomposting.

5. DISCUSSIONS

This chapter discusses the results (*see Chapter 4*) in three main sections: (*i*) the suitability test of three OSW materials (Bs, Fm and Bw) for vermicomposting of *E. fetida*; (*ii*) the single- and multi-factor effects on the survival rate of *E. fetida* of pH, EC and TAN; and (*iii*) the changes in physico-chemical properties of feeding substrates during monoculture and polyculture treatments of these OSW materials and the qualities of the products.

5.1. SUITABILITY TESTS OF ORGANIC SOLID WASTES FOR VERMICOMPOSTING OF *E. FETIDA*

5.1.1. Suitability test of fresh organic solid wastes

The three fresh OSW materials did not seem to be favoured feeding substrates for *E. fetida* because more than 95% of the earthworms died within 20-day vermicomposting tests (Fig. 4.1A). Similarly, Gunadi & Edwards (2003) reported death of *E. fetida* after 2 weeks in fresh cattle solids although physico-chemical properties were technically suitable for the growth of the species. In addition, Yadav *et al.* (2010) reported that 100% *E. fetida* died in fresh source-separated faeces not mixed with other OSW materials.

Fresh Bw was not accepted by *E. fetida* (<2.0%), even when its DM content was 27.6% and the pH was 5.3 (Table 3.1) which are in the tolerance range of this worm species (Domínguez & Edwards, 2004). Perhaps the high salt content (EC 9.1 mS cm^{-1}) was the limiting factor for the earthworms (Fig. 4.1C). Owojori *et al.* (2009) suggested a threshold for inorganic salts (4.0 mS cm^{-1}) above which the worms will be affected.

Fresh Fm had quite a low DM content (11.4%) (i.e. 89.9% moisture content), which could be the main reason for its refusal by most earthworms. The suitable DM content for *E. fetida* is reported to be ~20% (Edwards, 2007; Gunadi *et al*., 2003; Domínguez & Edwards, 2004; Yadav *et al*., 2010). Low pH (5.3) of the fresh Fm may also have affected worm survival.

Fresh Bs seemed to have 'acceptable conditions' of DM content (26%) and EC (3.7 mS cm^{-1}) for vermiculture. However, the pH 8.4 and high TAN content of 11.8 g kg^{-1} might be the reason for worm toxicity. NH_4^+ salts in the vermicomposted organic substrates should be <0.5% to avoid earthworm toxicity (Domínguez & Edwards, 2004; Edwards, 2007). In vermicomposting of a mixture of Bs with wastewater sludge, Garg *et al.* (2006) attributed the mortality of earthworms during the first 2 weeks of the process to NH_4^+ from the anaerobic conditions.

It is worth noting that the three parameters (DM, pH and EC) of the fresh OSW substrates did not change much after 20 days in the suitability tests compared with the control (without earthworms) and the initial substrate (Fig. 4.1B-D). Perhaps the

small quantities of surviving earthworms (<5%) which were present in the test substrates is the reason why these OSW materials had stable properties.

5.1.2. Suitability test of mixtures of fresh Bs and Fm

The high pH in fresh Bs (8.8) and the low DM content in fresh Fm (11.4%) were perhaps the reasons for the mortality of earthworms (*see 5.1.1*). Mixing of the two OSW materials might give more suitable physico-chemical parameters for vermicomposting of *E. fetida*, e.g. pH level in the range 5.0-8.0, DM content in the range 15-25%, and TAN content <0.5% (Domínguez & Edwards, 2004; Edwards, 2007).

The earthworm survival of >30% in the mixtures of fresh Bs and Fm after 30 days could indicate that the suitability of the Bs and Fm in mixtures is better than the individual fresh materials (<5.0% after 30 days). Similarly, Garg *et al.* (2006) used a mixture of Bs with wastewater sludge (1:1 by weight) to improve the yield of worm biomass. Bs has also been introduced in mixtures with cow dung, coirpith (a by-product of the coir industry) and weeds to improve the vermiculture process of *Eudrilus eugeniae* (Jeyabal & Kuppuswamy, 2001), while Sangwan *et al.* (2008) used sugar mill sludge substrate amended with Bs for vermicomposting of *E. fetida*. With the same purpose of Bs treatment, mixtures with vegetable-market solid wastes (Suthar, 2009) or with wheat straw, sugarcane trash and guar bran (Suthar, 2010) highlighted the impact of the bulking materials for the growth of *E. fetida* and its decomposition rate in Bs.

According to Suthar (2010), the biomass of individual worms increased by ~200% in a mixture of Bs and crop residues after 15 weeks, whereas increased biomass of *E. fetida* in the single Bs was only <150%. In a mixture of Bs and pressed mud (3:2 by weight), the gain in individual earthworm biomass was the highest (>150%) in comparison with the initial weight after 13 weeks, whereas in the single Bs, the gain was only ~115% after the same time (Sangwan *et al*., 2008).

In contrast, Garg *et al.* (2006) reported that fresh Bs was much better than its mixture with fresh solid textile mill sludge. The earthworms gained >500% in the fresh single Bs after 12 weeks compared with ~140% in the Bs mixture (2:3 by weight with the sludge). However, the lower pH level (7.4) of the single initial Bs of that study compared with other studies (>8.0) might explain the higher biomass gain in the single Bs than the Bs mixtures.

Depending on the pH levels of the combined substrate, the suitability of the four mixtures of fresh Bs and Fm (Mix1-4) was different for *E. fetida* (Fig. 4.2A). The lower initial pH levels of the four mixtures Mix1-4 (8.3, 7.5, 7.0 and 6.5) resulted in a higher number of earthworms (31.0%, 58.5%, 58.4% and 65.8%, respectively) after the suitability test. The pH difference was due to the ratio of the fresh Fm substance in the four mixtures. On the other hand, earthworm survival rate <30% at day 30 in the Bs-Mix4 could be explained by the high DM content at day 30 (>22%). Three other mixtures (Mix1-3) contained >55% surviving earthworms after 30 days because of the more suitable of the DM (<20%).

5.1.3. Suitability test of pre-treated Bs

Some previous studies concluded that vermicomposted feeds must be pre-treated to remove toxic factors for earthworms such as ammonium (NH_4^+) (Edwards, 2007), inorganic salts (Frederichson *et al*., 2007; Gunadi & Edwards, 2003), and anaerobic conditions (Garg *et al.*, 2006). In original form, the TAN content (~8-12 g kg^{-1}) in the fresh Bs might be a reason for the unsuitability of the substrate for vermicomposting. Consequently, the TAN content in fresh Bs should be reduced by pre-composting and acidification before use in vermicomposting.

5.1.3.1. TAN pre-treatment of fresh Bs material

The TAN content in fresh Bs was reduced significantly, which could be due to NH_3 volatilisation under pre-composting conditions (Fig. 4.3). Using the same methodology for the pre-treatment of fresh green wastes, Frederickson *et al.* (1997) gave the same explanation for NH_3 emissions during pre-composting. No significant lowering of TAN content or NH_3 concentration was found at day 5 compared with day 3 (P<0.05), which could indicate that the period of 3 days might be enough to eliminate part of the TAN content in fresh Bs. According to Frederichson *et al*. (2007), all pre-treatments should be kept at a minimum duration to avoid losses of N_{tot} (~5%) by NH_3 volatilisation.

The pH of Bs, which still remained at a high level of 9.0 after the 3-day pre-composting, can be explained by the occurrence of NH_3-N (~30% of TAN content). The addition of an acidic solution could be an efficient option to get a good substrate for earthworms under vermicomposting conditions (Tripathi & Bhardwaj, 2004b).

5.1.3.2. Suitability test of Bs3-HCl for vermicomposting

The four Bs3-HCl substrates were suitable for vermicomposting(Fig. 4.4A). A lower *E. fetida* survival rate was found in Bs3-A1 (~20%) and Bs-A2 (~60%) after four days because of the high pH level at the beginning (>8.0) (Fig. 4.2C), whereas ~95% of the surviving earthworms were found in Bs3-A3 and Bs3-A4 due to the lower pH (<7.5). According to Domínguez & Edwards (2004), *E. fetida* prefers acidic OSW materials than alkaline.

It seems likely that it was not pH but the corresponding NH_3 concentration that limited the survival rate of earthworms. The NH_3 concentration in the four Bs3-HCl varied from 9.4 to 270 mg kg^{-1} depending on the HCl dosage after HCl addition at the beginning, while the TAN content was the same in all (~3.3 g kg^{-1}) (Table 3.4). With the higher level of pH, the TAN content in Bs3-HCl was mainly converted to NH_3-N, which probably has more toxicity than NH_4^+-N.

EC might not have been a toxic factor for earthworms because the greatest numbers of *E. fetida* was found in Bs3-A4, which had the highest EC (9.1 mS cm^{-1}) among the four tested Bs3-HCl substrates at the beginning (Fig. 4.4D). According to Kaplan *et al.* (1980), an EC value of 12.8 mS cm^{-1} could be suitable for vermicomposting if the substrate (horse manure) is not contaminated by urine or NH_4^+ salts.

In summary, although there were many previous studies about vermicomposting of several OSW materials, it was very difficult to summarise these experiments in terms of 'suitable conditions for vermicomposting'. The above suitability tests for the three OSW materials, including Bs, Fm and Bw in fresh forms as well as the other modified forms (mixing, TAN-pre-treating and HCl addition) could give the general conclusion that these OSW materials are less suitable for vermicomposting by *E. fetida*.

5.2. SINGLE- AND MULTI-FACTOR TESTS FOR THE SURVIVAL RATE OF *E. FETIDA*

This section discusses the influences of of single- and multi-factors on the survival rate of *E. fetida*. Furthermore, a regression equation from the multi-factor test is suggested to contribute a prediction of the suitability for vermicomposting.

5.2.1. Single-factor influences on the survival rate of *E. fetida*

The tests of the influence of each single factor (pH, EC and TAN) on the survival rate of *E. fetida* were carried out separately to investigate the correlation of one parameter at a time on the activity of earthworms.

5.2.1.1. pH level and buffer system

The survival rate of *E. fetida* varied widely (from 0.1 hours to 800 hours) in the range of pH 2.0-9.0, and the activities were especially limited at pH <4.0 or pH >8.0 (<50 hours) (Fig. 4.5A). Citric acid seemed to be toxic for *E. fetida*. Whenever citric acid was a component of the buffer with Na_2HPO_4, the earthworms died faster, but the rate increased with the pH increasing in the range of pH2-7.

When citric acid was replaced by KH_2PO_4, the survival rate for the earthworms increased significantly (~800 hours). In the pH range between 5.0-8.7, the survival rates were similar in a range of 450-800 hours, with maximum survival at pH7 (Fig. 4.5B). This indicates that either the EC or the specific chemicals used for pH adjustment negatively influenced earthworm activity. This could explain the differences in the suggested pH optimum in a range of pH 5.0-8.0 from previous findings (Table 5.1). Therefore, the pH optimum for *E. fetida* seems to depend on specific salts in various OSW materials.

Table 5.1: Suggested optimal pH levels of different media used for toxicity tests with *E. fetida*

Medium	Conditions of pH test			Suggestion for optimal pH	Reference
	EC/ mS cm^{-1}	TAN/ mmol kg^{-1}	pH range		
Soils	na	na	na	7-8	Rivero-Hernandez (1991)
*Activated sludge	0.9-1.5	na	2.0-10	7	Kaplan *et al.* (1980)
Animal wastes	na	na	5.0-9.0	5	Edwards (1988)
Soils	na	na	5.0-9.0	5	Edwards & Bohlen (1996)
Organic wastes	na	na	4.0-9.0	5	Domínguez & Edwards (2004)
Vegetable wastes	na	na	5.0-9.0	5	Edwards (2007)
Citric acid/Na_2HPO_4	3.0	0	3.0-7.0	7	This study (2012)
KH_2PO_4/Na_2HPO_4	8.0	0	5.0-8.7	6-8	This study (2012)

*: Substrate contains 11% solids with pH 6.4 and uncontaminated by urine
na: data not available

5.2.1.2. Salt content (or electric conductivity parameter)

For the single influence of the salt content, the findings (Fig. 4.6) tend to support results from other studies (Table 5.2).

Table 5.2: Suggestions for salinity limitations in different media used for toxicity tests of *E. fetida*

Medium	Conditions of salinity test				Suggestion for salinity limit/g kg^{-1}	Reference
	Factor	Range/ g kg^{-1}	EC/ mS cm^{-1}	Adj. pH		
*Soil	KCl	0-20	na	na	< 5.0	Kaplan *et al.* (1980)
	NaCl	0-10	na	na	< 1.0	
	NaH_2PO_4	0-10	na	na	< 5.0	
	K acetate	0-10	na	na	< 5.0	
*Soil	NaCl	0-3.6	0.1-1.4	5.8	< 3.9	Owojori *et al.* (2009)
**Hm	na	na	1.5-3.0	na	EC<3 mS cm^{-1}	Hartenstein *et al.* (1979)
OSW	inorganic salts	na	na	na	<5.0	Domínguez & Edwards (2004)
***Pm	salts	na	na	na	<5.0	Edwards (2007)
H_2O	KCl	0-40	0-32.2	5.4	<10.0 (EC<16 mS cm^{-1})	This study (2012)
KH_2PO_4/ Na_2HPO_4	KCl	0-8.0	0-20.0	5.0-8.7	EC< 14 mS cm^{-1}	This study (2012)

*: Artificial soil (by DM)= 70% sand + 20% kaolin + 10% organic matter has 1.5 times its own weight of moisture
**: Horse manure (Hm) uncontaminated by urine
***: Fresh poultry manure (Pm)
na: data not available

All previous studies tested the effect of salts on earthworms, and all of them suggested that rather low salt content affects the worm's activity (<5%). For example, Kaplan *et al.* (1980) used KCl salt to vary the EC value in artificial soil for testing. However, they concluded that the KCl content in the medium should be <5.0 g kg^{-1}, two-fold lower as found in this study (10.0 g KCl L^{-1} or 16.0 mS cm^{-1}). It seems likely that not KCl but other salts may have a selectively stronger negative effect on the earthworms. In this study, the EC basically was defined by KCl and to a certain extent by the buffer system. A similar explanation may apply for the different limits for the salt content in previously tested media including organic compounds such as horse manure (Hartenstein *et al.*, 1979), organic waste (Domínguez & Edwards, 2004) and poultry manure (Edwards, 2007). Consequently, some specific salts seem to have different negative effects on earthworms, not only because of the EC value but also because of effects of the specific salts (e.g. NH_4^+). Unfortunately, there were no data on the parameter in those studies.

5.2.1.3. Total ammonium nitrogen (TAN)

For TAN ($NH_4^+ + NH_3$), all previous authors concluded that TAN content plays a major role in the survival rate of earthworms. But again, the data sets were incomplete to compare among those different studies and there were misunderstandings about the relative influence of TAN and NH_4^+ (Table 5.3).

Table 5.3: Suggestions for TAN limitation of different media used for toxicity tests with *E. fetida*

Medium	Conditions of TAN test				Suggestion for TAN limit/ mmol L^{-1}	Reference
	Factor	Range/ mmol L^{-1}	EC/ mS cm^{-1}	Adj. pH		
Animal waste	NH_4^+ salt	na	na	na	<71*	Edwards & Bohlen (1996)
OSW	NH_4^+ salt	0-300	na	na	<36*	Domínguez & Edwards (2004)
**Soil	CH_3COONH_4	0-130	na	na	<13	Kaplan *et al.(*1980)
H_2O	NH_4HCO_3	0-480	3-40	8.0	<30	This study (2012)
KH_2PO_4/ Na_2HPO_4 buffer	NH_4HCO_3	0-45	0-20	8.0	<1	This study (2012)
		0-45	0-20	7.0	<10	
		0-45	0-20	6.0	<40	

*: Value were calculated by mmol in kg fresh substrate (FS)
**: Artificial soil (by DM) = 70% sand +20% kaolin +10% organic matter has 1.5 times its own weight of moisture
na: data not available

In the TAN single-factor test, the earthworms were very sentitive to TAN at pH of 8.1. Even at low concentrations (<30 mmol L^{-1}), the survival rate of earthworms was <1.0 hour (Fig. 4.7). The results of the single-factor TAN test in aqueous solution are not comparable with those performed by others in solid medium such as artificial soil (Kaplan *et al*., 1980) or animal waste (Edwards & Bohlen, 1996).

TAN content contains <0.06% NH_3-N at low pH (<6.0), whereas NH_3-N comprises up to 5.6% of TAN content at pH8. Thus, NH_3 is perhaps the main negative influencing factor which dramatically affects the activity of earthworms instead of two multi-factors, TAN and pH level. Free NH_3 has been cited as one of the gaseous products during anaerobic decomposition of OSW materials, to explain the earthworm mortality (Frederickson *et al.*, 2007; Garg *et al*., 2009). Unfortunately, no previous study mentioned the limit of the NH_3 concentration on earthworm activity during vermicomposting.

As artificial soils or OSW materials contain a variety of buffers and differently loaded cation/anion exchange systems, it is difficult to adjust a physico-chemical parameter without influencing the soil and several organic components. In the aqueous test system, these problems do not exist, which is another advantage compared with a test system with soil and liquid phase. Furthermore, even with the pH controlled at a constant level, the single factor test of TAN showed a simultaneous influence on the survival rate of *E. fetida* of both parameters (EC and TAN) because as the TAN content increased, the EC value also increased.

5.2.2. Multi-factor influences on the survival rate of *E. fetida*

5.2.2.1. pH level and salt content

The two factors, pH and EC, negatively affected the earthworm survival rates when their values increased simultaneously (Fig. 4.8). The pH and EC optimum was at pH 6.0 and EC <10 mS cm^{-1}. This result is not in line with previous studies, which gave a pH optimum of ~5.0 (Domínguez & Edwards, 2004; Edwards, 2007) or 7.0-8.0 (Kaplan *et al*., 1980; Rivero-Hernandez, 1991) (Table 5.1). As discussed earlier (*see 5.2.1.1*), the KH_2PO_4/Na_2HPO_4 buffer used in this study is most likely different to the buffer system in substrates used in the other tests. As long as the TAN did not contribute to EC, EC levels up to 14 mS cm^{-1} did not affect the survival rate of *E. fetida* severely (Fig. 4.8). The EC limit is in line with findings of Kaplan *et al.* (1980), who tested several salts in urine-uncontaminated horse manure media for *E. fetida*.

5.2.2.2. pH level, salt content and total ammonium nitrogen

In the presence of TAN, a pH >7.0 strongly and negatively affected the survival rate of *E. fetida* (Fig. 4.9A-B). In almost all vermicomposted OSW materials, several TAN-containing compounds (e.g. urea salts) may cause earthworm toxicity. Kaplan *et al.* (1980) used urine-uncontaminated horse manure as the test medium and concluded that pH 7.0 was the optimum for *E. fetida*. On the other hand, the higher survival rate of *E. fetida* in the presence of TAN at pH <7.0 could also explain previous conclusions on the acidic pH optima (~5.0) for earthworms (Domínguez & Edwards, 2004; Edwards, 2007). Consequently, the simultaneous presence of the TAN might be the reason for the inconsistent pH optima for vermicomposting cited in previous studies.

Regarding the influence of NH_4^+-salts, Edwards & Bohlen (1996) concluded that the limit for NH_4^+-N in vermicomposted substrates is <72 mmol kg fresh $substrate^{-1}$. In

another study, Domínguez & Edwards (2004) concluded that NH_4^+-N contained in vermicomposted materials should be <36 mmol kg^{-1}. As a lower limit, Kaplan *et al.* (1980) concluded that the maximum concentration of CH_3COONH_4 for the acceptability of *E. fetida* was 13 mmol NH_4^+ L^{-1}.

The inconsistency of the TAN limits may be because those studies did not link the NH_4^+-parameter with the co-existence of the other multi-factors (e.g. pH, EC and NH_3) in the test medium. Additionally, no data on the parameters of test medium were given (Table 5.3). For the suitability for vermicomposting, the TAN limit is suggested to be <10 mmol L^{-1} when the pH level in OSW materials is acidic (pH <7.0), whereas alkaline OSW materials (pH >7.0) should not have any TAN-containing components when they are applied for vermicomposting.

5.2.3. Regression equation of multi factors for the prediction of OSW materials for vermicomposting

The regression equation [11] can be used as a tool to predict the potential of OSW materials for vermicomposting. The test was simplified by having only three main parameters (pH, EC and TAN) for several OSW materials. Furthermore, the prediction could suggest a suitable option for pre-treatment of several OSW materials before being treated by vermicomposting. Actually, all previous studies mentioned vermicomposting as a biological treatment for suitable OSW materials, but they only give separate optimal conditions in their properties for earthworms (Domínguez & Edwards, 2004; Somali, 2008). Furthermore, a lack of explanations is given for the choices of those OSW materials based on their initial properties (Table 2.3).

5.3. MONOCULTURE AND POLYCULTURE VERMICOMPOSTING OF *E. FETIDA* AND *D. VENETA*

The discussion in this section is divided into three parts, covering (*i*) the initial physico-chemical properties of the three fresh OSW materials and the pre-treatment for the vermicomposting suitability; (*ii*) changes in physico-chemical properties of the feeding substrates and the worm biomass and numbers during monoculture and polyculture; and (*iii*) qualities of all products after the entire process.

5.3.1. Initial characteristics of OSW materials and pre-treatments for vermicomposting suitability

5.3.1.1. Initial characteristics of OSW materials

The physico-chemical properties (pH, EC, DM, TAN and oDM) and the concentrations of elements (C_{tot}-N_{tot}-P_{tot}-K_{tot}) of the three initial fresh substrates (Bs, Fm and Bw) varied in comparison with each other due to the formation of the materials or the specific local areas (Albanell *et al.*, 1988; Haimi & Huhta, 1986).

The suitability tests on fresh Bs, Fm and Bw for vermicomposting (*see 4.1 & 5.1*) and the tests of the main influencing multi-factors (pH, EC, TAN and NH_3) on the survival

rate of *E. fetida* (*see 4.2 & 5.2*) showed that these three fresh OSW materials seemed not to be suitable for vermicomposting because of their initial characteristics in terms of pH level, DM or TAN content.

5.3.1.2. Pre-composting of fresh OSW materials to improve the suitability for vermicomposting

According to Gunadi *et al.* (2002), a pre-composting step could treat different types of OSW. Frederickson *et al.* (1997) and the authors suggested to keep the pre-composting step to 2-3 weeks to ensure that vermicomposting system operated at maximum efficiency. The goals of pre-composting could be NH_3 removal (Chan & Griffiths, 1988) or moisture reduction (Nair *et al.*, 2006) to become an efficient substrate for vermicomposting.

The pre-composted Bs seemed to be a more suitable feed for earthworms, since composting reduced TAN~70% from 9.1 g kg^{-1} to 2.5 g kg^{-1} (Fig. 4.12A). The pH level of the pre-composted Bs was high (~9.0) after 2 weeks of the pre-composting (Fig. 4.12A). This may be explained by the stable alkaline buffer in effluent of the bio-digestion process. The high pH level is also the reason for a high NH_3 concentration (>10%) in TAN components (Fig. 4.12A). The dry matter content in the pre-composted Bs increased during the pre-treatment because of evaporation of the moisture (Fig. 4.12B). The lower EC value in the Bs after 2 weeks (Fig. 4.12C) may be related to the NH_3 emissions. NH_3 emissions may go along CO_2 evolution in a liquid solid waste (Vandré *et al.*, 1997). As a consequence, the EC reduced. The low temperature of the pre-composted Bw (<30^oC) after 20 days showed that the substrate was satisfactory for vermicomposting because almost all excess heat from the substrate was released (Fig. 4.12).

5.3.1.3. Comparison of the physico-chemical properties of OSW materials

Single Bs substrate: The various sources of biogas effluent is the reason for the variations of each parameter for Bs (Table 5.4). Wide range of the parameters may explain the reported variability of the suitability of the Bs for earthworms in vermicomposting. The high DM content of the initial Bs feed for vermicomposting (30-45%) reported in some studies (Sangwan *et al*., 2008a; Suthar, 2010) might not be suitable for vermicomposting as the optimal range is 15-25% (Edwards, 2007). This higher DM content in the Bs (Garg *et al*., 2006; Sangwan *et al.*, 2008a; Suthar, 2010) might be due to the heating-dried treatment of biogas effluent discharge. Otherwise, the low DM content in Bs (<10%) (Ernst *et al.*, 2008; Jeyabal & Kuppuswamy, 2001) is because the solid parts of the biogas effluent were collected without the drying.

TAN was present in large amounts in single Bs because the biogas effluent was rich in N-containing components from animal slurry after anaerobic digestion. In previous studies, only Ernst *et al.* (2008) reported a high TAN content in single Bs (31.2 g NH_4^+ kg^{-1}). In the other studies, the lack of data on TAN content in single Bs limits the discussion for this parameter (Table 5.4). In this study, TAN content in Bs was low (<0.5 g kg^{-1}) because the TAN was removed by the pre-composting (*see 5.3.1.2*).

Table 5.4: Comparisons of physico-chemical properties and composition of Bs used as a feed for vermicomposting

Parameter	Jeyabal & Kuppuswamy (2001)	Garg *et al*. (2006)	Sangwan *et al.* (2008a)	Ernst *et al.* (2008)	Suthar (2009)	Suthar (2010)	This study (2012)
DM/ %	6.0-8.0	20-40	30.0	8.3	na	30-45	15.0
pH	7.0	8.3	8.1	8.4	7.4	7.4	8.1
EC/ mS cm^{-1}	na	na	na	na	na	na	6.3
C/N ratio	15.0	80.0	29.4	na	16.3	14.6	23.3
oDM/ %	na	na	na	72.1	55.6	55.6	87.6
C_{tot}/ %	27.3	41.6	46.4	na	29.0	29.0	43.8
N_{tot}/ %	1.8	0.5	1.6	6.5	2.0	2.0	1.9
P_{tot}/ %	0.8	0.5	0.6	na	0.3	0.3	0.9
K_{tot}/ %	0.9	0.5	1.7	na	1.3	1.3	0.6
TAN/ g kg^{-1}	na	na	na	31.2	na	na	<0.5

na: data not available

Single Fm substrate: The Fm feed for vermicomposting had a similar DM content and C_{tot} concentration as compared to other studies, but other parameters varied over a large range (Table 5.5). Again, it is quite difficult to make comparisons among these parameters as several data on EC, oDM, TAN and NH_3 concentration were not available in the other studies. TAN content in single Fm (4.8-8.2 g kg^{-1}) was higher than in other studies (1.2-2.4 g NH_4^+ kg^{-1}) (Shalabi, 2006).

Table 5.5: Comparison of physico-chemical properties and composition of the initial single Fm used as feed for vermicomposting

Parameter	Faechem *et al.* (1983)	Shalabi (2006)	*Yadav *et al.* (2010)	This study (2012)
DM/ %	na	13.0-18.0	15-25	12.1-21.1
pH	na	5.0-6.2	5.1-5.5	5.4-7.5
EC/ mS cm^{-1}	na	na	0.5-0.8	2.8-8.5
C/N ratio	na	12.0-23.0	9.5-11.5	15.9-19.0
oDM	na	na	na	92.0-94.3
C_{tot}/ %	44.0-55.0	45.0-48.0	40-43	45.8-54.7
N_{tot}/ %	5.0-7.0	3.4-4.6	3.7-4.5	2.1-2.9
P_{tot}/ %	3.0-5.4	1.8-3.2	0.9-1.3	0.8-1.0
K_{tot}/ %	1.0-2.5	1.6-3.4	2.6-3.0	0.1-0.3
TAN/ g kg^{-1}	na	1.2-2.4	na	4.8-8.2
NH_3/ mg kg^{-1}	na	na	na	35.5-47.9

* Value based on 48 samples
na: data not available

Combined substrates (BsBw, FmBw and BsFmBw): The mixture of the three single OSW materials could produce suitable feeds for vermicomposting because the chemical properties of the mixed feeds were adjusted more closely to the 'suitable levels' for earthworms than those of the single forms, in agreement with several previous studies (*see 2.3.2*). Both single Bs and single Fm were also mixed with other OSW materials (e.g. single Bw) or chemicals for several investigations of vermicomposting (Tables 2.8-9).

Combined BsBw was close to the optimal conditions for vermicomposting according to Edwards (2007) (Table 3.9). Yadav & Garg (2009) reported that mixing was an alternative for pre-treatment when single Bs could not be accepted directly by earthworms. Also, Suthar (2010) mixed single Bs with crop residues and the results showed that the bulky material played an important role in the rapid decomposition of the combined substrates. Suthar (2010) explained that the bulky material could make the feeding substrates soft and more acceptable for earthworms. Changes in its palatability probably promoted microbial colonisation, and most likely the combined substrate provided some readily available nutrients and better physical structure in the feed.

Like the combined BsBw, the two other mixtures of Fm (FmBw and BsFmBw) are suitable for vermicomposting because their characteristics were closer to optimal values (Table 3.9). Yadav *et al.* (2010) successfully mixed single Fm with other bulking materials to improve the acceptability of *E. fetida*. Also, according to Yadav *et al.* (2010), the mixing of fresh Fm with either Fm vermicompost or soil helped to prevent anaerobic conditions in the Fm substrates.

5.3.1.4. Prediction of vermicomposting suitability of OSW materials

According to the regression equation [11] (*see 4.2.3*) and based on the values in pH, EC and TAN of the fresh and pre-treated substrates of Bs, Fm and Bw (Table 3.9), the different survival rates of *E. fetida* were predicted via the worm survival rate (Table 5.6).

Spearman's rank correlation analysis showed no significant correlation between predicted survival rate (hours) and the number of surviving worms in substrate of experimental vermicomposting (%) (rho = 0.66, p=0.16). Although the predicted worms survival rates in pre-composted Bw, BsBw and BwFm were higher than in pre-composted Bs (Table 5.6), the numbers of surviving worms in the three substrates were observerd lower after vermicomposting. Perhaps, the less surviving worms in the three substrates because of increases in pH, EC & TAN of the feeding substrates during 12 weeks of vermicomposting (Fig. 4.16-18) (*see 5.3.2*).

Table 5.6: Expectation of the survival rate (± SD) of *E. fetida* in the fresh and pre-treated substrates of Bs, Fm & Bw and results of the observed surviving worm (± SD) after 12 weeks of vermicomposting

OSW material	pH	EC/ mS cm^{-1}	TAN/ mmol kg^{-1}	*Predicted survival rate/ hours	**Surviving worm/ % ± SD
Fresh Bs	8.8	6.3	642.9	0[a]	na
Fresh Fm	5.9	2.8	25.7	410	na
Fresh Bw	4.4	5.2	19.3	515	na
Pre-composted Fm	7.0	2.9	526.4	0[a]	0.0 ± 0.0
Pre-composted Bs	8.1	5.7	35.7	104	17.0 ± 0.2
Pre-composted Bw	8.0	4.6	26.4	195	2.0 ± 0.5
Combined BsBw	7.8	5.1	26.4	211	8.7 ± 2.1
Combined FmBw	7.4	4.6	25.7	258	10.2 ± 2.4
Combined BsFmBw	7.8	4.5	20.6	287	27.6 ± 11.3

*: The predicted worm survival rate in aqueous solution based on the parameters of substrate and the equation [11]
**: Results of surviving worms were observed in feeding substrate after 12 weeks of experimental vermicomposting
na: data is not available
[a]: The predicted values were negative (<0 hours) but the lowest value is considered zero (0)

5.3.2. Changes in physico-chemical properties during vermicomposting

5.3.2.1. Dry matter

Moisture is an important parameter to guarantee a sustainable vermicomposting process, as reported in many studies (Edwards, 2007; Garg *et al.*, 2006; Sangwan *et al.*, 2008, Suthar, 2009). Very high initial DM content (low moisture) results in early dehydration of the material, that hampers the biological processes, giving physically stable but biologically unstable compost (Shalabi, 2006). On the other hand, low DM content (high moisture) may result in anaerobic conditions due to water logging (Tiquia *et al.*, 1996).

By watering, the DM content in all vermicomposted substrates remained <40%, whereas in the controls without earthworms, DM content increased rapidly up to ~80% (Fig. 4.14). This may be explained by the evaporation of moisture and the leaking of excess water. Many previous studies reported that DM in vermicomposted substrates increased during the process (Domínguez & Edwards, 1997; Muyima *et al.*, 1994; Shalabi, 2006).

A significantly lower DM content in the vermicomposted substrates than in the controls of the same feeding substrate may be due to the presence of earthworms. Under their activity, the humus could retain added water in the vermicomposts, whereas the control substrates with large fragments and poor porous structures could not hold the added water. This effect was also observed in several previous studies (Atiyeh *et al.*, 2000; Shalabi, 2006). For example, Atiyeh *et al.* (2000) observed a clear difference in evaporation rates from feeds with and without worms. They attributed this to the vermicompost having a larger surface to volume ratio than the control, a property that enhances both aeration and evaporation.

5.3.2.2. Organic dry matter

A reduction of oDM content was observed in all vermicomposted substrates and composted controls during the 12 weeks of the treatment (Fig. 4.15). The reduction is due to emissions of CO_2 (Garg *et al.*, 2006; Tripathi & Bhardwai, 2004; Suthar, 2009; Yadav & Garg, 2008). Moreover, Yadav *et al.* (2010) noted that the rate of oDM content reduction depended on the degradable amount of C_{org} present in several OSW materials

The oDM content decrease in the four substrates (Bs, BsBw, FmBw and BsFmBw) was significantly higher as compared to the controls (Figs. 4.15A and 4.15D-F) and could be also explained due to the activities of earthworms. The aerobic microbial respiratory, even without the presence of earthworms could lead to a reduction in oDM content in the controls, but of course it was less than in the vermicomposted substrates with earthworms (Sangwan *et al.*, 2008). This is in line with Shalabi (2006) who explained the higher oDM content through leaching in the vermicomposted substrate. However, no leaching occurred in the treatment of this study.

An exception was the change in oDM content of vermicomposting of single Bw, which resulted in no significant difference in the parameter compared with the control (Fig. 4.15C), perhaps due to the low activity of surviving earthworms (<4%) in the substrate after 12 weeks of the process (Fig. 4.20).

5.3.2.3. pH level

Changes in pH level varied over a large range after the first 2 weeks because it depended on the initial pH of each substrate and the substrate acceptability of earthworms (Fig. 4.16). The pH level of Bs, FmBw and BsFmBw increased slightly (from 8.1 to ~8.3; 7.4 to ~7.7 and 7.7 to ~7.8, respectively), but a rapid increase in pH level of Bw (from 7.7 to ~9.0) and BwBs (from 7.8 to ~8.7) was observed after the first 2 weeks. Bw might still have had a number of anaerobic microorganisms present, which continuously decomposed the acidic organic substances to produce alkaline products (e.g. alcohol compounds, NH_3) after pre-treatment. For this reason, the higher the share of Bw in the four feeding substrates (Bw, BsBw, FmBw and BsFmBw) was, the higher the pH level was after the first 2 weeks of the vermicomposting process (Fig. 4.15D-F).

Many previous studies explained the pH increase by anaerobic decomposition at the beginning of vermicomposting process as well (Dominguez & Edwards, 2004; Edwards, 2007; Garg *et al.*, 2006; Sangwan *et al.*, 2008, Suthar, 2009). Shalabi (2006) attributed the increase in pH level after 2 weeks to the creation of aerobic conditions, leading to the decomposition of the volatile fatty acids in vermicomposted substrate (e.g. Fm).

pH levels in all vermicomposted substrates decreased from week 2 of the treatment, probably because of anaerobic processes in the OSW materials. Different authors attributed this typical pH decrease to the enhanced production of CO_2 and NO_3^-. In hot-rottening composting, the pH usually increases with treatment time. Perhaps, the most evident difference between hot-rottening composting and vermicomposting in

regard to pH is the different level of TAN. In hot-rottening, the TAN is higher and thus the nitrification potential is higher. Nitrification is a source of protons and may be the reason for the pH increase. Yadav & Garg (2009) also concluded that the pH change toward acidic conditions could be attributable to mineralisation of N_{org} and P_{org} into nitrites/nitrates and orthophosphates and bioconversion of the organic material into intermediates of organic acids.

Again, Bw showed a different response with a pH>8.0 at the end of the process. In Bw, the earthworms died in week 2 when the pH was >9.0 This indicates that the worms may play an active role in the pH decrease. Maybe the TAN is oxidized in the earthworm's gut but at higher pH levels, NH_3 is toxic.

5.3.2.4. Electric conductivity

Many previous studies defined a high EC (or salt content) as a negative factor for vermicomposting (Domínguez & Edwards, 2004; Hughes *et al*., 2009; Owjori *et al*., 2009), but did not explain the interaction between EC and the activity of worms. (Table 5.6-7).

The EC increased in all substrates treated by earthworms (Fig. 4.17) most likely because of the release of minerals in available forms (e.g. NO_3^-, PO_4^{3-}, NH_4^+ and K^+) from the organic matter. This is in line with most other vermicomposting studies, even with the different kinds of OSW materials such as sewage sludge (Khwairakpam & Bhargava, 2009), non-recyclable paper waste (Gupta & Garg, 2009) and mixtures of food industrial sludge and cow dung (Yadav & Garg, 2009). However, a few studies reported an EC decrease (Albanell *et al.*,1998; Mitchell, 1997; Nogales *et al*., 2005). Especially if leachate is formed ions may be washed away and may reduce the EC.

The significantly lower EC in the substrates without earthworms (control) may be due to the lower mineralisation rate of the material at any given time in the process (Yadav & Garg, 2009). As a consequence, potential ions were still part of the organic matter.

In the experiments, only in some treatments, mainly controls due to poor porous structures of materials, leachate occurred. The leachate was reused again for moistening the substrates and the EC generating ions were kept in the systems. The movement of earthworms in the vermicomposted substrates might improve the physical structure and the water-holding capacity of the materials (Frederickson *et al*., 2007).

5.3.2.5. Total ammonium nitrogen and ammonia

TAN and NH_3 are two important parameters for the understanding of the vermicomposting process.

Composting releases NH_4^+-N from the organic material by mineralization. Especially, during the first days easily degradable material such as proteins are a source of NH_4^+ (Edwards, 2007; Ernst *et al.*, 2008; Garg *et al.*, 2006). Depending on the pH, the equilibrium between NH_4^+-N and NH_3 with pK_a of 9.25, a certain amount of TAN is in

the form of NH_3. NH_3 is emitted during the early phase of composting. The NH_3 loss is higher in a hot-rottening system as compared to vermicomposting: NH_3 formation is a function of pH and temperature. Additionally, convection in a bigger hot-rottening windrow is high because of a chimney effect: air is heated up in the windrow. In a vermicompost, only moderate temperatures should be reached because of the temperature sensitivity of the worms. NH_4^+-N tends to be trapped in the material. As the NH_3 concentration is also positively related to the overall TAN content, the NH_3 concentration may increase to even higher levels as compared to the NH_3 in hot-rottening substrates.

In single Bs, the TAN content was low (<0.5 g kg^{-1}) after pre-treatment (Fig. 4.18A). It seems that the pre-treatment by composting may result in low TAN content (Gunadi *et al*., 2002).

In contrast, a high TAN content (~5.0 g TAN kg^{-1}) which remained in single Fm during the first 2 weeks of vermicomposting (Fig. 4.18B) might be due to the availability of NH_3 formed by anaerobic decomposition in the previous storage period which were cooler with a lower NH_3 emission than the hot-rottening process. Yadav *et al*. (2010) attributed the increase in pH to NH_4^+-N in single Fm to perhaps being produced from degradation of organic substances at the beginning of vermicomposting. Shalabi (2006) reported that NH_4^+-N in single Fm decreased rapidly during the first 10 days (from 1.2-2.4 g kg^{-1} to <0.2 g kg^{-1}), then remained stable during the rest of the process. The explanation for the sharp decrease was losses via the leachate, as it corresponded to the water content reduction during the same period.

As indicated earlier, not TAN but NH_3 seems to be the factor that highly influences the mortality of earthworms. Unfortunately, no data on NH_3 concentration was reported for the vermicomposted treatment in all previous vermicomposting findings.

A reduction in NH_3 concentration in single Bs during the process was observed (Fig. 4.19A) although TAN content was stable because of the decrease of pH (*see 5.3.2.4*).

NH_3 concentration was still high in single Fm (~40 mg kg^{-1}) after 2 weeks because the pH level and TAN content did not change over time. No further data on NH_3 in the single Fm from week 2 were obtained because all worms died a few hours after introduction (*see 5.3.3*).

The high pH level could also explain the high NH_3 concentration in other feeding substrates, including single Bw at week 12 (Fig. 4.19C), BsBw-control at week 12 (Fig. 4.19D), FmBw at week 2 (Fig. 4.19E) and BsFmBw-poly at week 2 (Fig. 4.19F). Whenever the NH_3 concentration was high (>10 mg kg^{-1}), earthworms were inhibited or died. Therefore, the parameter should be recorded to explain the mortality of earthworms and the suitability of OSW materials for vermicomposting (*see 5.3.3*).

5.3.3. Development of earthworms after vermicomposting

The earthworm population decreased by more than 50% in all vermicomposting during the 12 weeks (Fig. 4.20A-F). The highest mortality was in single Fm (100%), single Bw (~96%), combined BsBw (~90%), combined FmBw (~85%), single Bs

(~80%) and combined BsFmBw (~75%), respectively. The results were in line with this study defining NH_3 as a limiting parameter (*see 5.3.2.5*).

Some authors reported that either a lower DM content (<15%) (Frederickson & Knight, 1988; Shalabi, 2006; Yadav *et al.*, 2010) or a high oDM (>82%) (Frederickson & Knight, 1988; Yadav *et al.*, 2010) might negatively affect the population of worms.

In addition, the lower feeding rate might be the reason for gaining less earthworm biomass. Thus, earthworms might have died or had a limited growth, even in suitable physico-chemical conditions (Suthar, 2010). Gupta & Garg (2009) also concluded that when earthworms were fed below their maintenance level, they lost weight at a rate which depended upon the quantity and nature of ingestible substrates. According to Ndegwa *et al.* (2000), the highest bioconversion of the substrate into earthworm biomass was found at a daily feeding rate of 1.25 kg-feed kg-worm^{-1} day^{-1}, whereas Loh *et al.* (2005) concluded that this rate should be >0.75 kg-feed kg-worm^{-1} day^{-1}. In this study, the daily feeding rate was <0.3 kg-feed kg-worm^{-1} day^{-1} and might be a reason for the worm mortality.

More earthworms were found to survive in combined substrates (BsBw, FmBw & BsFmBw) (Fig. 4.20D-F) than in single substrates (Bw & Fm) because the mixtures were more suitable for earthworms. Suthar (2009) reached the same conclusion when the combined BsBw (in ratio 1:1 and 1:2 by fresh weight) gained a higher worm biomass than the single feeding of Bw. Similarly, a higher worm biomass was found in mixtures of Bs with wheat straw, sugarcane trash (Suthar, 2010) and wastewater sludge (Garg *et al*., 2006) than in the single Bs. Yadav *et al.* (2010) also reported that higher worm biomass in mixtures of fresh Fm and vermicompost than in single Fm.

The highest worm biomass was observed in the combined BsFmBw (Fig. 4.20F) due to the suitability of the initial physico-chemical properties. In the same line, Yadav *et al.* (2010) also reported that when soil and Fm vermicompost was added to fresh Fm in ratios 1:2:1 by weight, a higher amount of earthworm biomass was achieved compared with other mixtures of soil and fresh Fm or fresh Fm and Fm vermicompost (1:1), or soil, fresh Fm and Fm vermicompost (1:1:1). The authors concluded that the ratio of conversion of oDM into earthworm biomass might be an important parameter in earthworm growth.

5.3.4. Influences of polyculture treatment on vermicomposting of OSW materials

The data on physico-chemical parameters of all six feeding substrates during 12 weeks of vermicomposting treatment (*see 4.3.2*) showed that there was no significant difference between monoculture and polyculture treatment at any given time (Fig. 4.14-19). Shalabi (2006) also concluded that different earthworm species (*E. fetida* and *D. veneta*) did not cause any great differences in the physico-chemical properties of vermicomposted Fm in monoculture and polyculture treatment. Loehr *et al.* (1985) concluded as well no differences in the vermicomposting experiment of sludge with *E. fetida* and *E. eugeniae*.

Elvira *et al.* (1996) also concluded that although vermicomposting tests did not

present any advantage of polyculture over monoculture treatment, *E. andrei* had higher growth rates in the mixed cultures, whereas those of either *L. rubellus* or *D. rubida* decreased slightly in comparison with monoculture treatment. The lower biomass gain compared with monoculture treatment was explained by a negative effect of some earthworm species on others, with perhaps a thiamine-destroying factor in the faeces of *E. andrei* and *D. rubida* negatively affecting growth of the earthworms when ingested. Food competition seems to be another factor which can explain the different observed interactions. Furthermore, Loehr *et al.* (1985) measured the biomass increase of earthworms in monoculture and polyculture treatments with *E. fetida, E. eugeniae* and *P. excavatus* and then also concluded that there was no advantage with polyculture treatment.

Suthar (2008) reported similar findings in an experiment that combined an epigeic (*E. fetida*) and anecic (*L. mauritii*) species in terms of cow dung vermicomposting. Anecic earthworms create burrows in the decomposing sub-system, and cement it with mucus and other N-rich body secretions. Therefore, a burrow of earthworms attracts the decomposer community, especially bacteria associated with N mineralisation, due to its mucus-rich wall. The difference between this present study and that cited above may be that both *E. fetida* and *D. veneta* are epigeic species which might have the same decomposer community (the bacteria associated with N-mineralisation).

There was a significantly higher amount of earthworm biomass with polyculture treatment than monoculture treatment of BsFmBw (Fig. 4.20F). The physico-chemical properties of this vermicomposted substrate seemed to be more suitable for the *D. veneta* species than the *E. fetida*. Consequently, *D. veneta* was alive until the end of processing, whereas some *E. fetida* mortality occurred in the BsFmBw medium. However, the higher concentration of NH_3 in BsFmBw-mono (>35 mg kg^{-1}) than in BsFmBw-poly (<18 mg kg^{-1}) at week 2 (Fig. 4.19F) could indicate lower gain of worm biomass in monoculture than polyculture treatment (*see 5.3.3*).

5.3.5. Changes in elements in vermicomposted products

Feeding substrates of the five OSW materials (Bs, Bw, BsBw, FmBw and BsFmBw) were sieved into two fractions, with a size of Φ ≥4 mm, namely the incompletely digested substrate (IDS) and a size of Φ <4 mm, namely vermicompost (VC) after the 12-week vermicomposting. This section discusses the nutrient concentrations of these two products of OSW materials after storage for 6 weeks (IDS18 and VC18) and after 12 weeks (IDS24 and VC24).

Numerous previous vermicomposting studies reached the same conclusion, mentioning a relationship between earthworm activity and their mineralisation rate (Garg *et al*., 2006; Tripathi & Bhardwaj, 20004a; Edwards, 2007).

5.3.5.1. Total carbon

From different amounts at the beginning (33-44%), C_{tot} concentration decreased in all materials after treatment due to the loss of CO_2 as a respiratory product of aerobic microorganism and earthworm activity (Atiyeth *et al.*, 2000; Ernst *et al.*, 2008; Garg *et*

al., 2006; Sangwan *et al.*, 2008; Yadav *et al.*, 2010).

C_{tot} concentration in single Bs and combined BwBs (Fig. 4.21A-B) decreased less during the vermicomposting process, probably because Bs contained large fractions of degradable substances such as lignin compounds, cellulose and polyphenolic compounds, which were digested more slowly than the other components by earthworms and microorganisms. Comparing the reduction in C_{tot} concentration in fermented Bs to that in conventional Bs, Ernst *et al.* (2008) also explained the slow decreasing rate of C_{tot} by the existence of lignin compounds under the same vermicomposting conditions. A high amount of lignin-containing substances in the vermicomposted Bs may also explain the lower acceptability to earthworms in mixtures of Bs and coir pith/weeds, resulting in low multiplication rates during vermicomposting (Jeyabal & Kuppuswamy, 2001). Benitez *et al.* (2002) gave a similar explanation for lignocellulosic waste from the olive oil industry. It was accepted and digested by *E. andrei* only when the vermicomposted substrate was combined with other lignin-less material such as municipal wastes or animal slurry.

C_{tot} concentration in single Bw, combined FmBw and combined BsFmBw (Fig. 4.21A-B) was reduced by ~5-7%, much more than in single Bs and combined BsBw, perhaps caused by Fm and Bw components containing less degradable compounds than Bs. The lower amounts of C_{tot} in Bs-VC, BsBw-VC, and BsFmBw-VC after 24 weeks (the final products) in comparison with other three substrates and times (VC18, IDS18 and IDS24) could be explained by the continuous degradation of Bs components in the OSW materials by aerobic microorganisms instead of earthworms during storage (12 weeks). Furthermore, Yadav *et al.* (2010) reported that the reduction in C_{tot} concentration in vermicompost could go on for longer during storage.

The same amount of C_{tot} was found in all four products (IDS18, VC18, IDS24 and VC24) of single Bw and combined FmBw, which might be due to lower degradation of components in the materials during storage. Moreover, the results showed no significant difference between C_{tot} concentration in VC18 and VC24 for all OSW materials. This suggests that 12 weeks might have the same maturation of the vermicomposts in comparison with 6 weeks.

5.3.5.2. Total nitrogen

The increase in N_{tot} is most likely because of the overall loss of organic material. This mass loss results are higher for C and O from organic materials compared to N. CO_2 is emitted from the materials by respiration of microorganisms and earthworms in the substrate (Atiyeth *et al.*, 2000; Ernst *et al.*, 2008; Garg *et al*., 2006; Sangwan *et al.*, 2008; Yadav *et al.*, 2010). In hot-rottening systems, there is also a significant mass loss by gaseous H_2O. But in vermicomposting, the temperature is rather low and only some mass is lost as gaseous H_2O. It seems that the gaseous N losses via NH_3, N_2O or N_2 are smaller as compared to the C losses (Garg *et al*., 2006; Jeyabal & Kuppuswamy, 2001; Sangwan *et al.*, 2008; Tripathi & Bhardwaj, 2004). Maybe dead earthworms contributed as well to N_{tot} (Atiyeh *et al.*, 2000). According to Rodríguez-Canché *et al.* (2010), N_{tot} is ~10% of body weight (DM) in earthworms and could be a source of N_{tot} in vermicomposted products.

In this study, the N_{tot} concentration via earthworms can be estimated with 1.7-3.4 g N_{tot} kg substrate^{-1} (by DM). This would result in a theoretical N_{tot} increase from 0.2% to 0.4% if original masses were considered. In the case of high NH_3 emission, the N_{tot} concentration may decrease (Bernal *et al*., 1996; Martins & Dewes, 1992; Nogales *et al*., 1999; Yadav *et al*., 2010).

5.3.5.3. Total phosphorus and potassium

The gradual increases in P_{tot} and K_{tot} concentrations in all four vermicomposted products in comparison with those at the beginning (~20-50%) (Fig. 4.23-24) might be explained by a reduction in C_{tot} in form of CO_2 (Khwairakpam & Bhargava, 2009; Ndegwa & Thomson, 2001; Yadav & Garg, 2009; Yadav *et al.*, 2010). Another reason for the increase in P_{tot} and K_{tot} concentration could be P- and K-additions from earthworm mucus and their dead bodies, which contains ~1.0% of dried worm biomass (Chan & Griffiths, 1989; Rodríguez-Canché *et al.*, 2010).

In contrast to the increase in either K_{tot} and P_{tot} concentrations, Sangwan *et al.* (2008) observed a gradual decrease in K_{tot} concentration after vermicomposting of Bs and sludge and attributed it to leaching of K_{exc} and PO_4^{3-} by excess water during treatment. But leaching was not observed in this study.

Significantly higher P_{tot} and K_{tot} concentrations in the VC-products than in the IDS-products could be explained by much higher P_{sol} and K_{exc} in the VC-products (or worm casts) than in the IDS products (incomplete digested substrate).

During storage, no significant change in P_{tot} and K_{tot} concentrations were observed in the four vermicomposted products of all OSW materials either because the oxidation rate of organic materials in the absence of earthworms was slow or because the maturity of these products after 18 weeks was the same as after 24 weeks.

5.3.6. Maturity of vermicomposted products after treatment of OSW materials

5.3.6.1. C/N ratio

Numerous previous vermicomposting studies mention C/N ratio as an important parameter for determining earthworm assimilation capacity (Edwards, 2007; Gupta & Garg, 2009; Tripathi & Bhardwai, 2004). Moreover, the change in C/N ratio as a function of time is considered an important index widely used for the assessment of efficiency of the vermicomposting process and vermicompost maturity (Gupta & Garg, 2009). Also, according to Shalabi (2006), the decrease in C/N ratio reflects the degradation of OSW materials after vermicomposting treatments or the maturation rate of the vermi-/compost.

Five OSW materials (Bs, Bw, BsBw, FmBw and BsFmBw) had initial C/N ratios >20.0 (Fig. 4.25) which was reported as a ‘suitable characteristic’ for digestion by earthworms (Edwards, 2007; Ndegwa & Thompson, 2001). According to Ndegwa & Thompson (2001), the C/N ratio of the initial feeding substrates should be in the range 20-25 to be digested efficiently by *E. fetida*. However, the lower C/N ratio of

single Fm (18.0) at the beginning of vermicomposting was associated with 100% mortality rate of earthworms. This low C/N ratio in the initial Fm was due to the low C_{tot} concentration (36.4%) and the high N_{tot} concentration (2.1%) in its components.

The change in C/N ratio in all five feeding substrates during the treatments reflected the decomposition of these materials. The decrease in C/N ratio of these substrates could be explained by the same factors as the decrease of C_{tot} concentration (*see 5.3.5.1*) and the increase of N_{tot} concentration (*see 5.3.5.2*). This is in agreement with many previous studies on different OSW materials (Atiyeh *et al.*, 2000; Garg *et al*. 2006; Gupta & Garg, 2009; Sangwan *et al*., 2008).

The faster degradation rate after the initial 2 weeks of the vermicomposting process in all OSW materials could be explained by strong oxidation of the fresh substrates by the digestion of earthworms and microorganisms (Gupta & Garg, 2009).

The gradual decrease in C/N ratio of all five vermicomposted products and controls during the storage period from week 12 to week 24 could again be explained by the role of earthworms. In the absence of earthworms, the oxidation rate of organic matter was slower than in vermicomposting phase. However, a slow rate of oxidation continued in the maturing products at low C/N level (<15).

The C/N ratio of the vermicomposted substrates decreased more quickly than that of the controls at any given time because the OSW materials were digested simultaneously by both earthworms and aerobic microorganisms, whereas only the microorganisms acted in the controls. According to Gupta & Garg (2009), the change in C/N ratio is due to the loss of C as CO_2 through microbial respiration and a higher proportion of N_{tot} concentration in the vermicomposted products. Those authors also found a faster decrease in C/N ratio of the feeding substrates after vermicomposting treatment compared with that in substrates without earthworms (control) due to the residual effects of earthworms in the process.

The N added to earthworm biomass, mucus, nitrogenous excretory substances, growth-stimulating hormones and enzymes may be another reason for the lower C/N ratios in vermicomposted substrates compared with controls at any given time (Suthar, 2010; Tripathi & Bhardwaj, 2004b).

According to Gupta & Garg (2009), a decline in C/N ratio to <20 indicates an advanced degree of organic matter stabilisation and reflects a satisfactory degree of maturity of organic wastes. This low C/N ratio is needed because plants cannot assimilate N from the soil unless the C/N ratio of vermicompost in soil is ~20 or less (Edwards, 2007; Suthar, 2010; Tajbakhsh *et al.*, 2008).

Actually, five vermicomposted products (Bs, Bw, BsBw, FmBw and BsFmBw) achieved C/N ratio of <12. Some previous studies reported that C/N ratio of fresh OSW materials (e.g. rich N-containing substrates), which sometimes <20, cannot be used as an absolute indicator of maturity, as the C/N ratio of their vermicomposts should be significantly lower than 20 (Senesi, 1989; Tripathi & Bhardwaj, 2004). Shalabi (2006) also reported that Fm-vermicompost could not be used for improving soil fertility unless its C/N ratio was 10-12.

The C/N ratio of the five products decreased by over 60% in comparison with the

initial values, indicating the efficiency of the vermicomposting treatments and the role of earthworms in the rapid decomposition and mineralisation of the OSW materials.

5.3.6.2. Respiration activity and maturity of vermicomposts

Respiration activity for four days (AT_4) is another parameter to test maturity. Shalabi (2006) reported that the respiration index is a direct and reliable indicator of the stability of Fm-vermicomposts.

Generally, vermicomposted products (VC24) of all five OSW materials had a 50% lower AT_4 as compared to the composted products (controls) (Fig. 4.26). This indicated that the vermicomposting treatment of the OSW materials gave significantly more stable products ($P<0.05$). In studies examining the stability of cow manure vermicompost using the respiration index based on CO_2 production, less CO_2 was produced by vermicomposted substrates than the control (Atiyeh *et al.*, 2000). This was explained by earthworms, and the microbial activity they promoted, destroying most of the easily biodegradable substances rapidly (Shalabi, 2006). Additionally, Atiyeh *et al.* (2000) reported that the respiration index of vermicomposted substrate was sometimes higher than in the control due to the decay of dead earthworm biomass.

The respiration activity of all vermicomposts of the five OSW materials met the EU- and ASTM-threshold for the maturity of compost, but were higher than the Austrian and German-threshold.

5.3.7. Reduction in pathogens and sanitation requirement for vermicomposted products

Pathogens were detected in the fresh forms, with high concentrations of micro-organisms in all materials (Fm, Bw and Bs) (Table 4.2). Many studies agree that a large number of pathogens are present in OSW materials, especially in animal and human excreta (Aria & Domiguez, 2008; Edwards, 2007; Tripathi & Bhardwaj; 2004). Consequently, vermicomposts produced from these pathogen laden organic materials have to meet the requirements for hygiene safety. According to the WHO-guidelines for the safe use of wastewater, excreta and grey water, the pathogenic bacteria numbers must have a significant reduction (>2.0-3.0 $\log_{10}$ units) after sanitation treatment prior to application in agriculture (WHO, 2004b).

The pathogen reduction was the same or higher in vermicompost treatments as compared to the controls indicating that the earthworms seem to accelerate pathogen reduction in composts including *Salmonella* spp. (Table 4.3) *Enterococcus* spp. (Fig. 4.27), *E. coli* (Fig. 4.28), and somatic coliphages. This explanation seems to be in line with previous findings that earthworms may digest bacteria by the proteolytic activity of the enzymes present throughout their gut (Edwards, 2007), which would allow earthworms to assimilate amino acids and other compounds from the bacteria (Monroy *et al*., 2008; Monroy *et al*., 2009; Vaz-Moreira *et al*., 2008)).

This opinion was supported by Khwairakpam & Bhargava (2009), who also

concluded that reduction in pathogenic bacteria was because of elimination of the bacteria as they entered the food chain of the earthworm. A reduction of 85-98% in the numbers of total coliforms in earthworm excreta after one-week vermicomposting of pig slurry was attributed to the pathogens immediately affecting the composition of the micro-faunal community in vermicomposted substrates (Monroy *et al.,* 2008).

The removal of *Salmonella* spp. after only 2 weeks of pre-treatment of all six OSW materials (Table 4.3) was due to the heat produced from the thermophilic composting process. This concurs with Vaz-Moreira *et al.* (2008), who reported that pathogens in composts were reduced compared with those in several fresh OSW materials (e.g. domestic wastes, sewage sludge, poultry litter and municipal waste) after thermophilic composting treatment.

Salmonella spp. and somatic coliphages were not found again in all three products after vermicomposting of all five OSW materials (VC24-mono, VC24-poly and control24) (Table 4.3). Thus, this parameter could ensure that bacteria do not exist in vermicomposts to be applied in agriculture. According to Rodríguez-Canché *et al.* (2010), *Salmonella* spp. in sludge were reduced from >2400 to <3 MPN g^{-1} after 60 days of vermicomposting treatment with *E. fetida*. This met the Mexican Official Standard NOM-004-SEMANAT-2002 for agricultural use. However, the level of microbiological organisms in the control sludge was also low enough (<3 MPN g^{-1}) for the Mexican requirements due in part to the relatively high temperatures reached in the pre-composting period.

There was no difference of pathogen removal between mono- and poly-culture treatments. Khwairakpam & Bhargava (2008) also reported similar reductions of faecal coliforms and faecal streptococci between monoculture treatment (*E. fetida*) and polyculture treatment (*E. fetida* and *E. eugeniae*; *E. fetida* and *P. excavates*; *E. fetida*, *P. excavates* and *E. eugeniae*) of sewage sludge after 6.5 weeks.

In contradiction, Monroy *et al.* (2008) noted different reductions in bacteria numbers in vermicomposting treatments as affected by different worm species, *E. fetida* and *E. andrei* as well as *E. eugeniae* and *L. rubellus*. According to the authors, these differences might be due to the morphological and physiological characteristics of the digestive system of these earthworms. The oesophagus is longer in the Lumbricidae (*E. fetida*, *E. andrei* and *L. rubellus*) than in other families (Edwards & Bohlen, 1996). However, the gut typhlosole and the gut enzymatic array also vary with the species (Edwards & Fletcher, 1988), and the gut transit time is also species-specific (Monroy *et al.*, 2008), which could lead to the different reductions between monoculture and polyculture treatment.

5.4. GENERAL DISCUSSION

While some parts of the above approach were not fully covered in previous studies as well as in this study due to various limitations, a general overview of the complete vermicomposting process is given in the following sections.

In all practical experiments, the earthworm population decreased. For a stable process, earthworms have to replicate in the substrates. Otherwise a steady input of

fresh earthworm biomass is required. Hatlchings were found in most of the treated substrates but the treatment duration was too short to analyse the dynamic of the earthworm population. The performed experiments focused on the suitability of the material.

NH_3 toxicity seems to be the overall limiting factor for vermicomposting. This is a big challenge for vermicomposting as most of the organic wastes contain nitrogen in the form of N_{org} or NH_4^+. During decomposition of organic matter, a pH increase may result in toxic NH_3 concentrations. Suggested pre-treatments like pre-composting in a hot-rottening process are efficient but result in NH_3 emissions. For hot-rottening composting with N losses of up to 70% of TAN content, a short hot-rottening phase followed by vermicomposting will emit less NH_3 as compared to the hot-rottening phase. In this case the NH_3 emissions may be accepted as a necessary step to treat organic waste. However, for animal manure it is not clear if a pre-treatment followed by vermicomposting induces less NH_3 emissions as compared to a liquid slurry store.

6. CONCLUSIONS

Vermicomposting is a method used to treat OSW which produces a mature compost. But it has become evident that vermicomposting is very sensitive to NH_3 in the substrate. NH_3 inhibits earthworm growth and is toxic for worms. Basically all OSW contains TAN. Depending on the pH, NH_3 may negatively affect the worms. For a stable process it is necessary to either keep the pH below 7 (e.g. in bio-waste) or it is necessary to pre-treat the waste. This is especially true for effluent from biogas plants with high TAN contents and a pH >7.5. For these substrates, a pre-treatment is required, as was highlighted in this dissertation. Substrates are either pre-composted and stored with an open access to the atmosphere. Under such conditions, NH_3 is emitted. But such emissions are negative for the environment as NH_3 emissions may lead to NH_4^+ or NO_3^- losing resulting in acidification and eutrophication of ecosystems.

NH_3 can be captured by using stripping methodologies. But this makes the rather simple technique of vermicomposting more complicated.

Single- and multi-factor experiments to test the effects of one and several toxicity factors on earthworm and the regression equation [11] produced here are useful tools to identify the suitability of OSW materials for vermicomposting.

The electric conductivity (EC) of OSW materials does not appear to be a toxic factor for *Eisenia fetida*. The earthworms seems to be rather tolerant in a solution which has EC of up to 14 mS cm^{-1}. Furthermore, pH levels in the range 5.0-8.0 have no negative effect on *E. fetida* when TAN is not available. However, in the presence of TAN, there is a dramatic decrease in the survival rate of *E. fetida* with increasing TAN content as well as pH level. The pH- and TAN-parameters of feeding materials should be cited together to achieve an accurate prediction of OSW materials suitability for vermicomposting.

There was no significant difference between the physico-chemical properties of the feeding substrates including single forms (Bs, Fm and Bw) and combined forms (BsBw, BsFm and BsFmBw) in monoculture treatment which contained only *E. fetida* and polyculture treatment which contained a mixture of *E. fetida* and *D. veneta*.

Single Fm was not suitable for vermicomposting with either *E. fetida* or *D. veneta* because of high levels of TAN (~40 mg kg^{-1}) and organic dry matter (oDM, >94%).

Mixing of the three OSW materials (Bs, Fm and Bw) in proportions 1:1:1 by fresh weight might be an option to increase the gain of earthworm biomass. The highest amount was obtained from polyculture vermicomposting of combined BsFmBw as compared to the other five substrates.

Five OSW materials (Bs, Bw, BsBw, FmBw and BsFmBw) seem to be converted to mature vermicomposts with low C/N ratio (<10) and low oxygen requirements for

respiration after the 24-week vermicomposting treatment. The respiration index for four days (AT_4) of these five substrates met the EU-threshold for compost maturity.

Sanitation of OSW materials in terms of pathogens can be achieved by vermicomposting treatment. The vermicomposts of five OSW materials did not contain *Salmonell*a spp. while the numbers of *E. coli* and *Enterococcus* spp. were reduced strongly after the treatment. The numbers of the two bacteria in the five vermicomposts were also lower than in the controls confirming the efficiency of the biological treatment and the role of earthworms. Moreover, the pathogen numbers in the vermicomposts of the five OSW materials were below the WHO-threshold for sanitation for use in agriculture.

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8. PUBLICATIONS

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