Tampereen ammattikorkeakoulu

Development of Rapid Biodegradability Assays for Paper and Board Products

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THESIS
October 2021

Bioproduct and Process Engineering

TIIVISTELMÄ

Tampereen ammattikorkeakoulu
Biotuote- ja prosessitekniikka

HELEN, ERNO:
Development of Rapid Biodegradability Assays for Paper and Board Products

Opinnäytetyö 43 sivua, joista liitteitä 10 sivua

Lokakuu 2021

Opinnäytetyö tehtiin Delfort Specialty Papersin tuotekehitysosaston toimeksiannosta. Työn tavoitteeksi asetettiin päivittäiseen käyttöön sopivan, nopean biohajoavuustestin kehittäminen. Testiä tarvitaan, koska standardinmukaiset biohajoavuustestit ovat noin puolen vuoden mittaisia ja Delfortilla haluttiin pystyä valikoimaan ennalta lupaavimmat näytteet standarditesteihin lähetettäväksi. Testiä tullaan käyttämään Delfortin tuotekehitysyhteisössä useissa yrityksen toimipisteissä ympäri maailman, joten työn kieleksi valittiin englanti.

Tehtävänantoon vastattiin kehittämällä kaksi testiä. Delfortin Tervakosken tehtaalla toimiva R&D-tiimi toivoi “boksia” – laboratoriokompostoria, jossa biohajoamista voidaan testata samankaltaisissa olosuhteissa kuin standarditesteissä. Toiveisiin vastattiin kehittämällä kompostointitesti, joka pyrkii asettamaan testatut näytteet biohajoavuuden sujuvuuden mukaiseen järjestykseen kuukauden kestävän testauksen myötä. Lisäksi kehitettiin sellulaasientsyymitesti, joka pyrkii tekemään saman kahdessa tunnissa.

Opinnäytetyössä tutustuttiin kattavasti kompostointia sekä biohajoavuutta käsittelevään kirjallisuuteen ja tutkimustietoon. Hankitun tiedon perusteella laboratoriokompostoriin pyrittiin kehittämään lignoselluloosalle tehokas kompostisubstraatti. Työn myötä jouduttiin toteamaan myös käytännön vaatimukset – sienirihmastojen tai erikoisten kiinalaisten kiviainesten hankkiminen osoittautui käytännön kannalta kestämättömäksi.

Korona-aika hankaloitti projektin läpivientiä merkittävästi. Käytännön tutkimusta kehitetyillä menetelmillä oli tarkoitus tehdä Delfort Tervakosken laboratoriossa, mutta tehtaan johdon asettamien koronarajoitusten vuoksi suunnitelma peruuntui. Kehitettyjä menetelmiä ei ehditty testata lainkaan ennen opinnäytetyön julkaisua.

Ilmeisiä jatkotutkimuksen kohteita jäi runsaasti: laboratoriokompostorin haihduttaman kosteusmäärän mittaaminen ja kastelukäytännön ohjeistaminen mittausten perusteella, kompostorin näytetaskujen tarkoituksenmukaisuuden toteaminen käytännössä, entsyymitestissä käytettävän entsyymiliuosmäärän optimointi kokeilemalla sekä entsyymitestin nopean näytteistyksen kannattavuuden arviointi olivat ennalta suunniteltuja testauskohteita, jotka jäivät koronarajoitusten vuoksi tekemättä. Samasta syystä opinnäytetyön raportti muuttui luonteeltaan kirjallisuuskatsauksen omaiseksi; teoriaa käsitellään runsaasti, koska omaa tutkimusta ei saatu mukaan.

Asiasanat: biohajoavuus, disintegraatio, selluloosa, paperi, kartonki

ABSTRACT

Tampereen ammattikorkeakoulu
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Thesis of 43 pages, including 10 pages of appendices

Lokakuu 2021

This thesis work was commissioned by the Research and Development team at Delfort Specialty Papers' Tervakoski factory. The aim was to develop a rapid biodegradability test suited for daily laboratory use. Such a test is necessary because standardised biodegradability tests take up to six months to run and the team wanted to be able to benchmark for the most promising product candidates to apply certifications for. The work is presented in English, because the test is to be deployed in several of the company's locations around the world.

The commission was met with two testing methods. The R&D team requested a “box” – a laboratory composter that duplicates the conditions of standard tests. Hence, two composting units were built and a method for the use of these was developed. The composter test benchmarks six samples for readiness to biodegrade in one month's time. In addition, a test method utilising cellulase enzymes was developed, aiming to do the same in two hours.
Literature and research papers on composting and biodegradability were broadly studied in the research phase of the work. The learnings were applied in developing a compost substrate that performs well at lignocellulose degradation. During the work, practicalities also came into play – procuring fungal rhizome cultures and exotic mineral conglomerates proved unpractical, so compromises had to be made.

The prevailing Corona pandemic hindered the work significantly. The new methods were going to be tested by the author at the laboratory at Delfort Tervakoski, but unfortunately factory management ended up declaring coronavirus-related preventative measures that barred outside personnel from the factory premises. Thus, the methods developed saw no real-world testing before the publishing of this thesis.

Many needs for further research remain: measuring the evaporation of moisture from the laboratory composter to draw up a watering regimen, evaluation of the workability of sample envelopes, optimisation of the volume of enzyme solution in the enzyme test and evaluation of the usefulness of rapid sampling in the enzyme test were all planned, but ended up cancelled due to Corona preventative

measures. This report ended up concentrating on the theoretical basis of the subject because of lack of bespoke experimental data.

Keywords: biodegradability, disintegration, cellulose, paper, cardboard

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1 INTRODUCTION

An over-arching theme in the zeitgeist of the current generation of people inhabiting the globalised section of planet Earth is environmentalism. The author would willingly argue it to be the dominant trend-setting force already today, and knowing the roadmaps for the future, such as the United Nation's 2030 Sustainable Development Goals, it is clear that the dominance of environmentalism will only grow in the coming decades.

As a producer of bulk consumer products, Delfort Specialty Papers are riding the cutting edge in the necessity to adapt to current and future challenges posed by regulation in the march to sustainability. This thesis work was commissioned to find new methods to approximate the performance of paper products in standardised biodegradability tests. Such tests and certifications of conformity are the day-to-day manifestation of environmentalism s increasing effect on production.

The need for this work grew from Delfort finding out that some product formulations unexpectedly failed to disintegrate in standardised tests. Such failures are expensive, since applying for certification is not only expensive, but also time-consuming: a full suite of biodegradation tests can take up to six months to run.

A key requirement for the solutions to be developed in this thesis work was speed. The author was advised to think in terms of rapid benchmarking rather than trying to replicate standard tests. Two methods were developed in response; both the Enzymatic Test and the Composting Test, described in their respective chapters, are designed to benchmark up to six different product formulations against each other, producing results in one month in the case of the Composting Test and in a matter of hours in the case of the Enzymatic Test. The practical application of these methods would be to test a series of different furnishes of a single product deemed to be applied for certification and finding ones that clearly outpace the rest in biodegradation terms.

1.1 On environmentalism

The resurgence of environmentalism is a fascinating modern phenomenon that deserves a moment of consideration. A timeline for modern environmentalism is often drawn out starting from the 1962 publication of Rachel Carson's book Silent Spring. The book raised a timely and very public concern about the widespread use of DDT, juxtaposing the perceived technological progress of mankind – often cited in the era as "better living through chemistry" after the chemical company DuPont s advertising slogan (Urban Dictionary 2021) – with the painfully evident abscence of regard to consequences in the ecosystem. The short-sightedness and the heavy stakes at play allowed for plenty of momentum for the environmentalist movement, and a fertile soil for even lofty ideas such as James Lovelock's famous Gaia theory to flourish.

Lovelock's and Lynn Margulis' NASA-initiated exploration of atmospheric gases as a "beacon of life" was first published in 1974. By 1979, the subject had ballooned to a wholesome concept of the Earth as a living superorganism, codified in the book Gaia: A New Look at Life on Earth and redefining the canon usually reserved to be the domain of the great naturalists such as Darwin. (Radford 2019). A little over a decade had been enough time for environmentalist thought to win such acceptance that Lovelock's poetic, if not downright animistic or even transcendental framing of "all is one" was readily welcomed and endorsed by mainstream academia.

1980s saw the Green Movement grow from a scientific and popular concern and grassroots activism into an established political worldview. An important milestone was the work of Gro Haarlem Brundtland's commission, inaugurated already in December 1983 on UN Secretary General's urgent request. The commission's final report, published in 1987, spoke in now-familiar terms of Gaian interconnectedness and the need to harness the Green Movement's momentum when stating the goals of the commission:

[…] recommend ways concern for the environment may be translated into greater co-operation among developing countries and between countries at different stages of economical and social development and lead to the achievement of common and mutually supportive objectives that take account of the interrelationships

between people, resources, environment, and development. (United Nations 1987, 5.)

The decades that have passed since environmentalism has been lifted to serve as mankind's figurehead have changed the world in corresponding ways. In the year 2021, we have come to a point where it is necessary to test products in development to make sure they decompose to a standard. It is timely to accept that the hippies won, adapt and overcome!

2 BIODEGRADATION PRINCIPLES

This chapter aims to help Delfort's R & D community better understand the subject of biodegradation in a wholesome way. In discussions with Delfort Tervakoski R & D staff, it was mentioned multiple times that there is not a great deal of knowledge about this subject matter in-house. This chapter will draw the difference between biodegradation in nature and compost, focus on the dynamics involved in the process of composting and consider the compostability challenges of actual paper products.

The microbiology of biodegradation in nature is a wide subject due to the multitude of different climates and conditions it takes place in and also due to biodegradation – in essence recycling of resources – being a key process of ecology. Biodegradation happens everywhere, because it needs to. Nobody needs to doubt its function, for if biodegradation didn't work, life on Earth would have been suffocated by a layer of tree leaves! The biosphere is keen to recycle everything thrown at it, wetting with the rain, tearing with the wind, irradiating with the sun and throwing the pieces down to the microbes. Given long enough time, everything will eventually be decomposed.

From the vantage point of product certifications, the mechanical aspect described above as a part of the grand scheme of biodegradation is not considered at all. Therefore, there is little sense in giving it further thought, although one might linger on the thought that a concept of "engineered wear limit" for a paper product would be a useful design parameter that could give products an edge in certification testing. Standardised testing methods for aerobic and anaerobic biodegradability in soil and municipal waste treatment settings are very similar across all standardisation bodies: products are tested for disintegration via composting over a shorter time period of 12 weeks, complete biodegradation over four to six months by quantitatively measuring the formation of carbon gases CO_2 and CH_4 from sample and control, and finally testing germination of plant seed in the compost substrate resulting from the second stage against a control (SFS 2001, 11; ASTM 2018, 4). In addition, presence of heavy metals and other contaminants are analysed, and the pass thresholds for this is where any regional differences can be observed.

Anaerobic processes are outside the scope of this work. We will now take a deeper look at aerobic, soil-driven biodegradation, often referred to as composting.

2.1 Composting

The age-old practice of composting is a way to harness the natural biodegradation processes, optimise them and monitor their development. It is also a way to call forth the entire parade of soil life to show itself – a most handy way of studying the microbiological foundations of biodegradation.

2.1.1 Natural composting progression

It took some effort to find a piece of literature that would aptly define composting. A brilliantly concise definition is to be found in Steven Solomon's 1993 book Organic Gardener's Composting, where he defines composting as "enhancing the consumption of crude organic matter by a complex ecology of biological decomposition organisms." This is an apt description, because even though biodegradation does happen everywhere, in a compost the activity is enhanced by bringing a great amount of biodegradable materials together. Having the word "complex" in the definition is also justifiable. The colloquial concept of the law of the jungle is an apt mindset to lean on when looking at the phases of compost activity.

A freshly built compost pile is a very unnatural state of affairs, and nature responds to it in an explosive manner. The pile contains a lot of simple sugars and proteins in its constituents, providing ample food for mold fungi and bacteria that start colonising the compost. The first phase is called the Mesophilic Phase, referring to the reign of mesophilic bacteria that thrive in ambient temperatures. The initial decomposers begin multiplying rapidly, and as generations of microbes pass each other, the accumulating remains of dead microbes become fodder for more microbes. (Diaz 2007, 32.)

As microbial life accumulates unchecked in the fresh compost, the heat produced by microbes also accumulates and begins to build up in the compost. Among the

microbes, there are thermophilic species adapted to higher temperatures in the range of 35 - 65 °C. These species thrive on the feedback loop and soon outcompete the mesophilic species completely, beginning the Thermophilic Phase of composting. (Diaz 2007, 32.) It is worth noting that also in fungi there are thermophilic species, growing most abundantly in temperatures of 35 - 55 °C.

The Thermophilic Phase then continues until the compost finally runs out of easily digested material. The end of the Thermophilic Phase is marked by a decrease in temperature in reaction to lessening microbial activity. As temperature returns to ambient levels, the mesophilic species re-colonise the compost, beginning the Second Mesophilic Phase. This time, they find a different landscape: the simple carbohydrates in the compost pile have been eaten. Natural selection will then side with the microbes that can produce enzymes for the degradation of complex carbohydrates that make up starch, cellulose, chitin and such materials. (Diaz 2007, 34.)

The humble compost pile is very relevant as a subject of scientific inquiry in the 2020s. In the days of first tentative steps towards putting humans on Mars, it is good to know that since only less than one percent of the species of life found in compost piles can be presently grown in cultures for study (Diaz 2007, 34), there is an abundance of undiscovered species doing incredible feats and begging for scrutiny right in the back yard!

2.1.2 Industrial composting

Moving from the humble compost pile to industrial composting arrangements is a big step, and a surprisingly recent one. While evidence of large-scale centralised composting has been found all along the history of agriculture-practicing human settlements (Diaz 2007, 7-8), the first attempts at systematically applying techniques to improve the composting process are products of the modern era. In the Indore region of colonial India, Sir Albert Howard and his team developed and improved the Indore Process starting in 1933 (Diaz 2007, 8). The process laid down guidelines for the size of the pits used for composting, the practice of stacking different feedstocks and a regimen for turning the compost – originally

only twice within a composting run of six months, which would have meant the process to be mostly anaerobic.

Another early effort was the Beccari process, invented by it's namesake in Italy in 1920. This process already incorporated aeration via air vents built into the purpose-made airtight cells. (Diaz 2007, 10.) The Beccari process was developed primarily for reducing odor nuisance from composting, and by design only worked aerobically for the second part of the two-part processing. The performance of the aeration was likely not very great.

Aerobic composting does away with the formation of methane, which could be a fire hazard, and greatly increases effectiveness. The classic method of aerating a compost pile was mechanical agitation, i.e. turning of the compost in backyard composting setting, or using rather massive augers, screws or other agitators in an industrial setting. Modern industrial composting practices have since the 1970s (Diaz 2007, 41) applied active aeration as a key part of the process. Active aeration refers to pumping air into the compost pile. The design goal in an active aeration arrangement is to ensure sufficient gas exchange in the entire compost pile to remove the produced CO_2 and replenish O_2 without mechanical agitation.

Active aeration can be designed to flow two ways: air can be pumped either into the compost pile, or out of the bottom of the compost pile. The latter arrangement is particularily interesting, as the exhaust can be filtered to deal with odors (Diaz 2007, 71) and even used to produce energy with heat exchangers (Brown 2014, 41). At the most sophisticated level, aeration can be utilised to counter the heat of the compost ultimately rising too high for thermophilic bacteria and so extending the maximal effectiveness of the Thermophilic Phase of composting at will.

The preference for thermophilic species is simply a question of efficiency: high temperatures are caused by high amount of active microbes per unit of volume. Preferring thermophilic species is therefore akin to preferring production chicken hybrids that are taller and slimmer – more will fit in a given area, giving more production. With that, we have now arrived at the status quo of modern industrial

composting; automation-driven, actively aerated processes that are tuned to favour thermophilic microbes and are possibly using heat capture to utilise biologically generated heat energy.

2.2 Biodegradation of lignocellulose in nature

As stated previously, composting is not strictly a natural phenomenon, but rather a technique engineered by man. In the natural order, a pile-up of organic matter is rare; autumnal fallen leaves in a deciduous forest is the closest the natural order regularly comes to composting. The multi-phase progression and the dynamics implied in composting are therefore not readily applicable to nature; rather, the prevailing conditions are steady and similar to the Second Mesophilic Phase and Maturation Phase of composting.

During literature review for this thesis work, the author was surprised to learn that science has thus far not accurately described the mechanism that decomposes the lignin component of wood. The main biological actor identified to be present when lignin decomposition takes place are the white rot producing fungi belonging to the species Basidiomycetes, but not a single enzyme has been identified that is capable of degrading lignin (Käärik 1983, 45). It is likely that lignin decomposition is ultimately a co-operative effort by multiple microbial species. This acts to underscore the complexity of natural systems.

Biodegradation of the cellulose component is much more straightforward and the biological activity is well understood. While only a limited selection of fungi can attack the hardy cell walls of wood cells with cellulases, once breached there are an abundance of bacteria and mold fungi that break down the cell contents and surrounding hemicellulose components with amylase and xylanase enzymes (Diaz 2007, 36-37, 39-40). Brown rot producing Basidiomycetes fungi are mentioned as being particularily effective at attacking cell walls (Käärik 1983, 45). An ecologically revolutionary discovery of the 1970s is also at play in the process: decaying wood hosts nitrogen-fixing bacteria to such an extent that the French experimenter Jean Pain found his wood chip piles to produce compost that rivalled commercial nitrogen fertiliser despite almost no nitrogen being present in the

source material (Brown 2014, 27; Käärik 1983, 44). This discovery double-underscores the importance of wind-felled branches and whole trees to the forest ecosystem's nitrogen infeed.

2.3 Special considerations regarding biodegradation of paper and board in composting

Products of the paper and board industry, although largely consisting of wood fibre and oftentimes lignin, are much more complex in their total furnish. Biodegradability testing standards commonly state that products made from materials of natural origin – probably referring to something like a straw hat – are exempt from testing, since they are expected to decompose. Paper and board products are nevertheless expressly included in biodegradability testing.

In the base papers and board plies, inorganic fillers are routinely added, and the likely coating layers sandwiching the wooden core of the paper will contain inorganic pigments at upwards from 80 % of the coating's mass (Paltakari 2009, 60). Inorganics are not biodegradable. The test procedures outlined in this work are taking this into account by using the ash content of samples as a correction in calculations. However, standardised tests do not contain this compensation in, for example, a requirement of a certain percentage of sample mass to have been lost in dry weight evaluation or as basis of the calculation to find how much CO_2 should be produced to pass the sample. This means that by definition, any paper or board product incorporating a high amount of inorganic fillers is going to have a harder time to pass biodegradability tests.

The coating process, also known as surface sizing process, is designed to evenly and completely coat the surfaces of the paper web. The result is in all practical senses a laminate product, where the outer ply presents something resembling an unpassable plastered wall to the soil life that encounters it. The plaster will yield over time to moisture and mechanical stress, but it is easy to see some plausibility to the hypothesis that the standard pigment coating may cause an initial delay to the biodegradation process. If that would be the case, it would cause a direct hit to test results in standardised tests that set a time limit for disintegration.

Finally, as previously explained, the degradation of lignin is observed to require complicated interactions between several species of fungi, and the "smoking gun" of the process still escapes science (Käärik 1983, 45). It is likely that a biodegradability test arrangement does not present a varied enough ecology of species to allow for degradation of lignin. Therefore lignin-rich product formulations can be expected to perform less well in tests, albeit having the signature ecological brown texture.

3 THE ENZYMATIC TEST

3.1 The case for the enzymatic test

In the natural order, cellulose is broken down by cellulase enzymes excreted by fungi. The enzymatic biodegradability test's rationale is to "cut to the chase" of enzymatic activity against the sample by immersing the sample in a standardised enzyme solution and monitor the accumulation of breakdown products in the water over time. The author's hypothesis is that the structure of the paper, particularily the coating layer, acts as a barrier to enzyme activity and the effect can be observed as a delayed onset of the inevitable rise in concentration, as well as the rate of change in concentration normalised to the actual amount of cellulosic material present in the sample.

An analogous case for testing already exists in enzyme science: the Dinitrosalicylic Acid (DNAS) Method (Miller 1959) is used to measure enzyme activity, i.e. the success of a particular enzyme over another (Taipalus 2021). This test could be applied to address Delfort's needs, yielding a very precise and repeatable measurement of the progress of decay. However, assaying with a tight temporal resolution, as would be necessary to secure data on the inital delay and the change over time, would be very laborous with the DNAS method as it is commonly performed. The DNAS method is presented here as a potential future development of the enzymatic test, but for practicality and efficiency, it was decided to rather use the feedback from an electric conductivity (EC) meter to monitor the progress.

Rapid assaying was one of the development goals for Delfort's test. The enzyme test is promising to be very fast and reliably repeatable. Using the EC meter makes it possible to non-invasively take readings in real time, yielding enough temporal resolution to measure the onset delay.

3.2 Development of the enzymatic test

The author is a keen proponent of the Lean ideology. The foremost goal in the development of the test was to apply this ideology to produce a method that is first and foremost effortlessly usable. The procedure was iterated several times to remove unnecessary steps and opportunities for operator error were eliminated as thoroughly as possible.

Work on the enzymatic test began by contacting AB Enzymes, a leading producer of industrial enzymes. After discussions with Mr. Pasi Taipalus, a Senior Application Engineer at AB Enzymes, the author secured samples of two enzyme products deemed useful for the application. The samples were substantial enough in quantity to serve for many years of active testing, and mr. Taipalus gave a tentative promise that if the test was adopted for use across Delfort's worldwide operations, the enzyme products could be procured by Delfort in suitable amounts, i.e. in amounts less than a whole IBC container at a time. Mr. Taipalus also tipped the author on the DNAS test. (Taipalus 2021.)

The products to be used for the testing are designed for use in the pulping stage of the papermaking process. Their preferred windows for pH and temperature, presented in table 1, reflect this.

TABLE 1. Preferred conditions for Ecopulp products (AB Enzymes 2020).

Product	Temperature	pH
Ecopulp R	40 - 65 °C	4,5 - 7,5
Ecopulp L900	30 - 60 °C	4,0 - 6,0

In the spirit of keeping the test maximally feasible to use, it was decided not to follow these recommendations. Arranging the elevated temperature to the multiple separate sample cells with heated sand would require a lot of heated sand; arranging a closed space where the ambient air could be heated would similarly add complexity and take up a great deal of laboratory space, which would have been unmanageable at the laboratory at the Tervakoski mill. Room temperature on the other hand is constant and ubiquitous. It was therefore ruled that the test will be performed in room temperature. The need to lower the pH of the enzyme solution was to be tested during initial tests. However, due to the pandemic situation prevailing during this work, the opportunity of using the laboratory at Tervakoski was barred from the author, so the planned initial testing was not performed.

The initial plan of action with regards to measuring enzyme activity on the sample was to use the industry standard Dinitrosalicylic Acid test. This test uses a colorimetric reagent that produces a colour of varying intensity corresponding to the amount of reducing sugars present in the sample. With a colorimeter, the intensity is then quantified according to a standard curve. (Miller 1959, 426.) Practicalities once again intervened; a special quality of the enzymatic test would be that it allows for a tight temporal sampling resolution, possibly quantifying the delay from the beginning of sampling to the time when enzyme activity is seen to ramp up and thus giving a measurement of how much a given coating is impeding on the activity. The mental image of tens of cuvettes being filled in rapid succession per every sample, complicated calculations of results as the sample water volume is reduced with each sampling and ultimately ending up with a colorimeter run of possibly over a hundred cuvettes quickly rendered this avenue unpassable, chiefly for humanitarian reasons.

A less selective means of measuring dissolved sugars and other matter would be to measure electric conductivity. Since selectivity is lost, a control sample would be needed, being similar to the active sample, but without the enzyme. Two reasonably priced, handheld, low concentration EC meters were procured and the final procedure for taking measurements was drawn up, again putting emphasis on usability.

A pair of sample cells are to be set side by side for each product to be tested, with the active, enzyme-containing cell on the left and the control cell with just UHP water on the right. The cells have lids on to minimise evaporation and possible atmospheric contamination from CO_2, breath, sneezes or dust. Both cells should contain a similar piece of the product. The EC meters are attached to a holder that can be operated with one hand. At sampling intervals, the meters are dipped into each pair of sample cells in quick succession and EC readings are taken into the provided spreadsheet with the free hand. In the spreadsheet, the results are taken into adjacent columns that are oriented similarly to the meters, i.e. active on the left and control on the right. It is expected that the paper samples in both cells will dissolve constituents such as coating pigments into the water, but on the enzyme side, the activity of the enzyme will produce a more rapid rise in EC. By subtracting the figures from the control side from the active side figures,

a result for enzyme activity is produced. These results are plotted on a graph to visualise differences in enzymatic breakdown of cellulose between the samples.

3.3 Experimentation

Due to the coronavirus pandemic prevailing at the time of this work, the author was not able to use the laboratory at the Tervakoski mill. Some experimentation was nevertheless conducted at the author's home. Initially, to justify the purchase of a low concentration EC meter, the author tried to conduct measurements of electrical conductivity increase in the presence of a sample and the enzyme with a standard multimeter. This proved futile; the meter was unable to get a reading from an enzyme solution prepared with UHP water. Adding salt did increase the conductivity to measurable levels, but the lack of temperature compensation caused a constant drift that once again hampered experimentation. As a last resort, the author constructed electrodes with a large surface area placed close to each other, but this did not help the situation.

After the aquisition of proper EC meters was agreed upon and completed, the author tested measuring again and this time usable results were recorded, exhibited in table 2. The series shows three measurements: the enzyme (active) results and control results are as explained above, and represent the data used to calculate test results. A third series is from a cell without a lid, testing whether atmospheric contamination is going to be an issue. This series was not recorded for the full length of the test, since the figure didn t seem to be increasing. It should be noted that the meter used gives measurements in tens of µS / m. The sample tested was of regular office paper.

The results are promising; the active sample is increasing in EC quicker than the control sample, and the series with no paper is showing that atmospheric contamination is not contributing towards the EC increase in the previous two.

TABLE 2. Results from first test of the enzyme method.

Time [min]	Enzyme [µS / m]	Control [µS / m]	Enzyme, no paper [µS / m]
no sample	10	0	10
2 min	20	10	10
4 min	20	20	10
6 min	30	20	10
8 min	40	20	10
10 min	40	20	10
12 min	40	30	
14 min	50	30	
16 min	50	30	
18 min	50	30	
20 min	50	40	

3.4 Operation of the Composting Test

Detailed instructions for the operation of the enzymatic test were prepared as a manual for laboratory employees. This manual is included as Appendix 1 of this thesis. Figure 1 presents an overview of operation.

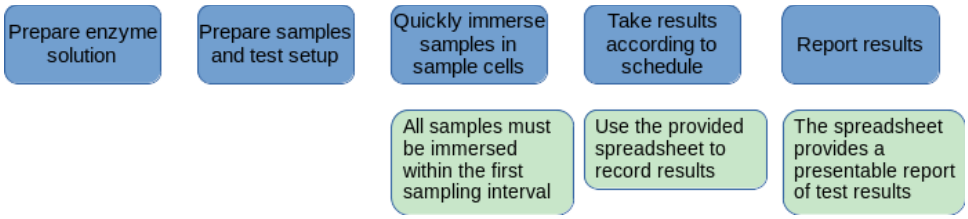

FIGURE 1. Overview of the operation of the Enzymatic Test.

4 THE COMPOSTING TEST

4.1 The case for the Composting Test

Standardised compostability tests all work by actually composting the samples. Hence, it makes sense to benchmark for real-world test performance using the same method as is used in the real world. Testing for composting by composting makes sense, but takes an impractically long time. A composting-based approach to testing was preferred by Delfort Tervakoski R&D, so a composting based test was developed.

Delfort's laboratory composting test attempts to make composting quick and results are taken once a week, so already in two weeks, the test will have produced useful comparative data. It is possible that weekly sampling will prove too frequent in practice to produce measurable differences; if this is the case, the test is designed so that sampling interval can be increased without any changes to the reporting spreadsheet.

4.2 Development of the Composting Test

4.2.1 Practicalities and design goals

The author is a keen proponent of the Lean ideology. The foremost goal in the development of the test was to apply this ideology to produce a method that is effortlessly usable. The procedure was iterated several times to remove unnecessary steps and opportunities for operator error were eliminated as thoroughly as possible.

The necessity for this work stems from standard compostability tests taking a long time to run. For product development, this alone makes outsourcing the tests less than optimal. The long time spans also make compostability tests very expensive to source from commercial laboratories. The primary goal for the test developed here is the ability to provide results on the rate of biodegradation as quickly as possible.

It is very difficult to put nature in a box. The complex progression of soil life in a compost cycle incorporates innumerable species of bacteria, fungi and bigger

living organisms, as explained in Chapter 2. The real design challenge of the compost test is finding a compost mix that facilitates the breakdown of all papers and cardboard – not at all a homogenic spectrum of products, also covered in Chapter 2. It is likely that lignin-heavy furnishes will suffer a performance hit in the laboratory composter, but then again, the case will be similar in an actual composting test.

In industrial composting, the use of active aeration is a given. Apart from upholding aerobic conditions in the process, it is used as a process control, modulating the aeration intensity to manipulate the heat output of the compost. This is necessary, because industrial composting attempts to upkeep the conditions favoured by fast-acting thermophilic microbes. The laboratory composter will implement forced aeration to promote faster composting, but in this case upholding thermophilic conditions is not the goal, so there will be no feedback loop and the throughput will not need to be high.

The efficiency of a force-aerated composter also depends on the ability of the design to distribute oxygen evenly to the whole of the compost mass. To this end, the laboratory composter discussed here was initially planned to have a silo-like taper towards the bottom, where the air enters. This was ultimately abandoned in favour of having a larger volume of compost substrate in the cells to maximise the total amount of moisture in the cell and therefore allowing for more time between waterings. Oxygen distribution is enhanced in the final design by a water lock on the exhaust side, causing a slight overpressure in the cell which helps to saturate the entire contents with fresh air.

The composting test should be usable for both finished products and lab sheets. The latter mode can be used to test different raw materials and recipe changes, which was a goal set at the initial talks about this project. Because of this, the standard rectangular laboratory sheet former size of 165 x 165 mm was used as the basis for the sample dimensions for the composting test. At 8 x 8 cm, the laboratory sheet yields four pieces, which is enough to fill all four time slots in the test.

The required area of the composting vessel is dictated by multiples of the sample size. The volume of the composting vessel was kept such that mixing of the compost can be performed in a regular ten liter bucket. This design goal goes towards usability of the test: preparing the compost takes little room and can be performed even if laboratory space is at premium. The apparatus should be easy to transport and store, and ideally should be stackable for storage. Draw hoods in particular are premium laboratory space, so the design should ideally allow the test to run in any available space.

An ideal moisture level in a compost is said to be between 45 to 65 % (Brown 2014, 43). Water is a prerequisite of life even down to the microbial kind. A regimen of rehydration should be established to keep the compost at passable moisture level; this is particularily acutely necessary because aeration will be constantly pumping moisture out. This watering will become a routine effort for the laboratory staff, so it should also be designed with usability first.

4.2.2 Physical construction

For the containment of the compost cell, a regular home storage box from a reputable and dependable supplier was chosen. This solution is both cost effective and high quality: the boxes are manufactured in Sweden by either injection or pressure molding techniques, having no seams and hence being guaranteed airtight. The specifications of the plastic have been provided, being a food grade stock that withstands heat up to +120 C. Boxes even come with a tight-fitting lid that has locking latches. Using food grade plastic and aquarium grade adhesive makes for a guaranteed chemically inert containment for the compost. Ample selection of box sizes are available. The size was chosen so that the surface area of compost is enough to hold three by four samples of 8 x 8 cm side by side with some clearance to each other.

Aeration is produced with a standard aquarium pump that transfers roughly 60 L of ambient air per hour into the compost cells via an oblong air stone at the bottom of the silo. The pump deployed is a two-sided membrane pump with two pressure outlets that are fed at the opposing phases of the membrane stroke. This makes for an excellent equalisation between the two cells in the amount of flow they

receive. The pump is also very quiet when the mounting makes use of the pump s provided vibration dampener feet. The pump has got adjustable flow, which for the composter can be turned all the way down, reducing the excursion of the membrane and thus extending its service life to the maximum. The manufacturer of the pump recommends membrane replacement every 12 months of constant duty at minimum excursion.

At the end of the aeration cycle, purge air from both cells is collected into a single water lock constructed out of 12 / 16 mm flexible hose. The hose is transparent to ease filling with water. The water lock serves two functions: the back pressure it builds before allowing pressure to be released builds similar overpressure conditions in the compost cells, further helping aeration, and the escaping air is passed through a water filter that will capture some of the possible microbial exhaust leaving the compost. The water lock should not be relied upon for thorough filtering, so it is advisable to always run the composters in a draw hood or cabinet.

PICTURE 1. Two laboratory composters.

PICTURE 2. The water lock.

Three different products (or two products and a reference) can be loaded into a single compost cell. There are four replicate samples, cut into 8 x 8 cm squares, of each three products. Samples are laid into the composter forming a matrix as per figure 1. When setting up the experiment, the operator places four strips of 600 µm plastic mesh material across the compost cell. The samples are then laid onto the strips, and another similar mesh strip is laid on the top of the samples. The top strip is a good place to label the samples; if nothing else, it s a good idea to mark the strips with the projected date they are to be removed and weighed. Finally the strips are lightly covered with compost.

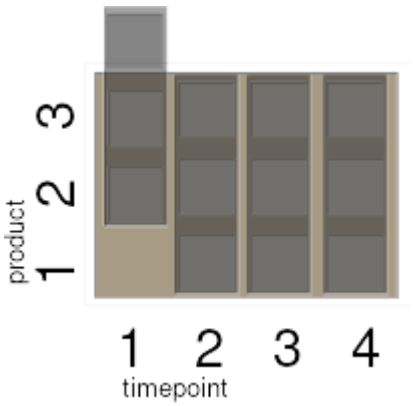

FIGURE 1. Sample arrangement in a composter cell.

4.2.3 Compost substrate design

Designing the compost makeup is where the compost test gets its advantage. However, there are considerations beside performance that come to play, and this makes for a design challenge. The compost constituents must be easy to aquire, perpetually available in controlled quality for a reasonable price. It is also important that building a new batch of compost can be done in a systematic way that promotes continuity of the results over compost batches, and more importantly still, the procedure must be effortless for the staff. While considering these practicalities firsthand, the compost should also be optimised for speedy biodegradation.

Chapter 2 exhibits the biological succession of the compost microecology in more detail. The stage where wood fibre most effectively decomposes is the late stage of composting, the Maturation Phase. This phase is characterised by mild conditions and the relative calm of there being no easily available food and none of the microbes depending on it. In these conditions, fungi and bacteria are allowed to do their hard work of breaking the more difficult carbon chains of nature, such as those of starches, cellulose and lignin, which are found in paper and board products.

In this sense, the job at hand is thankful: late stage living compost is available as a component of many (albeit more premium) commercial planting soil mixes. However, the infeed for the production of this compost has likely been lightweight, leafy and green material – likely, because in the scheme of commercial compost production, it would slow down turnover to have chunky, woody material in the

process. If the infeed doesn't contain chunky wood, the compost won't favour cultivation of the kind of soil life that biodegradation of paper would prefer. The author did contact a manufacturer to ask about their infeed constituents, but did not receive a final answer. The discussion did lend credibility to the manufacturer's continued monitoring of quality, so from an engineering standpoint their product makes for an ideal basis for the laboratory compost. The specifications are available, which makes it possible to develop a fine-grained control of moisture into the compost batch building procedure. The product is easy to aquire – a 40 liter bag conveniently fills the two laboratory composters – and the price is very modest.

To make sure the compost also hosts the fungi that attack wood fibre and possibly even lignin, the fungi should be introduced into the compost, along with a helping of their preferred substrate. The initial idea was to find out about possibilities of aquiring particular, wood-decaying species of fungi from a supplier. The species (Stereum hirsutum and Armillaria mellea) are discussed in more length in chapter 2. However, supply practicalities ultimately made fungal supplementation unpractical, and due to limited time there was no motivation at this time to explore this option further with other suppliers.

During research into compost supplementation options, the author discovered a recent study by a Chinese-Indian research team that found remarkable benefits to lignocellulose degradation with the application of medical stone, a natural mineral conglomerate that could be described as young rock (Wang et al. 771, 2017). Medical stone contains a number of biologically available micronutrients and it's porous structure offers a welcoming habitat for microbial life. There was an attempt to secure medical stone from two Chinese suppliers, but supply practicalities once again rendered this supplementation option unpractical.

In the end, it was decided to supplement the compost with biochar in an attempt to glean some of the claimed efficacy of medical stone. Like medical stone, biochar is a porous material that promotes microbial activity by increasing available surface, and also similarly to the inorganic structure of the stone, biochar is biologically inavailable carbon, so in itself it will be an inert structural component in the compost ecology. To mimic medical stone's function as a

source of micronutrients, the biochar is soaked with commercially available hydroponics micronutrient fertiliser.

4.2.4 Adjusting to account for inorganic material in papers

As has been previously discussed, the spectrum of paper grades is anything but homogenous in furnish. In the composting test, measurements are by weight, and hence it is necessary to adjust the recorded weights to account for the amount of non-composting material within the sample's furnish. If this was not done, a product with less coating would have an innate advantage against a more heavily coated sample, for instance.

Ash content is a standard measurement in the paper industry, given as a percentage of a paper's grammage that can be considered to be non-combustible matter that remains after a paper sample is burned. Conveniently, it can often be acquired from on-line measurements performed automatically at the paper machine, so it can be presumed that a number is already available for the samples to be tested.

The ash content of the sample will be taken to pass for the amount of non-composting inorganics contained within the dry mass of the sample. If a representative ash content figure is not available for the sample, it should be obtained following the standard test procedure defined in ISO 1762 / TAPPI T 211. This procedure defines 525 °C as the combustion temperature and aims to preserve the inorganics that would be broken down at higher temperatures.
The ash content figure is entered into the supplied spreadsheet for reporting results of the test, and will be automatically taken into account in calculations. The mechanism is to calculate the given ash percentage from the first weighing of samples, and subtract the result from all remaining weighing results.

4.3 Operation of the Composting Test

Detailed instructions for the operation of the composting test were prepared as a manual for laboratory employees. This manual is included as Appendix 1 of this thesis. Figure 3 presents an overview of the operation procedure.

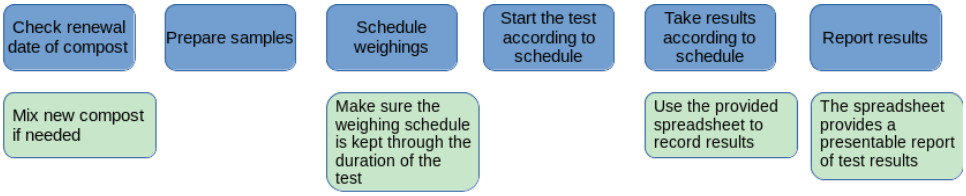

FIGURE 3. Overview of the operation of the Composting Test.

5 CONCLUSIONS

This work was conducted in the midst of the Covid-19 epidemic. An unfortunate consequence of the epidemic was that the author was not able to conduct research work at Delfort Tervakoski s laboratory as planned. This came to be in a somewhat dramatic manner; the author came to the factory to collect keys to have free access to a designated office and the laboratory, but in less than an hour, the management decided to escalate Covid measures, barring access to the premises from outsiders entirely.

The author had prepared to do testing on product samples that had already been tested by other laboratories to establish whether the result could have been anticipated by the newly developed tests. The Covid lockout made such work impossible. Hence, this thesis work had to be completed without any experimental data to show for the newly developed tests. Some aspects of the tests also remain untested – most importantly, the degree of evaporation from the laboratory composters was not tested, and the watering regimen specified in the instruction manual is pure guesswork. The laboratory staff were instructed to perform testing when the test is first run.

During the work, the author made the point about inorganic coatings being suspect to hindering biodegradation. This was received as a novel, important idea. The Enzymatic Test was then designed to yield temporally tight measurements that may be able to identify samples where the coating acts as a barrier to enzymatic action on the cellulose of the sample. The tight sampling interval required by this does make the test more laborous for the staff, however. Further research should be conducted to see if the tight sampling regimen yields useful data, or whether it would be more efficient to sample less frequently.

In general, the author finds the concept of biodegradability testing questionable to some speculative degree. The performance of a particular product will depend greatly on the suitability of the compost substrate to that particular product's furnish. One can speculate that while most compost substrates are likely to contain microbes that readily process bioplastics, it would be difficult to formulate a substrate that contains necessary fungi to breach the walls of wood cells and

process lignin. From this, it can be assumed that any paper product containing mechanical pulp is going to have a hard time passing biodegradability tests, even though on the forest floor, a piece of corrugated board would surely disintegrate equally quickly to a biodegradable plastic material. Similarly, one could speculate that a laboratory offering certification testing could set itself a competitive advantage by offering clients testing with a tuned substrate that will yield favourable results for the client s product.

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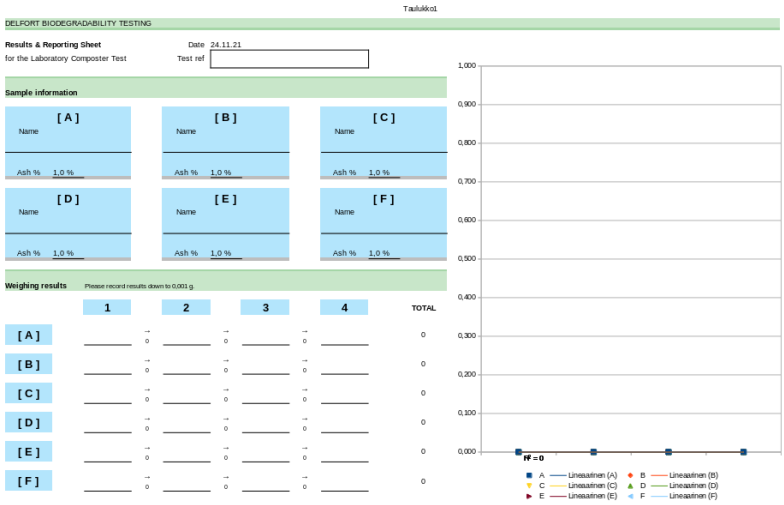
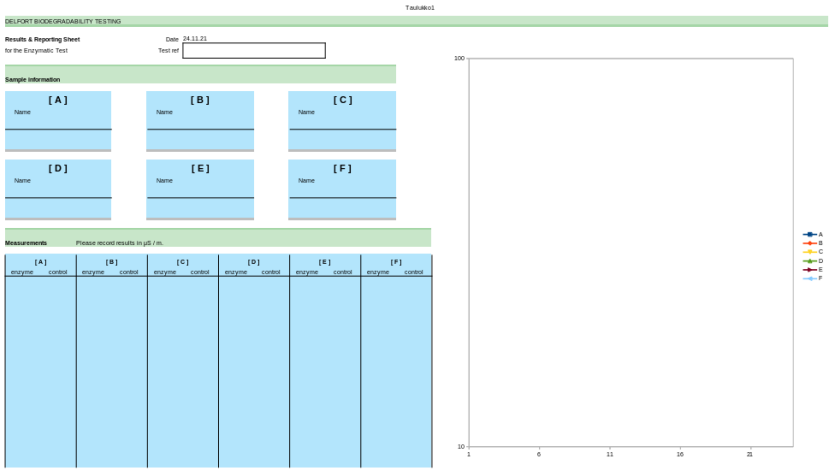
APPENDICES

Appendix 1. Screenshots of reporting spreadsheets



Results reporting sheet for the Composter Test



Results reporting sheet for the Enzymatic Test

Appendix 2. Instructions for the use of Delfort Biodegradability Tests as provided to the laboratory staff

Contents

1 ENZYMATIC BIODEGRADABILITY TEST

1.1 Introduction

The Enzymatic Biodegradability Test was developed to rapidly assess biodegradability performance of paper products. The test procedure involves putting a precisely measured sample piece of paper into a cellulase enzyme solution and repeatedly measuring electric conductivity at set intervals according to the procedure. The enzymatic test was developed to serve as a quick and easy method that doesn't require sample conditioning or the use of special equipment.

The cellulase enzyme causes the cellulose of the paper to begin breaking down into smaller carbohydrate molecules, which dissolve in the water, increasing the conductivity of the water. To take into account the natural dissolution of paper in water, a second sample is immersed in water without the enzyme present. From successive measurements, the rate of change is deduced.

The test can be run for any length of time; the activity of the cellulase enzyme remains constant. Two hours is a recommended period.

1.2 Health and Safety Considerations

Enzymes are proteins that can cause acute and violent allergic reactions in people. All standard laboratory safety precautions must be observed when working with enzymes. Skin contact in particular should be avoided. The test should be conducted in a draw cabinet.

Enzyme products in liquid suspension form are used in this test. They are easier to handle than dry products due to not dusting. The likely vectors of exposure to these enzymes is if an aerosol should be formed by pressurised air, or should droplets of the liquid be flung at the operator from, for example, accidentally dropping a pipette. The operator is advised to be wary of these dangers and work carefully.

Of particular importance is to remember that even in the solution prepared according to this instruction, the enzyme is present in high concentration.

The MSDS of AB Enzymes Ecopulp product is included as an appendix to this document.

1.3 Preparations

The necessary equipment for setting up the enzymatic test are shown in table 1.

Sample cells	two per sample
Plastic mesh	two per sample
Bottle	1 l
Pipette	for 0,5 ml
EC meters	two, for low concentrations
Timer	
ECOPULP enzyme	0,5 ml / 10 samples
Measuring glasses	two, for 0,1 l volume

Table 1. Equipment and materials necessary for the enzymatic test

1.3.1 Preparing the enzyme solution

The enzyme solution is prepared one liter at a time. One liter of enzyme solution is enough to perform 10 test runs. It is not advised to prepare the solution in other sizes, because routine preparation is a key point in the test being repeatable and stable over time.

- Measure one liter of UHP water with a measure that provides reasonable accuracy and good repeatability, such as a measuring cylinder. Pass the water into a clean bottle with a cap.
- Quantitatively and repeatably draw 0,5 ml of enzyme suspension from the stock bottle and add to the liter bottle. Cap the bottle and shake to mix. Mark the bottle.

The enzyme solution is now ready. Before using the solution, remember to let it acclimatise to the experiment temperature, since electric conductivity is affected by temperature.

1.3.2 Preparing the test

The enzyme test can be performed on up to ten samples simultaneously using one liter of enzyme solution as prepared earlier. For every sample to be tested, two test cells are needed. A test cell is a common plastic food container that comes with a cover that has an access hole for the EC probe and plastic mesh to contain the sample. Cells must be covered with lids to reduce evaporation. The plastic

mesh makes for easier unloading of the wet sample after the test has run its course, particularily if the paper sample tends to disintegrate when wet.

For each sample, fill one sample cell with the enzyme solution and another with UHP water. Use separate measuring glasses for enzyme solution and water.

Set up the test in a draw cabinet. For each sample to be tested, place two sample cells next to each other. Mark one as containing the enzyme solution and the other as containing water. Rinse the probes on the EC meters with UHP water. Label the EC meters or take other precautions to make sure cross-contamination from using a meter across the enzyme and water cells does not take place.

Finally, ensure that you can commit to monitoring the test intensively for the duration of the test.

1.4 Test Procedure

The test procedure requires constant attention. Do not begin the test if you cannot commit to tending to the test for the prescribed duration.

If two EC meters are not available, the enzyme and water control series must be sampled in separate test runs to prevent cross-contamination.

Have your samples at hand placed in an orderly fashion so that you can quickly place the correct samples to their designated cells. Remove lids.

Start the timer.

Working quickly, place samples into cells using tweezers. Be careful not to cause cross-contamination. Using tweezers in both hands, one hand for enzyme samples and one for the controls in water, is recommended for efficiency. Put lids on the cells when finished.

When the timer reaches your chosen sampling interval (five minutes is recommended), systematically begin taking readings using the EC meters. Make sure not to cause cross-contamination by accidentally putting the meter designated for the enzyme samples into the water-containing control cells. Record readings into your notes or directly into the spreadsheet.

Continue taking a round of readings at your chosen sampling interval for the duration of the test.

1.5 Results

A spreadsheet is provided for the interpretation and reporting of results of up to six samples at once. Results should be entered in µS / m as reported by the EC meter. After recording all results into the spreadsheet, a graph is produced, showing the development of the difference in electric conductivity

between the enzyme sample and the control sample over time. The greater the difference at the end of a test run, the more readily the sample can be observed to have been acted upon by the cellulase enzyme. Graphing the results over time also makes it possible to observe any possible delay before cellulase activity ramps up, hinting at the structure of the paper presenting hindrances to cellulase activity on the wood fibre.

2 AEROBIC LABORATORY COMPOSTOR TEST

2.1 Introduction

The laboratory composting test was developed to rapidly assess biodegradability performance of paper products. The test arrangement is similar to standardised tests for compostability in that a biological substrate such as soil is present, bringing with it biodegrading soil life. However, this test obtains results solely by weighing the samples. CO_2 respiration is not monitored. The test borrows forced aeration from the toolbox of industrial composting to speed up running the test, and the soil mix is enriched with biochar loaded with micronutrient solution to promote microbial activity.

The operation of the test does not infer a great burden of labour, but there are several key points to master in both the operation of the test and the interpretation of results. Personnel working the test are recommended to study and understand this documentation. In particular, preparing the soil mix is a key point when setting up the test.

2.2 Health and Safety Considerations

Always wear gloves when handling soil or working with the samples during the test. Soil might contain microbes that cause serious health problems.

The laboratory composter should be operated in a draw cabinet or hood. Exhaust air from the composter may contain pathogenic microbes.

2.3 Preparations

2.3.1 Sample preparation

Obtain ash content figures for the papers being tested. Ash content figure in percentage of dry weight is used to approximate the amount of inorganic and hence non-composting constituents in the paper. Ash content should be determined by combustion at 525 °C according to ISO 1762 or TAPPI T 211

standard: make sure not to use ash content figures obtained from 900 °C combustion according to ISO 2144 or TAPPI T 413.

The test requires four sample pieces cut into 8 cm squares for each product to be tested. The pieces should be allowed to acclimatise to standard conditions for four hours as per ISO 187. The pieces should then be weighed individually. If the mass of one piece is found to be significantly out of line with the other three, a new sample should be obtained. The initial weights do not need to be recorded.

2.3.2 Composter preparation

Volumetric mixing of compost components, initial watering, filling the composter, topping up water lock.

A composter cell takes 5,5 liters of compost mix. Mixing is performed one cell at a time to ensure the constituents are present in similar proportions in every cell. The necessary equipment are listed in table 2.

Bucket	10 liters
Stick / Spatula	for cleaning bucket
Pitcher	1 l with 1 dl markers
Measuring glass	1 l or 500 ml
Pasteur pipette	for 0,5 ml
Soil mix	5 l / cell
Wood pellets	0,5 l / cell
Biochar	0,5 l / cell
Micronutrient mix	0,5 ml / cell

Table 2. Equipment and materials necessary for preparing compost mix

In the mixing procedure, the pitcher is used for solid ingredients and the measuring glass for liquids. It is not necessary to use purified water for the procedure. Using a bucket with a lid makes it possible to mix the contents by tumbling and shaking if operator strength and diligence permits; an alternative approach is to use a stick or spatula to stir the mixture.

- Start by measuring a volume of 0,6 l of biochar into the pitcher. Pour the biochar into the bucket.
- Using the pipette, draw 0,5 ml of micronutrient solution and put into the measuring glass. Flush

the pipette with water and put the flushing also into the measuring glass.
- Fill the measuring glass to 0,2 l with water and pour into bucket.
- Allow liquid to seep into biochar before continuing. When no more water will seep, add a volume of 0,6 l of wood pellet into the bucket using the pitcher.
- Using the pitcher, add a volume of 6 l of soil mix into the bucket. Tumble or use a stick to mix after adding every pitcher.
- Using the measuring glass, add 0,4 l of water to the bucket. Tumble or mix thoroughly. This will bring the moisture content of the mix to the preferred level of 65 %.
- Once the mix is thoroughly mixed, pour the mix into the composter cell. With gloves in hand, even out the surface completely flat and pat gently to compress. Use the spatula to completely empty the contents of the bucket.
- Close the lid to retain moisture. Mark a date on the lid ten weeks ahead of the day the compost was prepared. This is the 'best before date' for the compost cell.

The compost cell is now ready. Once prepared, a compost cell can be maintained for up to ten weeks if watering schedule is adhered to.

2.3.3 Watering the compost

The laboratory compost is a habitat of soil life and hence dependent on water. The aeration promotes removal of moisture from the composter, so it is very important to periodically water the running composters.

Watering should be performed twice per week at regular intervals. The first watering of the composters should be done two days after setup. On every watering, simply pour 0,2 l of water in through the watering port on the lid.

2.4 Test Procedure

2.4.1 Starting a test run

Choose the day of the week and time of day for weekly sampling and tending to the composter. You will need to commit to tending to the test once per week at this same time. You should strive to be somewhat punctual, down to within one hour, so it's good practice to find a time that is guaranteed free week to week.

The starting procedure explained here must be done two days prior to your chosen time.

Check from the markings on compost cells that the compost is not due for a re-mixing. The projected end of your test run must not overshoot the ‘best before’ date marked on the lid by more than a week.

Strips of plastic mesh are provided with the laboratory composter. These are used to contain the samples in soil and allow for easy removal of samples during weekly taking of results.

- After acclimatisation of samples to standard atmosphere as per ISO 187 for four hours, the samples are laid onto four strips of plastic mesh in the manner as demonstrated in image 1.

Image 1. How to insert samples into sample strips

- Another strip of plastic mesh goes on the top. If you need to make markings on the samples, the top strip is a good place to attach labels.
- Remove a small amount of soil from the composter cell. Place the four strips holding three product samples each into the composter as shown in image 2.

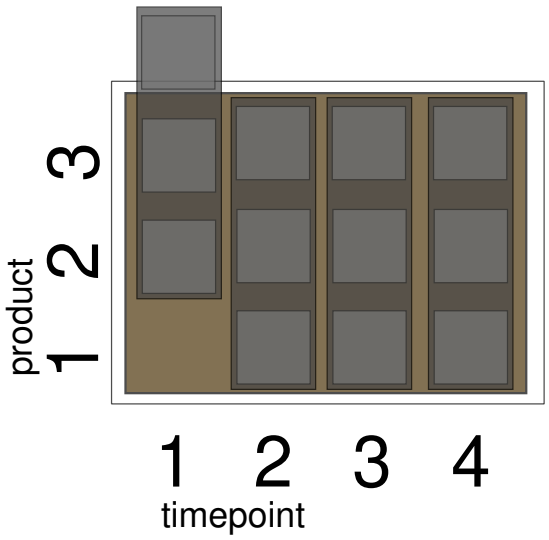


Image 2. Layout of compost cell

- Finally, cover the strips with a layer of soil using the small amount removed in the previous step. Close the cell cover and set up the other cell in a similar manner. Top up the water lock up to the mark with tap water and make sure the cell covers are latched shut so that exhaust air makes air bubbles in the water lock.

The test is now underway. Return to take the first results in two days, at your chosen time.

2.4.2 Taking results weekly

It is crucial to adhere to the set schedule for results taking.

The samples will be oven-dried and weighed. This is an operation where repeatability and precision are paramount. The results of weighings should be recorded with accuracy of 1 mg.

- The four plastic strips that enclose the samples are separate, because they are to be removed for analysis one after another in successive weeks. Double-check that you are removing the correct strip.
- Remember to wear gloves when handling the strips. Remove the strip for the week from the compost cell. It is of no consequence if some soil mix follows with it. Close the lid of the compost cell.
- Be careful not to mix the multiple strips that you may remove at the same time. Once a strip is opened, do not touch the samples with your hands, even with the gloves on; instead, use tweezers.
- Dry the samples according to ISO 638. Record the dry weight obtained as the weekly result for the sample.

This procedure must not experience delays, or the week-to-week integrity of measurements may be disrupted. After recording the weighing results, the samples can be discarded.

2.5 Reading and Reporting Results

The provided spreadsheet produces output suitable for weekly reporting. A rough estimate of the inorganic, non-composting fraction in the sample is calculated from the first recorded result after two days of composting. This fraction is subtracted from the following results. A single graph is produced, showing the observed loss of mass normalised to the first measurement. Thus, in the graph all samples begin at the same point, and differences are easy to see.

The composting test can be adapted to longer-term assays simply by sampling at longer intervals. The spreadsheet is agnostic to the sampling interval.