Biorenewables Development Centre

Plants • Processes • Products

Report developed for:

Plate2Plate

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Analysis and microbial assessment of liquor derived from IVC treatment of food waste

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Executive Summary

Plate 2 Plate Compost is a Leeds based company which makes compost from domestic food waste. Plate 2 Plate Compost uses multiple in-vessel-composting (IVC) systems to convert the food waste into valuable, nutritious compost material. As a by-product of this process, several thousand litres of liquor are produced annually from their IVCs, at a rate of around 40 L every two days. Plate 2 Plate is interested in investigating if this waste liquor could be used and sold as liquid fertiliser.

The BDC was commissioned to analyse the liquor and to investigate techniques to reduce microbial growth in the liquid so it can be bottled safely. Several techniques were trialled, including filtration, autoclaving and pasteurisation. It was found that autoclaving was the most effective technique for stopping microbial growth.

Compositional analysis indicated that the liquor may be viewed as a useful source of available nitrogen, phosphorous and potassium. Micronutrient analysis indicated that the majority of those associated with healthy plant growth were in the range found in other commercially available liquid fertilisers when diluted to their working strengths. The exception was molybdenum, which is an order of magnitude lower than that typically seen in the other fertilisers used for comparison, under the conditions tested.

The liquor was determined as being acidic, with a pH of 3.5. This may be too acidic for the majority of plants, but may prove useful for acid-loving (ericaceous) plants. It may be worth confirmation and further assessment, with the addition of lime, for example, to bring the pH nearer to neutral to generate a general-purpose plant feed.

It should be noted, however, that the nature and composition of the input food waste material will have a bearing on the composition and pH of the resulting liquor. If the input material is likely to vary considerably, these compositional studies should be viewed as a snapshot or example of likely future output, rather than actual, reproducible levels.

This information will now allow Plate 2 Plate to potentially grow their business by introducing a new product and reducing their own waste production.

Contents

1. Introduction

Plate 2 Plate Compost uses multiple in-vessel-composting (IVC) systems to convert food waste (predominantly fruit-based) into valuable, nutritious compost material. As a by-product of this process, several thousand litres of liquor are produced annually from their IVCs, at a rate of around 40 L every two days.

Plate 2 Plate currently disposes of the compost liquor but would like to know whether it is suitable for use as a liquid plant fertiliser. The company has raised a number of concerns with regards to microbiological aspects of the liquor. Firstly, how could they reduce the microbial load of the liquor in order to prevent gas build up during product storage. The second concern relates to the presence of the human pathogens, *E. coli* and *Salmonella* spp. The Biorenewables Development Centre (BDC) was commissioned to carry out the following work in order to help guide the client in deciding the product's potential:

- Compositional analysis of the liquor to assess micronutrient content as well as nitrogen, phosphorus and potassium (NPK) content and pH to determine suitability as a liquid plant fertiliser.
- Investigate the use of potential treatments (filtration, autoclaving and pasteurisation) to help reduce the microbial load and eliminate any *Escherichia coli* and *Salmonella* spp present. Microbiological analysis will be performed before and after the treatments and include total aerobic mesophilic plate counts and specific pathogen tests for *E. coli* and *Salmonella* spp.

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2. Methodology

2.1. Liquor analysis for pH and NPK content

A sample of IVC liquor was sent to NRM Laboratories (www.nrm.uk.com) for analysis of pH and nitrogen, phosphorus and potassium (NPK) content.

2.2. Liquor micronutrients analysis

Inductively coupled plasma-mass spectrometry (ICP-MS) was used to assess the content of a range of micronutrients plus potassium and phosphorous in the IVC liquor. A digestion vessel was set up containing 50 mL of IVC liquor, to which 4 mL of concentrated trace metal grade nitric acid and 1 mL of 30% hydrogen peroxide were added. A further vessel was similarly set up as a blank (experimental control) containing no liquor, only nitric acid and hydrogen peroxide. The digestion vessels were sealed and placed into the digestion microwave, with a thermocouple inserted into one of the vessels to monitor the temperature of the liquid inside. The samples were heated to 200 °C over a period of 30 mins and maintained at that temperature for 15 mins. The

samples were then allowed to cool and removed from the microwave. Two dilutions were made (dilution factors were 16.3 and 1630) using distilled water, 10 mL of each was then taken for analysis along with two 10 mL aliquots of the blank.

Dilutions of the Environmental Standards calibration set available from Agilent (Santa Clara, USA) were also analysed to generate a calibration curve for each element against which the samples could be measured.

Analysis was carried out using an Agilent 7700x ICP-MS system, operated in helium collision cell mode to reduce potential interference. The output for each sample was compared to each calibration curve and the result multiplied by the dilution factor to produce the concentration of each element in the samples.

2.3. Microbiological analysis

2. 3. 1. Filtration

The IVC waste liquor was filtered using a coarse filter (Whatman number 1) and associated Buchner flask to remove large particles before membrane filtration was attempted. Filters with 0.4 µm and 0.2 µm pore sizes where used for the membrane filtration, however, the method was unsuccessful, as the filters became clogged. From this it was concluded that filter sterilising would not be a suitable method for reducing microbial load.

2. 3. 2. Autoclaving, Pasteurisation, re-pasteurisation.

Aseptic techniques (i.e. procedures carried out under sterile conditions) were used throughout. Tests were carried out in triplicate to ensure the results were scientifically robust.

For each test, 200 mL of liquor was added to sterile 500 mL bottles. Three vessels were left untreated, three were autoclaved at 121°C for 30 minutes and six were pasteurised in a water bath. For the pasteurisation step, the samples were placed into a waterbath which had been pre-warmed to 70°C. Once the temperature of the samples had also reached 70°C (15 minutes) they were held at this temperature for 1 hour. For the two-stage pasteurisation, three of the pasteurised samples were incubated at 30-35 °C and agitated at 150 rpm for 7 days, then re-pasteurised as above. It was hoped that the incubation period would encourage any surviving spores to germinate into vegetative cells which would then be more susceptible to the second pasteurisation.

Each of the untreated/treated liquors were then tested using the following methods:

Total aerobic mesophilic plate counts (TAPC)

TAPC were carried out for all samples. Serial dilutions of the samples were created using maximum recovery diluent (MRD) up to a dilution of 10^{-6}; 1 ml of each dilution was then transferred to individual petri dishes. Molten plate count agar (PCA) was then added to the petri dishes, which were mixed by gentle manual swirling. Plates were incubated at

30-35 °C for 2 days and the colonies counted in order to determine the total colony forming units (CFUs) per mL of sample plated.

Spore survival

A 1 mL aliquot from each bottle was put into a flask containing 100 mL of tryptic soy broth (TSB) and placed in a shaking incubator at 30-35 °C for 2 days at 150 rpm. Following this, the broth was examined for turbidity (evidence of microbial growth). If turbidity was observed, it indicated that microorganisms were still present and viable within the samples. This step was initially carried out to determine if spores had survived the treatments and was performed on the untreated (control), one stage pasteurisation and autoclaved samples.

E.coli testing

A 1 mL aliquot from each bottle was put into 20 mL of MacConkey broth and placed in a shaking incubator at 37 °C for 2 days at 150 rpm. A loopful of broth was then streaked onto Eosin Methylene blue agar (EMB) and incubated for 2 days at 37 °C. Any colonies present were examined to see if they exhibited morphology typical of *E. coli*.

Salmonella testing

A 1 mL aliquot from each bottle was put into 20 mL of buffered peptone water and placed in a shaking incubator at 37 °C for 2 days at 150 rpm. Following this, 1 mL of each incubated peptone broth was added to 100 mL of Rappaport Vassiliadis Soya (RVS) broth and incubated for 2 days at 37 °C. A loopful of broth was then streaked onto *Salmonella* Chromoselect (SCS) agar and incubated for 2 days at 37 °C. Any colonies present were examined to see if they exhibited morphology typical of *Salmonella* spp.

3. Key findings

3.1. Compositional analysis

3. 1. 1. NPK analysis

The analysis of the IVC liquor carried out by NRM Laboratories gave the results shown in Table 1:

Table 1: NPK analytical results

Determinant	Value (% w/w)
Total nitrogen	0.59
Ureic nitrogen	<0.1
Ammoniacal nitrogen	<0.1
Nitric nitrogen	<0.1
Water soluble phosphorous as P_2O_5	<0.1
Water soluble potassium as K_2O	0.27

In addition, the BDC assessed the liquor for phosphorous and potassium content using ICP-MS analysis. This suggested the presence of:

- Phosphorous: 565122.9 ppb (equivalent to 0.0565%)
- Potassium: 648896.3 ppb (equivalent to 0.0649%)

While the ICP-MS data is in approximate agreement with the analysis carried out by NRM, it is not possible to determine what proportion of the levels detected by ICP-MS are water soluble forms of the two elements.

3. 1. 2. Micronutrient analysis and their role in plant growth

The results of the ICP-MS micronutrient analysis, along with their roles in plant growth, are shown in Table 2:

Table 2: Micronutrient content of IVC liquor as determined by ICP-MS and their roles in plant growth (information taken from https://www.cropnutrition.com)

Element	Concentration (ppb)	Role in plant growth
Boron	1324.0	Boron is one of the most important micronutrients affecting membrane stability and cell wall formation and is thus essential for the growing points of the plant. It improves seed set under stressful conditions. Boron-deficiency symptoms first appear at the growing points and are more pronounced during drought periods when root activity is restricted. Note caution around toxicity through over-application.
Copper	105.3	Copper is closely linked to vitamin A production as well as ensuring successful protein synthesis. Copper activates enzymes and catalyses reactions in several plant-growth processes and ensures adequate chlorophyll production. Other metals in the soil, such as iron, manganese and aluminium, affect the availability of copper for plant growth. Many vegetable crops show copper hunger, with leaves that lose turgor and develop a bluish-green shade before becoming pale (chlorotic) and curling.
Iron	8151.1	Iron is essential for crop growth and food production. Iron is a component of many enzymes associated with energy transfer, nitrogen reduction and fixation, and lignin formation. Iron deficiencies can be caused by imbalances with other micronutrients, e.g. copper, molybdenum and manganese. Deficiency may be displayed as pale leaf colour (chlorosis).
Manganese	2791.8	Manganese functions primarily as part of enzyme systems in plants, activating several important metabolic reactions and playing a direct role in photosynthesis. Manganese accelerates germination and maturity while increasing the availability of phosphorous and calcium.
Molybdenum	3.5	Molybdenum is required for the synthesis and activity of the enzyme nitrate reductase and is vital for the process of symbiotic nitrogen fixation by Rhizobia bacteria in legume root modules. Molybdenum availability increases as pH goes up, the opposite of most other micronutrients.
Nickel	106.0	Nickel is important in plant nitrogen metabolism. Nickel deficiencies are not commonly seen in crop plants since the critical amount typically required is approx. 1.1 parts per million (ppm). Some deficiencies have been observed in nursery plants and tree crops, where mouse ear (small curled leaves and stunted growth) is observed.
Zinc	5247.2	Zinc is involved in protein synthesis and growth regulation. A deficiency will give rise to shortened internodes and stunted leaf growth. Deficiency symptoms first appear on younger leaves since zinc is less mobile within the plant.

3. 1. 3. **pH**

The pH of the IVC liquor was determined by NRM Laboratories to be 3.9.

3. 1. 4. **Comparison of IVC liquor with proprietary fertilisers**

Table 3 shows a comparison of the composition of IVC liquor with commercially available liquid fertilisers:

Table 3: Comparison of IVC liquor with liquid fertilisers.

Component	Amounts (% w/w)				
	IVC liquor	**Black Magic All Purpose Liquid Feed**	**Miracle-Gro All Purpose Liquid Feed**	**Westland Houseplant Feed**	**Solufeed Ericaceous Plant Feed**
Total nitrogen	0.59	0.070	0.03	0.03	0.012
Ureic nitrogen	<0.1	0.017	0.017	0.016	0.0013
Ammoniacal nitrogen	<0.1	0.018	0.0156	0.004	0.0107
Nitric nitrogen	<0.1	0.035	nd	0.01	0
Water soluble phosphorous as P_2O_5	<0.1 (0.0565)*	0.013	0.012	0.0088	0.0044
Water soluble potassium as K_2O	0.27 (0.0649)*	0.042	0.023	0.029	0.009
Copper	0.0000105	0.00002	0.00001	0.00065	0.000017
Iron	0.0008151	0.0003	0.00017	0.00015	0.00006
Manganese	0.0002792	0.0001	0.00006	0.00005	0.000034
Molybdenum	0.0000004	0.00001	0.000006	0.000015	0.000001
Zinc	0.0005247	0.00002	0.00001	0.00003	0.000017
Boron	0.0001324	nd	nd	nd	0.000015
Nickel	0.0000106	nd	nd	nd	nd

Commercially available fertiliser values are given for working strength dilutions. Nd=not determined. *Figures in brackets denote data determined by ICP-MS for phosphorous and potassium.

3.2. **Microbiological analysis**

The microbial load, denoted as CFUs per mL, and selective culturing results are shown in Table 4.

Table 4: Microbiological analysis results.

Treatment	CFUs per mL*	*SCS* agar	EMB Agar	Spore survival
Untreated	1.08×10^6 (1.76×10^5)	Growth-typical morphology observed	Growth-typical morphology **not** observed	Growth observed
Autoclaving	6.00 (6.00)	No growth observed	No growth observed	No growth observed
Pasteurisation	2.03×10^6 (9.98×10^5)	No growth observed	No growth observed	Growth observed
Two stage-pasteurisation	6.67×10^5 (8.41×10^4)	No growth observed	No growth observed	NA

* The standard error of the mean is given in brackets.

The untreated liquor was found to have a mean TAPC of 1.08×10^6 (1.08 million) CFUs per mL. Typically, antimicrobial treatments are judged on their ability to reduce microbial numbers by log reductions. For example, a one log reduction (from 10^6 to 10^5) is equivalent to a ten-fold reduction, a two-log reduction (from 10^6 to 10^4) is equivalent to a 100-fold reduction. The pasteurisation treatment did not lead to a log reduction indicating that this method was not suitable. The two-stage pasteurisation did show a log reduction in the number of microorganisms, however, it was only a one log reduction meaning a significant number were still present within the liquor. The autoclave treatment was the most successful with a six-log reduction being achieved.

Colonies exhibiting morphology typical of *Salmonella* spp (pink/red colonies) were present on the untreated sample's SCS agar plates - see Figure 1. It should be noted that further analysis would need to be carried out in order to confirm if these colonies were in fact *Salmonella* spp. No microbial growth was observed on the SCS agar for any of the treated samples suggesting these methods were successful at killing any potential *Salmonella* spp present.

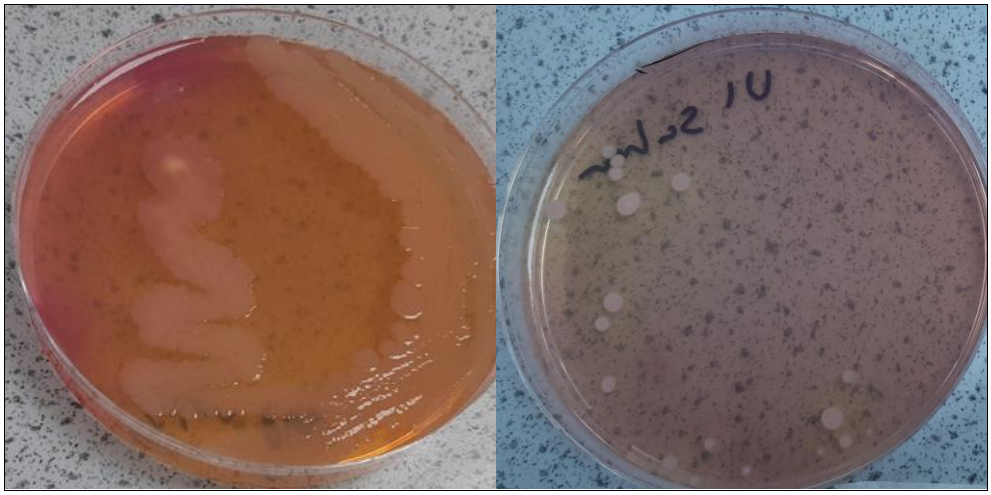

Figure 1: Salmonella Chromoselect agar. Left- shows morphology typical of *Salmonella* spp. Right -Colonies from the untreated sample.

Growth was observed on all three EMB plates for the untreated samples, however, the morphology was not typical of *E.coli* colonies grown on EMB agar, see Figure 2. No growth was observed on any of the other EMB plates.

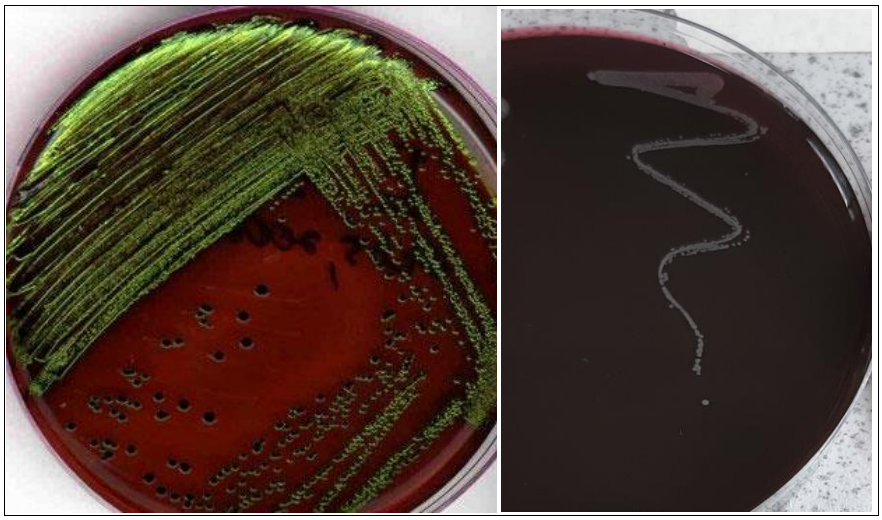

Figure 2: EMB agar. Left - shows morphology typical of *E. coli*. Right - Colonies from the untreated sample.

4. Conclusions

The compositional analysis indicated that the liquor can be viewed as a useful source of available nitrogen, phosphorous and potassium as well as a range of essential micronutrients. The analysis, however, did not determine the level of water soluble phosphorus present, being below the detection limit of the NRM Laboratory assessment. Plate 2 Plate may wish to consider further testing around this aspect.

Regarding the content of micronutrients important for healthy plant growth, the majority are in the range found in commercially available liquid fertilisers when diluted to their working strengths, apart from molybdenum, which is an order of magnitude less than that typically seen in the other fertilisers used for comparison.

The liquor was determined as being acidic, with a pH of 3.5. This may be too acidic for the majority of plants, but may prove useful for acid-loving (ericaceous) plants. It may be worth further assessment, with the addition of lime, for example, to bring the pH nearer to neutral to generate a general-purpose plant feed.

It should be noted, however, that the nature and composition of the input food waste material will have a bearing on the composition and pH of the resulting liquor. If the input material is likely to vary considerably, these compositional studies should be viewed only as a snapshot or example of likely future output, rather than actual, reproducible levels.

The autoclaving treatment successfully reduced the microbial load of the compost liquor to a level which should significantly prolong the shelf life of the liquor. Although the pasteurisation methods used in this study were not successful at significantly reducing the microbial load, pasteurisation may still be an option if a higher hold temperature was used. This may also be a more cost effect treatment as it does not require the purchase of an autoclave. Further work centring around pasteurisation temperatures is recommended. With regards to the pathogen testing, as *E.coli* was not isolated from the untreated liquor it could not be confirmed if any of the treatments were able to remove this pathogen. As presumptive *Salmonella* spp was isolated from the untreated liquor and not the treated samples it indicates that these treatments may potentially remove this pathogenic genus.

5. Next steps

The BDC hopes this short study has been a useful evaluation of the potential use of IVC liquor. We are happy to discuss the findings of this study with you, to help identify the next steps in bringing the IVC liquor to market as a safe, useful plant fertiliser.

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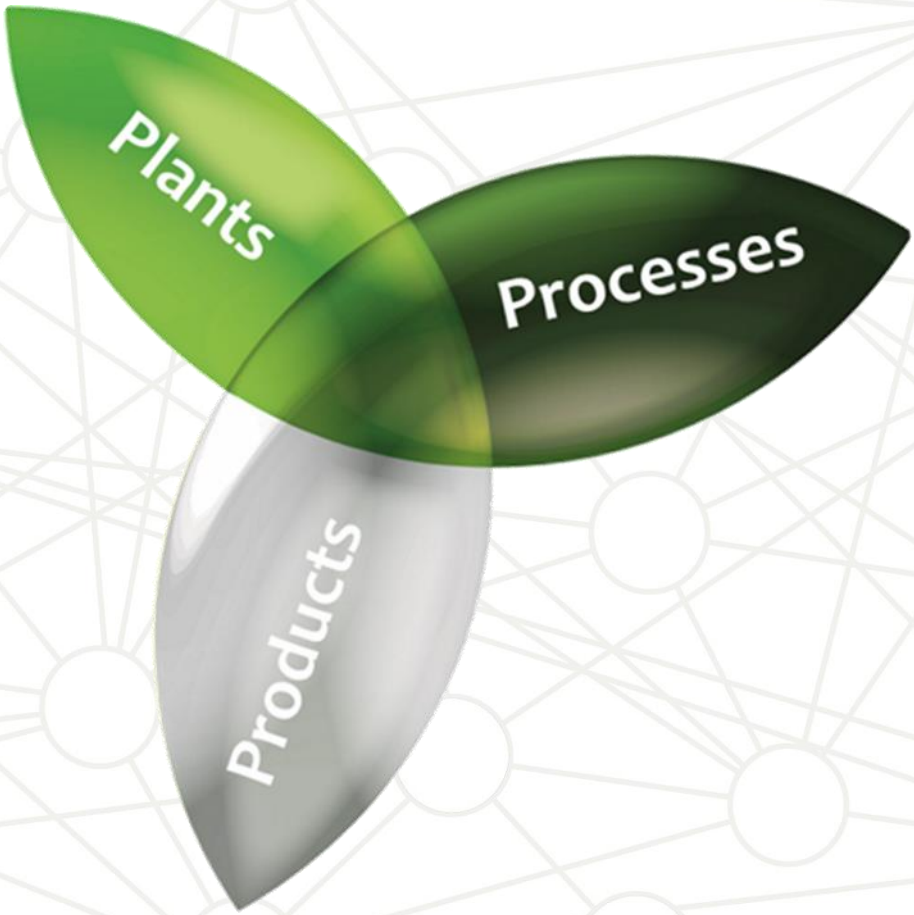

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