Biorenewables Development Centre

Plants • Processes • Products

Report developed for:

Ekolive s.r.o.

KET4CP TR111 / CMRC 3523

Date: April 2021

Plant growth trials using products derived from bioleaching

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1. Project brief and aims

This work forms part of the KET4CP-funded project with Ekolive s.r.o. It aims to determine whether the leachates produced from their bioleaching process might find value as fertilisers.

The BDC undertook an 8-week plant growth trial using basil (as an indicator crop) grown in coir compost watered with varying dilutions of preparations of humic acid, leachate and concentrated leachate.

2. Methodology

2.1. Materials – preparation and characterisation

All materials were provided by Ekolive s.r.o. or Bay Zoltán Nonprofit Ltd, and comprised:

- Humic acid preparation
- Leachate
- Concentrated leachate

Samples of the leachate and concentrated leachate were sent to NRM Laboratories Ltd for analysis, to assess the water-soluble levels of the macronutrients N, P and K. There were insufficient volumes of humic acid to send for analysis.

2.2. Basil as an indicator plant

Quick-growing, indicator plants such as basil are used to determine the effect of the various soil treatments, as described below. In addition to being relatively fast growing, basil is a good indicator of the state of the growth medium, for example, if pH levels and/or nutrients are unfavourable.

2.3. Plant growth trial set-up

2. 3. 1. Germination trials

Coconut fibre growth medium (coir compost) was purchased from Amazon Ltd (https://www.amazon.co.uk/COCO&COIR) and rehydrated according to the manufacture's instructions.

The rehydrated mixture was distributed equally between 30 plant growth tray inserts and the bottom of each insert covered with a clean plastic bag to prevent water run-off being taken up by neighbouring inserts when resting in plant growth trays.

The filled inserts were equilibrated overnight in a Panasonic MLR-352 PE plant growth cabinet set to 22°C, before basil seeds (https://www.diy.com/departments/basil-seed) were sown, evenly spaced at a rate of 25 seeds per insert.

Three inserts each (a total of 75 seeds) were watered with 10 mL of one of the following:

1. Water
2. Leachate (100%)
3. Leachate (50%)
4. Leachate (10%)
5. Concentrated leachate (100%)
6. Concentrated leachate (50%)
7. Concentrated leachate (10%)
8. Humic acid (100%)
9. Humic acid (50%)
10. Humic acid (10%)

The prepared inserts were returned to the growth cabinet set to 22 °C, 16h days. Germination numbers were scored at the end of one and two weeks after sowing.

2. 3. 2. **Plant growth / health assessment**

After germination scoring, seedlings were then thinned to 10 per insert and incubated in the growth chamber for a further 6 weeks under the same conditions as germination. Each insert received a further 10 mL application of the relevant treatment type at 4 weeks after sowing.

Plants were assessed on a weekly basis for the following attributes:

- Overall health (poor / normal / good)
- Number of true leaves emerged
- Number of seedlings remaining
- Evidence of disease (e.g. appearance of leaf spots, mould or mildew)
- Evidence of plant stress / nutrient deficiency (e.g. yellowing leaves)
- Plant height (at weeks 4 & 8)
- Plant dry weight (at week 8)

Plant dry weight was determined by harvesting all aerial tissue from plants grown in each soil type and drying at 50 °C until the weight decreased and remained constant. Root material was not assessed since it was not possible to remove all traces of the coir compost, which would have affected the data.

3. Key findings

3.1. Material characterisation

Table 1 shows the results of the characterisation studies of the leachate and concentrated leachate carried out by NRM Laboratories Ltd.

Table 1: Characterisation of bioleachate products as carried out by NRM Laboratories Ltd. Quantification falling below the limit of detection is given by '<0.10'.

	Leachate	Concentrated leachate
Total nitrogen (%w/w)	<0.10	<0.10
Ureic nitrogen (%w/w)	<0.10	<0.10
Ammoniacal nitrogen (%w/w)	<0.10	<0.10
Nitric nitrogen (%w/w)	<0.10	<0.10
Water-soluble P as P_2O_5 (%w/w)	<0.10	<0.10
Water-soluble K as K_2O (%w/w)	0.28	0.32
pH	4.4	4.5
Density (g/mL)	1.013	1.017

Unfortunately, the levels of nitrogen (all forms) and water-soluble phosphorus were below the limit of detection. Low levels of water-soluble potassium were observed, however. Of note is the acidic pH of the leachate products, which may need buffering or neutralising, depending on the market/application Ekolive s.r.o. focuses on.

For commercially available fertiliser and composts, prominently featured on packaging or product information will be **the N-P-K ratio**, i.e. the percentage that the product contains by volume of nitrogen, phosphorus and potassium. A 25-4-2 formulation, for example, will contain 25% nitrogen, 4% phosphorus, and 2% potassium. All fertilisers/composts will contain at least one of these components; if any is missing, the N-P-K ratio will show a zero for that nutrient; for example, a 12-0-0 fertiliser will contain nitrogen but no phosphorus or potassium.

To put the leachate N-P-K amounts into perspective, Table 2 below gives examples of the N-P-K levels of several commercially available fertilisers.

Table 2: Examples of the N-P-K levels of commercially available fertilisers

	Miracle Gro Continuous Release Plant Food	Elixir Gardens Growmore All Round General Purpose Fertiliser	YaraMila COMPLEX fertiliser	Dr Earth Organic Tomato, Vegetable & Herb Fertilizer
Total nitrogen (%w/w)	17.0	7.0	12.0	4.0
Ammoniacal nitrogen (%w/w)	9.3	4.9	7.0	-
Nitric nitrogen (%w/w)	7.7	2.1	5.0	-
Water-soluble P as P_2O_5 (%w/w)	6.5	6.4	11.0	6.0
Water-soluble K as K_2O (%w/w)	11.0	7.0	18.0	3.0

ICP-MS analysis of the leachate, conc leachate and humic acid (see Supplementary Data 1) indicated the values of several trace elements required for healthy plant growth. Table 3 indicates the values obtained for these trace elements and their role in plant growth and health (NB boron was not included in the ICP-MS standards set used for calibration).

It is important to refer to local legislation regarding the levels of trace elements permitted in materials intended for application as a fertilizer.

Table 3: Micronutrient content of leachates and humic acid samples as determined by ICP-MS and their roles in plant growth. N.D. – not determined; LOD - limit of detection; 1000ppb = 1mg/kg (information taken from https://www.cropnutrition.com). Results represent an average concentration of three technical replicates.

Refer to Supplementary Data 1.

Element	Concentration (ppb)			Role in plant growth
	Leachate	Conc leachate	Humic acid	
Boron	N.D.	N.D.	N.D.	Boron is one of the most important micronutrients, affecting membrane stability and cell wall formation. It is essential for the growing points of the plant, improving seed set under stressful conditions. Deficiency symptoms first appear at the growing points and are more pronounced during drought periods when root activity is restricted.
Copper	230	1653	41	Copper is closely linked to vitamin A production as well as ensuring successful protein synthesis. It activates enzymes and catalyses reactions in several plant-growth processes, ensuring adequate chlorophyll production. Other metals, such as iron, manganese and aluminium, affect the copper availability. Many vegetable crops show copper hunger, with leaves that lose turgor and develop a bluish-green shade before becoming pale and curling.
Iron	195109	289888	<LOD	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 appear as pale leaf colour.
Manganese	20689	31312	98	Manganese functions primarily as part of enzyme systems in plants, activating several important metabolic reactions, with a direct role in photosynthesis. It accelerates germination and maturity while increasing the availability of phosphorus and calcium.
Molybdenum	124	2641	<LOD	Molybdenum is required for 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. Its availability increases as pH increases (opposite to many micronutrients).
Nickel	<LOD	<LOD	<LOD	Nickel is important in plant nitrogen metabolism. Deficiencies are not commonly seen in crop plants since the critical amount typically required is ~1.1 ppm. Some deficiencies have been observed in nursery plants and tree crops, where small curled leaves and stunted growth are observed.
Zinc	1437	2951	25	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.

3.2. Germination

Figure 1 shows the results of germination at one and two weeks after sowing.

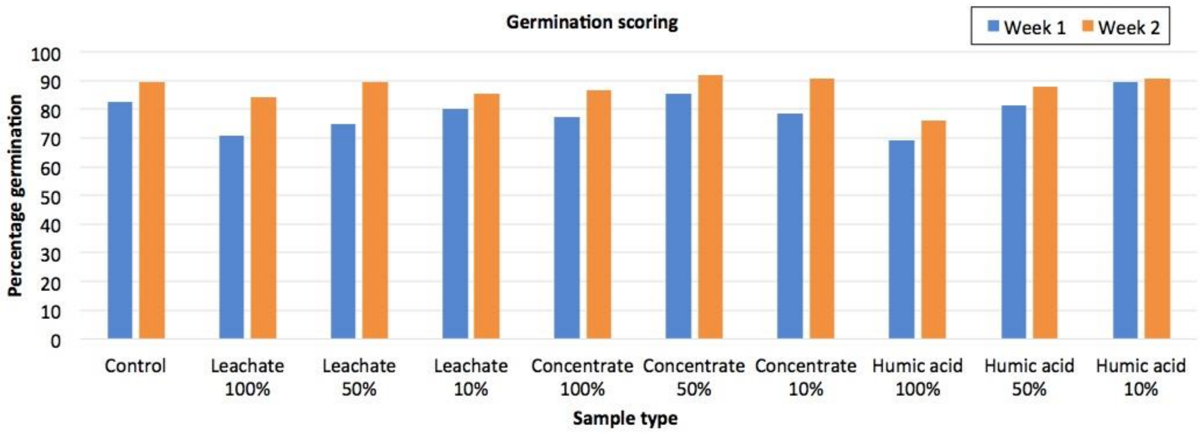


Figure1: Germination results at one and two weeks after seed sowing

The data shows that, generally, seeds germinated well on coir compost supplemented with all treatment types, and comparable to the control seeds (only water provided) with perhaps the exception of seeds watered with humic acid 100% which germinated at a slightly lower frequency than the control seeds.

3.3. Plant growth and development

3. 3. 1. General plant health

Appendix 1 shows images of the seedlings at 3 - 8 weeks after sowing. Generally plants were green and healthy throughout. While seedlings growing in leachate 100%, conc. leachate 100% and humic acid 100% supplemented coir were clearly larger than the control plants by week 8, there was some yellowing of leaves / leaves having a lighter green colour in the leachate 100% seedlings at weeks 7 and 8, suggesting that they may have been staring to be depleted of some nutrients by that stage.

3. 3. 2. Plant height

Figure 2 shows the average seedling heights measured at weeks 4 and 8 after sowing.

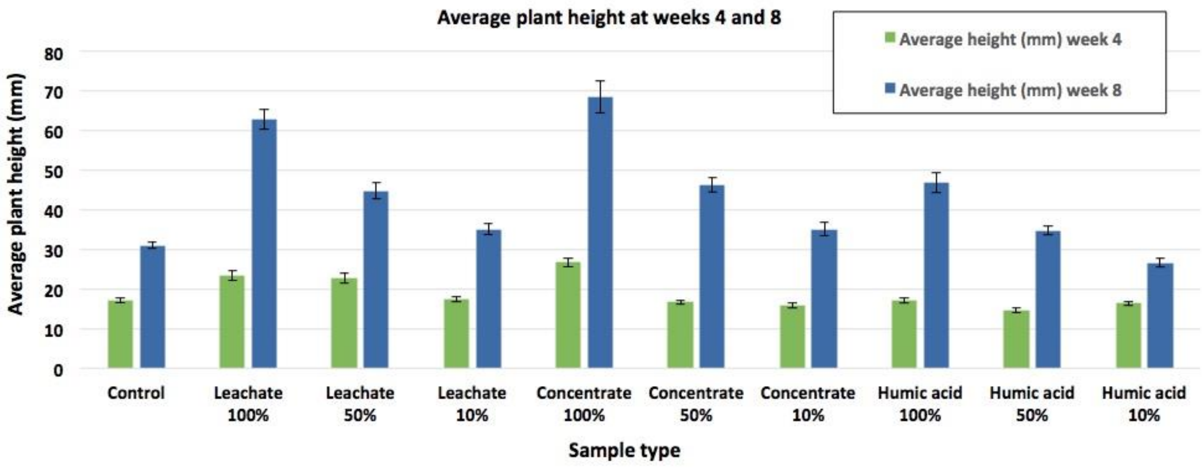


Figure 2: Average plant heights measured at 4 and 8 weeks after sowing

Seedlings grown on most treatments managed to reach a greater height than the control seedlings, apart from those grown on humic acid 10%.

3. 3. 3. Dry weight of aerial tissue

Figure 3 shows the dry weights of aerial material harvested at week 8 for each treatment type.

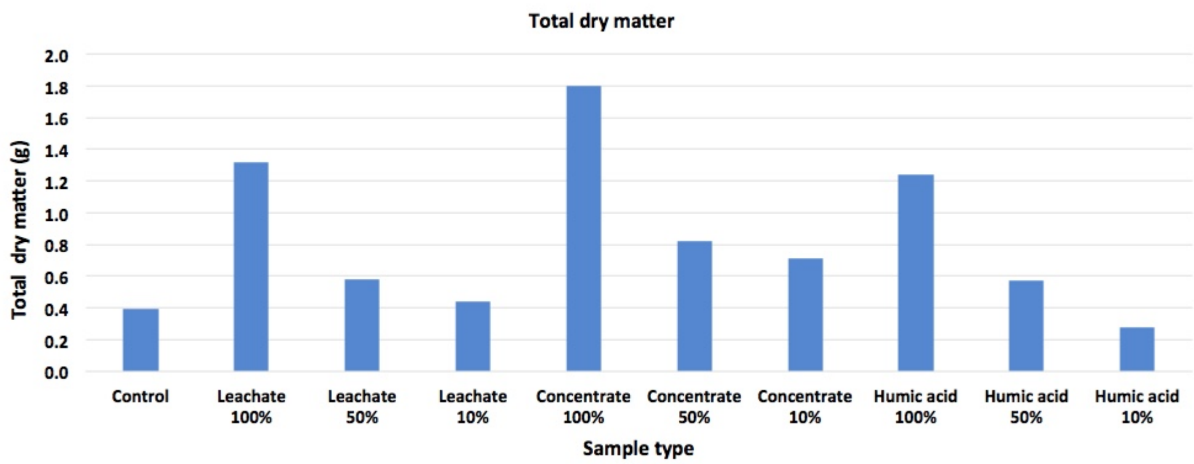


Figure 3: Dry weights of aerial material harvested at week 8.

It was not possible to assess the roots of seedlings in this study, but seedlings supplemented with leachate 100%, conc leachate 100% and humic acid 100% had significantly higher dry weights than the control seedlings.

3. 3. 4. Emergence of true leaves

Figure 4 shows the rate of emergence of true leaves of seedlings grown in each treatment type at weeks 4 and 8 after sowing.

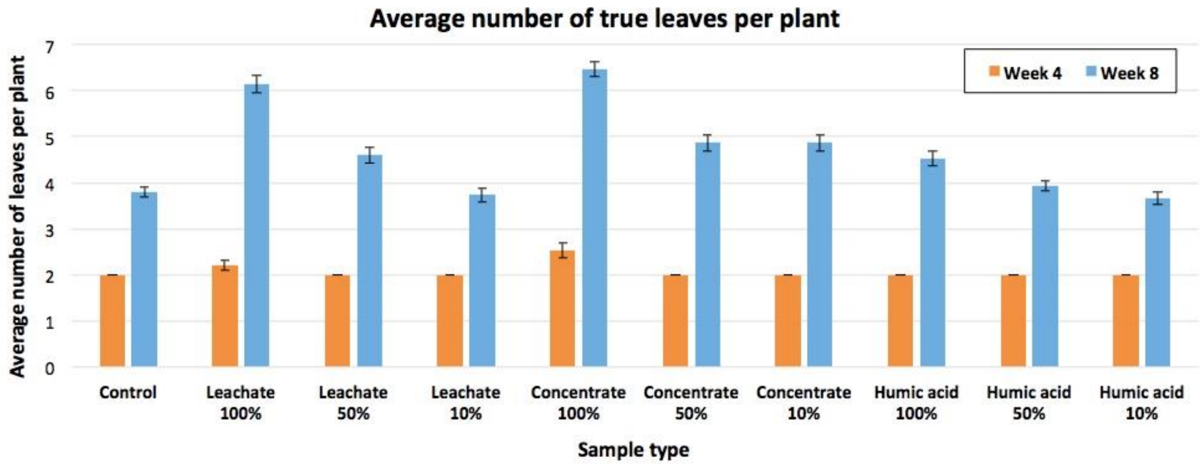


Figure 4: Average number of true leaves per seedling grown in each treatment type.

There was little difference between treatment types at week 4: all had reached the two true leaf stage, with several seedlings supplemented with conc leachate 100% reaching the four leaf stage. All seedling types had, on average, reached the same development stage as the control or better by week 8, with seedlings supplemented with leachate 100% and conc leachate 100% progressing furthest.

4. Conclusions

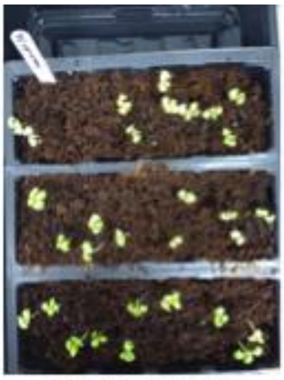
Taking the data for plant height, dry weight, development stage reached and general health observations, it is clear that the leachate 100%, conc leachate 100% and humic acid 100% samples show promise for application as a plant fertilizer. Humic acid 100% appeared to have a small detrimental effect on germination frequency under the conditions tested, but those seedlings that emerged successfully benefitted from its application and developed further than the control seedlings. It may, therefore, find use as a fertilizer supplement once seedlings are established.

The analysis of N, P and K by NRM Ltd, indicated that the levels of these nutrients were low in the leachate and conc leachate. ICP-MS analysis of the leachate, conc leachate and humic acid suggested that there are useful levels of micronutrients essential for plant growth, however levels of these and other metals (such as lead, cadmium, chromium, and mercury) should be assessed relating to local regulations to ensure that upper permitted limits are not exceeded.

5. Appendix 1: Seedlings at 3 - 8 weeks after sowing

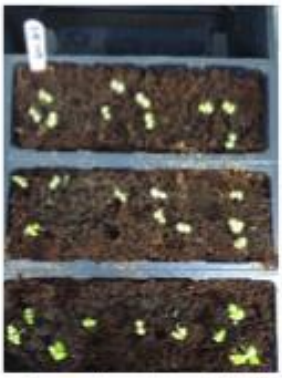
Week 3:


Leachate 100%


Leachate 50%

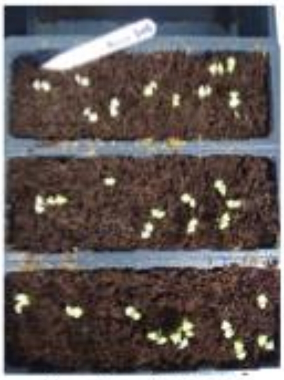

Leachate 10%


Conc. leachate 100%


Conc. leachate 50%


Conc. leachate 10%


Humic acid 100%


Humic acid 50%


Humic acid 10%


Control

Week 4:


Leachate 100%


Leachate 50%


Leachate 10%


Conc. leachate 100%


Conc. leachate 50%


Conc. leachate 10%


Humic acid 100%


Humic acid 50%


Humic acid 10%


Control

Week 5:

Leachate 100%

Leachate 50%

Leachate 10%

Conc. leachate 100%

Conc. leachate 50%

Conc. leachate 10%

Humic acid 100%

Humic acid 50%

Humic acid 10%

Control

Week 6:

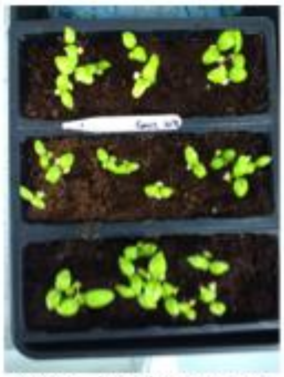
Leachate 100%

Leachate 50%

Leachate 10%

Conc. leachate 100%

Conc. leachate 50%



Conc. leachate 10%

Humic acid 100%

Humic acid 50%

Humic acid 10%

Control

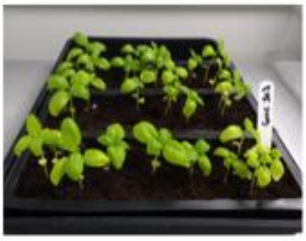
Week 7:

Leachate 100%

Leachate 50%

Leachate 10%

Conc. leachate 100%



Conc. leachate 50%

Conc. leachate 10%

Humic acid 100%

Humic acid 50%

Humic acid 10%

Control

Week 8:

Leachate 100%

Leachate 50%

Leachate 10%

Conc. leachate 100%

Conc. leachate 50%

Conc. leachate 10%

Humic acid 100%

Humic acid 50%

Humic acid 10%

Control

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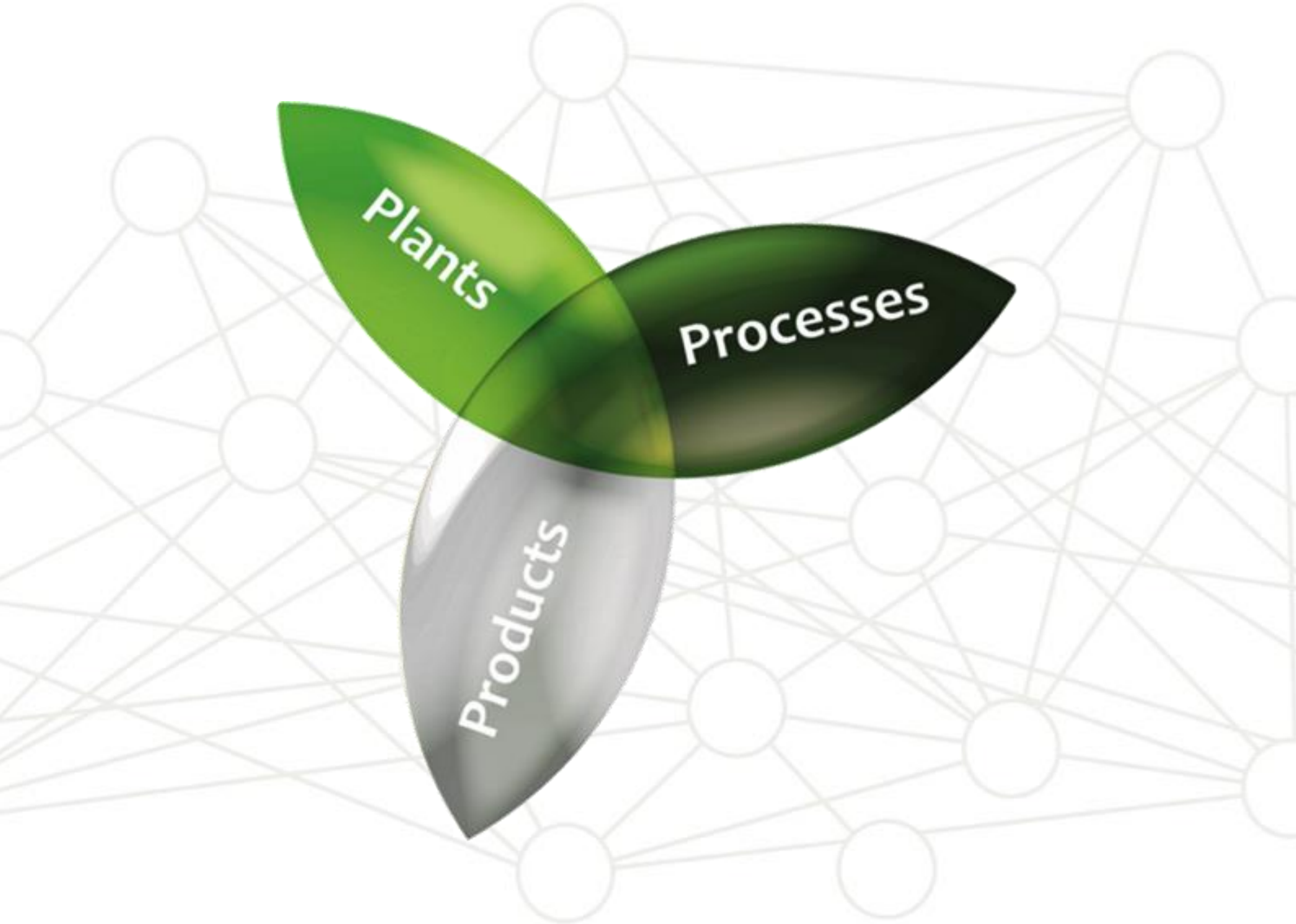

Biorenewables Development Centre

Biorefining centre developing ways of converting plants and wastes into products

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