BIO-BASED FERTILISERS: FROM COMPOSITIONAL CHARACTERISATION TO PLANT RESPONSE

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WASTE-TO-FERTILIZER STRATEGY

MEAT AND BONE MEAL (MBM) AS A RESOURCE

MEAT-INDUSTRY BY-PRODUCT

A route to reuse animal residues in fertilizer production.

PROTEINS AND AMINO ACIDS

Protein-rich material that can release amino acids by hydrolysis.

PLANT NUTRIENTS

Contains nitrogen, phosphorus, calcium and organic matter.

WHY HYDROLYSIS? MBM proteins are poorly soluble in water. Alkaline hydrolysis releases amino acids for use in fertilizer formulations.

AIM OF THE STUDY

To convert MBM into and effects on two granulated organo-mineral fertilizers and assess their nutrient composition, safety, nutrient availability, cucumber growth.

Hydrolysis optimisation → F1 and F2 formulation → Product characterisation → Cucumber tests

MBM FEEDSTOCK: PHYSICAL CHARACTERISTICS

RAW MATERIAL CHARACTERISATION

Table 1. *Physical and chemical properties of the meat and bone meal feedstock*

Parameter	pH	Moisture *(%)*	Volatile matter *(%)*	Ash *(%)*	Protein *(%)*	Lipids *(%)*
Meat and bone meal *(MBM)*	6.25±0.31	4.80±0.14	38.6±1.9	28.1±1.1	54.6±4.9	8.38±0.33

FEEDSTOCK SUITABILITY

- The material combined a protein-rich organic fraction with a substantial mineral phase.
- Its physical properties were suitable for chemical hydrolysis and subsequent granulation.

LOW MOISTURE

4.80% moisture supports stable storage and handling before hydrolysis.

MINERAL-RICH FRACTION

Ash accounted for 28.1%, consistent with the high bone-derived Ca-P fraction.

ORGANIC FRACTION

38.6% volatile matter and 8.38% lipids confirm a substantial recoverable organic component.

MBM FEEDSTOCK: NUTRIENT AND SAFETY PROFILE

MACRONUTRIENTS

Table 2. *Macronutrient content in the MBM material*

Element	N_{tot} *(%)*	C_{tot} *(%)*	P_2O_5 *(%)*	K_2O *(%)*	CaO *(%)*	MgO *(%)*	Na_2O *(%)*	SO_3 *(%)*
MBM	8.73 ±0.78	36.3 ±3.6	11.6 ±2.3	0.443 ±0.066	13.7 ±2.7	0.290 ±0.043	0.658 ±0.099	0.999 ±0.150

MICRONUTRIENTS

Table 3. *Micronutrient content in the MBM material*

Element	Cu *(mg/kg)*	Fe *(mg/kg)*	Mn *(mg/kg)*	Zn *(mg/kg)*
MBM	10.3±1.5	180±27	14.5±2.2	91.1±13.6

TOXIC ELEMENTS

Table 4. *Toxic elements content in the MBM material*

As *(mg/kg d.m.)*	Cd *(mg/kg d.m.)*	Cr *(mg/kg d.m.)*	Ni *(mg/kg d.m.)*	Pb *(mg/kg d.m.)*	Hg *(mg/kg d.m.)*
0.230 ±0.035	0.068 ±0.010	0.306 ±0.046	1.03 ±0.16	1.09 ±0.16	<0.001

KEY RESULT

The feedstock supplied 8.73% N, 11.6% P_2O_5 and 13.7% CaO, while every quantified toxic element remained far below the applicable limits for EU fertilising products.

MICROBIOLOGICAL QUALITY OF THE FEEDSTOCK

MICROBIOLOGICAL SCREENING

Table 5. *Microbiological screening of MBM material*

Indicator	Experimental result	Regulatory / safety interpretation
Total number of microorganisms	<100 CFU/g	Very low aerobic count
Fungi + yeasts	<100 CFU/g	Below the detection threshold used
Coliforms	<10 CFU/g	Very low hygiene-indicator count
Escherichia coli	Within the legal limit	≤1,000 CFU/g required
***Salmonella* spp.**	Not detected in 25 g	Absence in 25 g required

REGULATORY COMPLIANCE

E. coli complied with the ≤1,000 CFU/g criterion and Salmonella was absent in the required 25 g test portion.

✓ MICROBIOLOGICALLY SAFE FOR FERTILIZER PROCESSING

PROCESS IMPLICATION

Low initial contamination reduces biological risk; alkaline hydrolysis and exothermic neutralisation provide additional sanitisation barriers.

BOX–BEHNKEN EXPERIMENTAL DESIGN

INDEPENDENT VARIABLES AND CODED LEVELS

Table 6. *Factor levels used in the Box–Behnken design*

Factor	Symbol	−1	0	+1
KOH concentration (%)	X_1	10	30	50
Temperature (°C)	X_2	20	60	100
Solid:liquid ratio (m/m)	X_3	1:1	1:2	1:3

RESPONSE SURFACE METHODOLOGY

- Quadratic model fitted to the experimental amino-acid data.
- Utility profiles used to identify the process optimum.

15
EXPERIMENTAL RUNS

1 h
HYDROLYSIS TIME

3
CENTRE-POINT REPLICATES

Y_1
TOTAL FREE AMINO ACIDS *(mg/kg)*

OPTIMISATION OBJECTIVE

Maximise amino-acid release from MBM while identifying the combined effects of alkali concentration, temperature and slurry dilution.

HYDROLYSIS OPTIMISATION

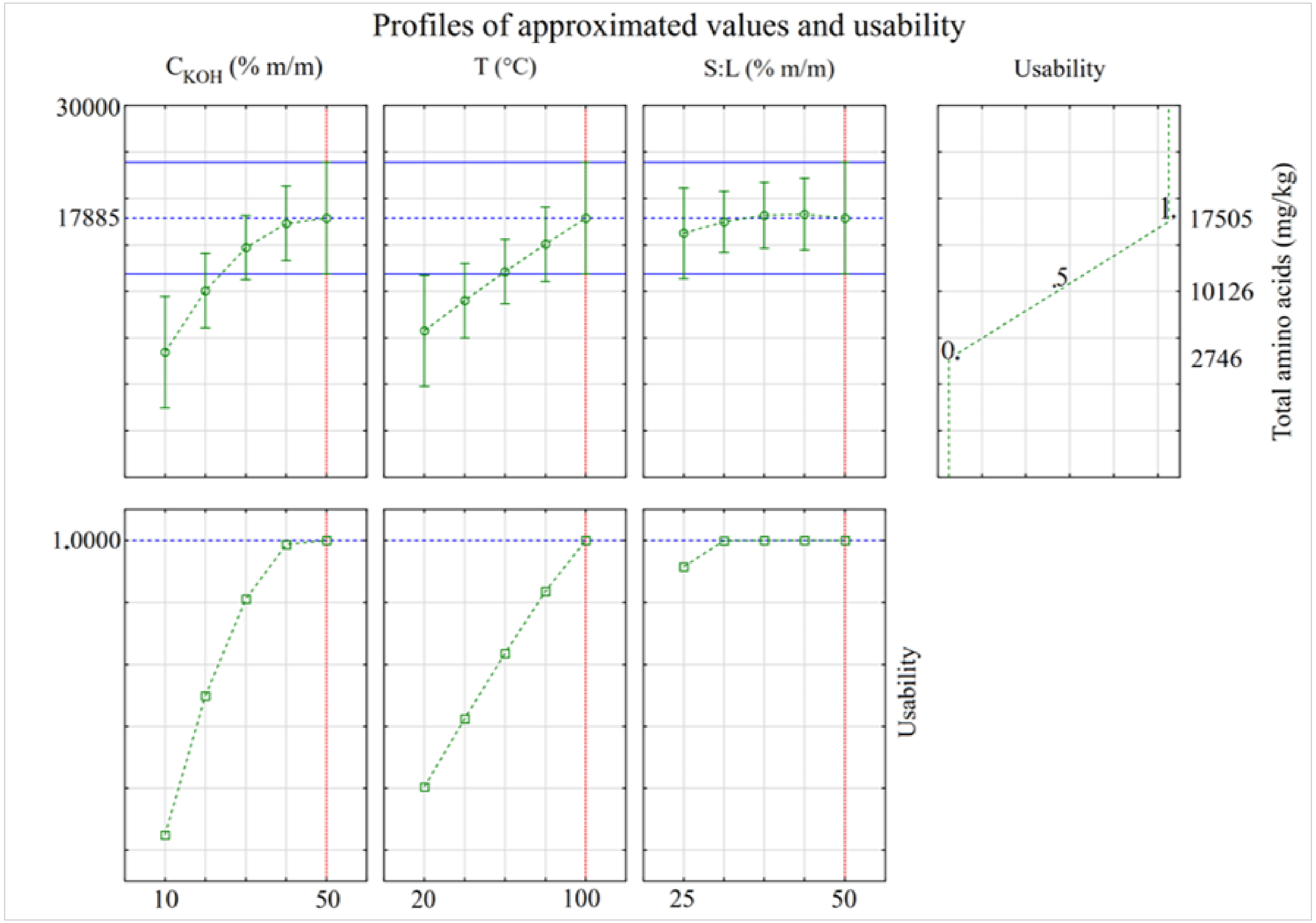


Figure 1. *Profiles of approximated values and overall usability for the hydrolysis variables*

17,505±721 mg/kg

The highest experimental total amino-acid content.

EXPERIMENTAL MAXIMUM

50% KOH, 100°C and S:L = 1:2

18,090 mg/kg

mg/kg - model-predicted maximum

PREDICTED OPTIMUM

50% KOH, 100°C and S:L = 1:1.

STATISTICAL VALIDATION OF THE RSM MODEL

ANOVA FOR THE QUADRATIC MODEL

Table 7. *Key results for analysis of variance (ANOVA) and regression coefficients for the quadratic RSM model*

Term	F-value	p-value	Interpretation
X_1: KOH (L+Q)	30.2	0.00161	Significant
X_2: temperature (L+Q)	27.1	0.00207	Significant
X_3: S:L (L+Q)	0.609	0.579	Not significant
$X_1 \times X_2$	11.9	0.0182	Significant interaction

$R^2 = 0.9673$

VARIANCE EXPLAINED BY THE MODEL

DOMINANT EFFECT

The quadratic KOH term was significant ($p=0.0228$), demonstrating a non-linear response and the need to optimise rather than simply maximise reagent concentration.

SYNERGISTIC INTERACTION

The KOH×temperature interaction was significant: high alkali was most effective when combined with high temperature.

MODEL–EXPERIMENT AGREEMENT

Predicted and measured maxima differed by only 3.3%.

AMINO ACID

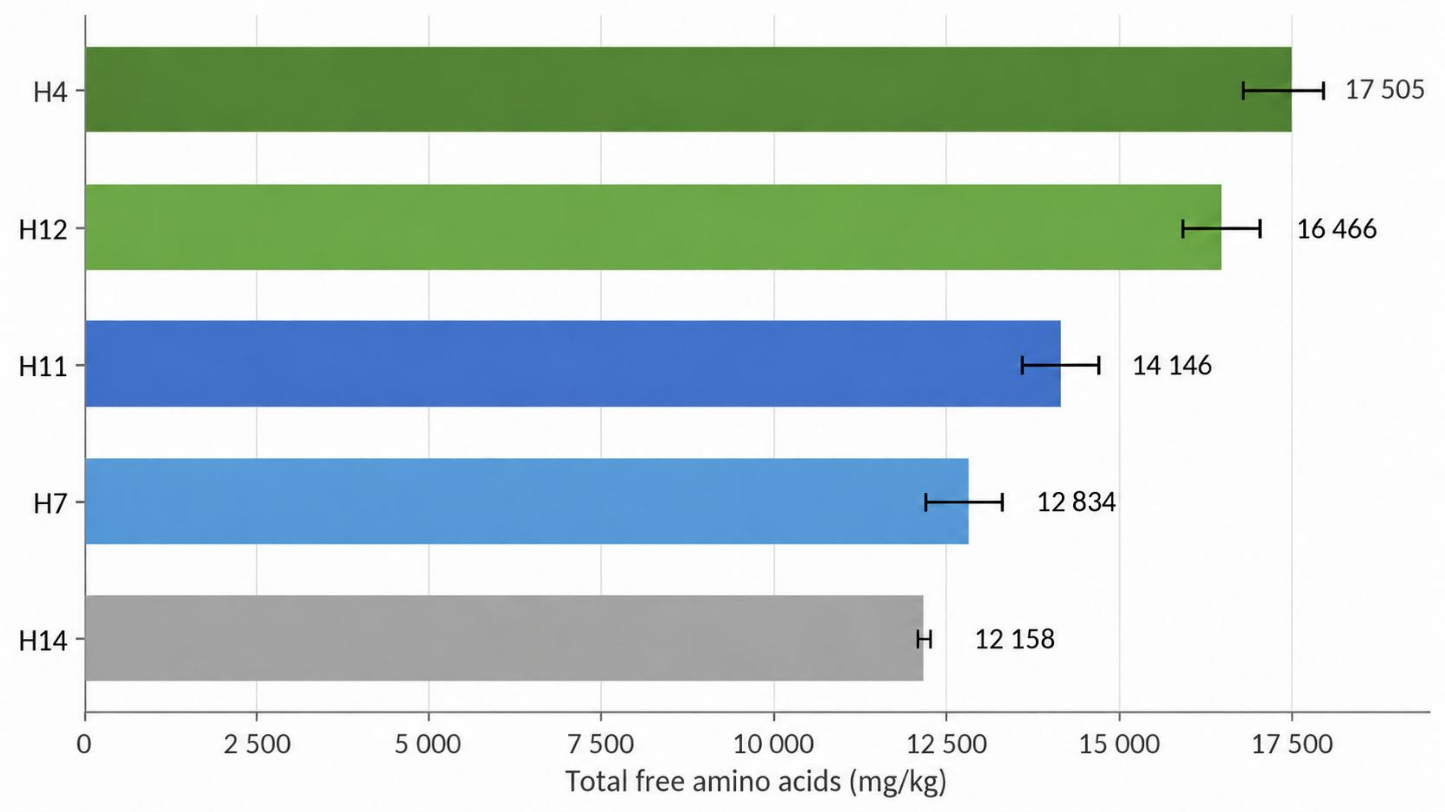


Figure 2. *Key results for Hydrolysates with the highest total amino-acid contents.*

HYDROLYSIS CONDITIONS

Hydrolysate	**KOH** *(%)*	**T** *(°C)*	**S:L** *(m/m)*
H4	50	100	1:2
H12	30	100	1:3
H11	30	100	1:1
H7	50	60	1:1
H14	30	60	1:2

Hydrolysis time: 1 h for all variants.

BIOACTIVE COMPOUNDS WITH THE HIGHEST LEVELS

Compound	**Main role in plants**
Arginine	Nitrogen source; precursor of stress-related polyamines
Lysine	Supports osmoprotection and stress-response pathways
Threonine	Supports seedling elongation and root development
Serine	Precursor of nucleotides, amino acids and phospholipids
Methionine	Supports growth and tolerance to water deficit

HYDROLYSATE PHYTOTOXICITY SCREENING

SELECTED CUCUMBER GERMINATION RESULTS

Table 8. *Selected variants for biometric response of cucumber seedlings to MBM hydrolysates*

Group	Stem length *(cm)*	Chlorophyll *(mg/m^2)*	Root length *(cm)*	Fresh mass *(g)*	Dry mass *(g)*
W	3.27±0.39	512±79	38.4±9.9	8.88	0.351
H3	6.02±0.50	527±41	46.1±12.8	11.2	0.439
H4	6.53±0.95	544±46	44.5±9.7	10.9	0.449
H10	7.09±0.59	517±70	51.4±6.9	12.5	0.478

+117%

H10 STEM LENGTH VS CONTROL

+41%

H10 FRESH MASS VS CONTROL

+36%

H10 DRY MASS VS CONTROL

NO PHYTOTOXIC SIGNAL

Chlorophyll content remained close to the control range. H10 also increased root volume by 24% and surface area by 14%.

FERTILIZER PRODUCTION ROUTE

1 MBM FEEDSTOCK

Protein- and mineral-rich meat and bone meal.

2 ALKALINE HYDROLYSIS

50% KOH; 100°C; S:L = 1:1; 1 h.

3 NEUTRALISATION

Hydrolysate adjusted to pH 6.0.

4 MICRONUTRIENT ENRICHMENT

Cu and Mn salts added.

FORMULATION-DEFNING STEP

The neutralising acid determined the final phosphorus-to-sulfur balance.

F1 H_3PO_4

Phosphoric acid neutralisation → P-rich formulation.

F2 H_2SO_4

Sulfuric acid neutralisation → S-rich formulation.

5–7 GRANULATION AND FINISHING

Dry peat : zinc lignosulfonate=20:1 → rolling for 15 min → drying for 48 h → sieving to the 2.5-5 mm fraction.

TWO COMPLEMENTARY PRODUCTS

Identical finishing steps produced comparable granule morphology while retaining the acid-dependent P/S differences.

GRANULE MORPHOLOGY

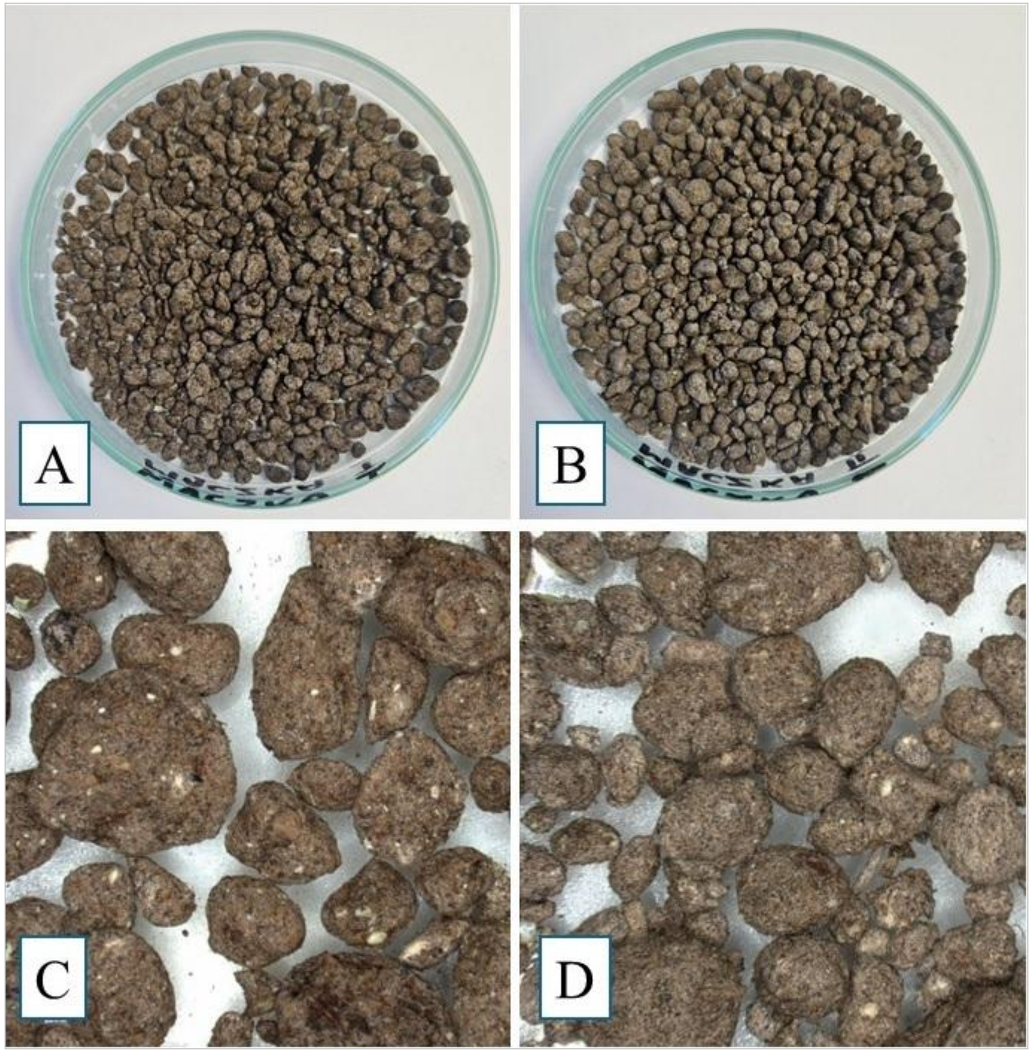


Figure 3. *Granular MBM-based fertilizers: F1 (A, C) and F2 (B, D)*

FORMULATION IDENTIFICATION

F1 - PANELS A AND C

Granules neutralised with phosphoric acid (H_3PO_4).

F2 - PANELS B AND D

Granules neutralised with sulfuric acid (H_2SO_4).

MORPHOLOGICAL OBSERVATIONS

- Both formulations showed similar dark-brown colour and particle-size distribution.
- The compact structure confirmed successful incorporation of organic and mineral components.
- Irregular surfaces were attributed to the peat binder.
- Small white inclusions were consistent with residual bone fragments in MBM.

FERTILIZER MACRONUTRIENT COMPOSITION

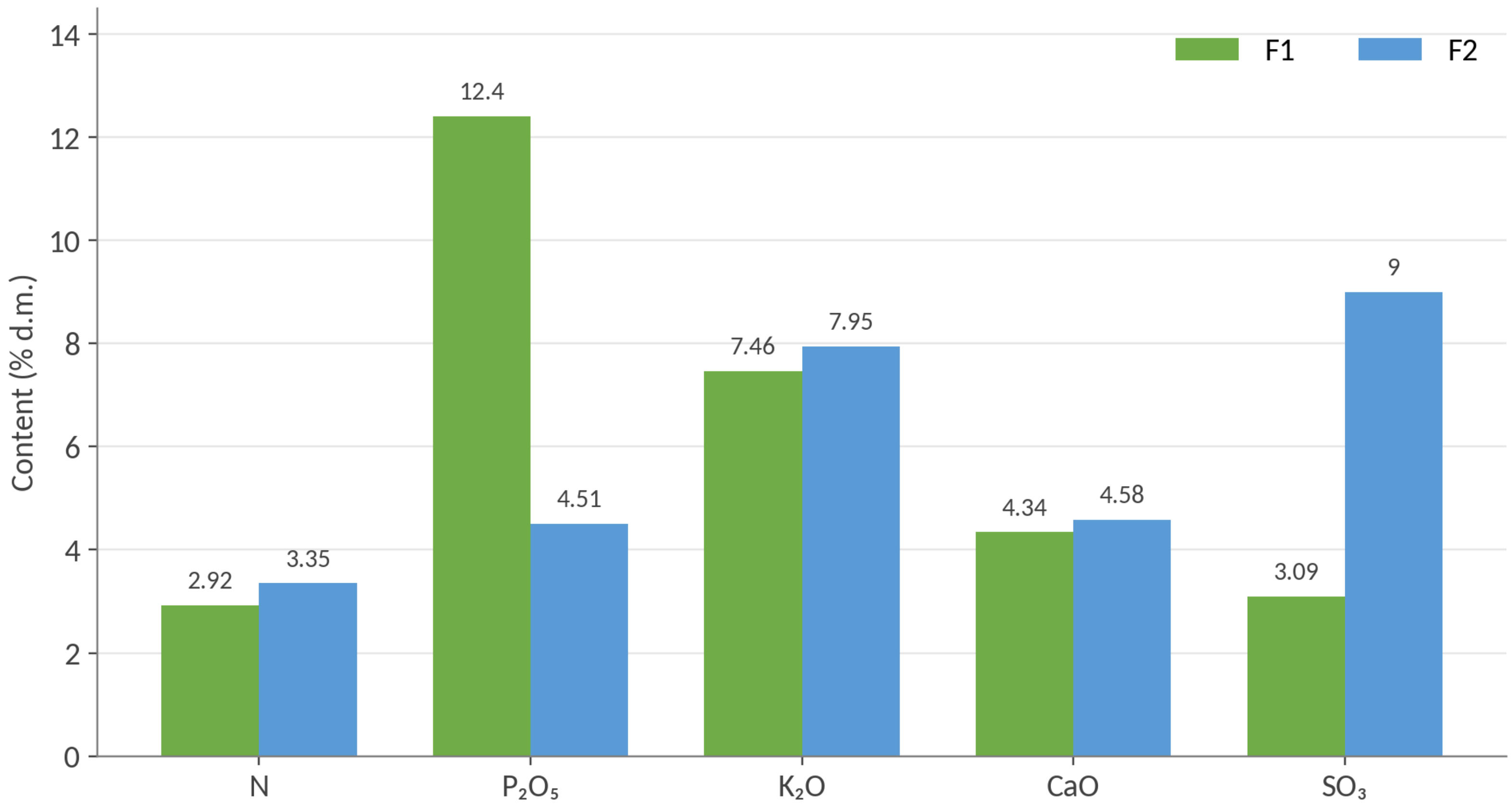


Figure 4. *Major macronutrients in formulations F1 and F2*

F1 - PHOSPHORUS-RICH

12.4±2.5% P_2O_5 and 3.09±0.62% SO_3.

F2 - SULFUR-RICH

4.51±0.90% P_2O_5 and 9.00±1.80% SO_3.

SIMILAR BASE NUTRITION

Both products contained about 7.5-8.0% K_2O and 4.3-4.6% CaO.

F2 N:P:K ratio = 1:1.35:2.37 more balanced

MICRONUTRIENTS AND TOXIC-ELEMENT SAFETY

MICRONUTRIENTS

Table 9. *Micronutrient content in fertilisers F1 and F2*

Material	Cu *(mg/kg)*	Fe *(mg/kg)*	Mn *(mg/kg)*	Zn *(mg/kg)*
F1	7,999±1,560	867±130	7,539±1,508	1,212±242
F2	8,368±1,674	443±66	8,309±1,662	1,423±285

TOXIC ELEMENTS

Table 4. *Toxic elements content in fertilisers F1 and F2*

Material	As *(mg/kg d.m.)*	Cd *(mg/kg d.m.)*	Cr *(mg/kg d.m.)*	Ni *(mg/kg d.m.)*	Pb *(mg/kg d.m.)*
F1	<LOD	0.110±0.022	1.50±0.23	13.0±1.9	11.1±1.6
F2	<LOD	0.030±0.005	<LOD	14.0±2.0	11.0±0.7

<LOD - below the limit of detection.

✓ ALL MEASURED TOXIC ELEMENTS BELOW APPLICABLE EU LIMITS

COMPOSITIONAL DIFFERENCES

F1 contained approximately twice as much Fe, whereas F2 showed slightly higher Cu, Mn and Zn.

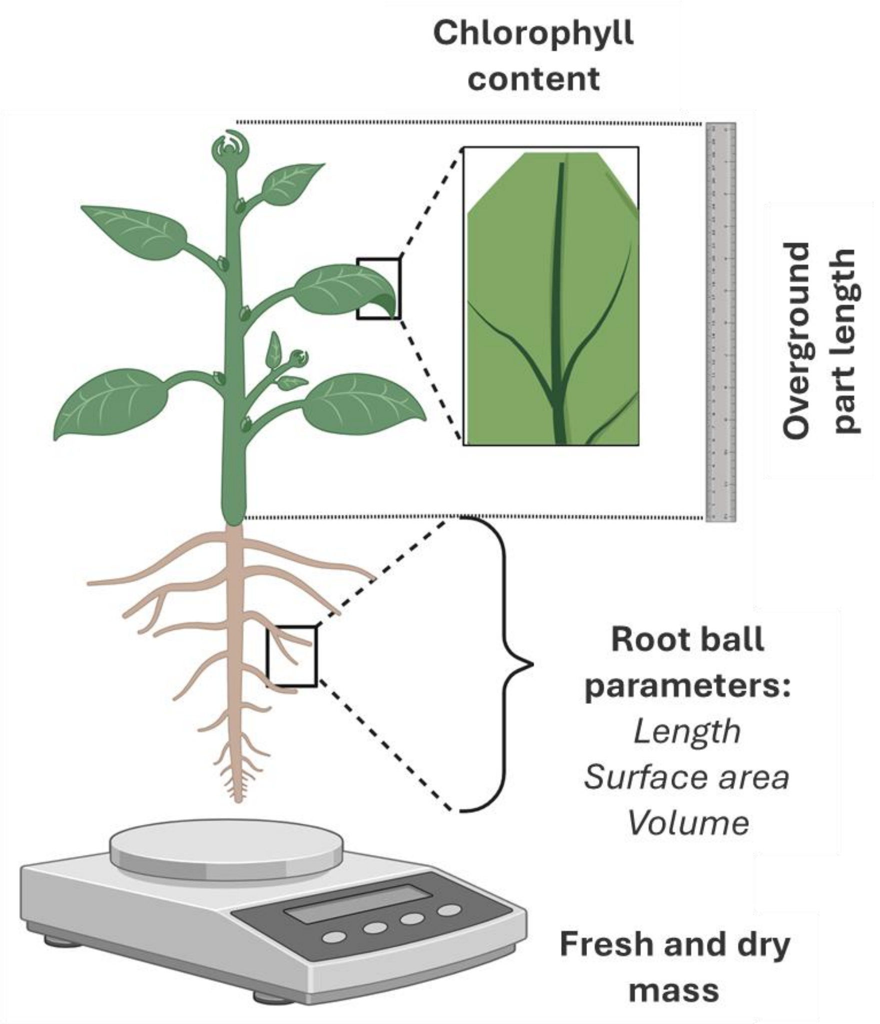
POT TRIAL DESIGN

EXPERIMENTAL CONDITIONS

Test plant	Cucumber (*Cucumis sativus L.*, Cornichon de Paris)
Substrate	Soil:sand = 2:1 (v/v); pH 5.5
Cultivation	10 d pre-growth+20 d after fertilisation=30 days
Environment	22-24°C; 2,400 lux; 16 h light/8 h dark; 60% moisture
Replication	Three replicates per treatment

TREATMENTS

- **W:** unfertilised control.
- **REF:** commercial organo-mineral fertilizer.
- **F1 and F2:** 25%, 50% and 100% of cucumber N requirement.



CUCUMBER GROWTH AND FLOWERING

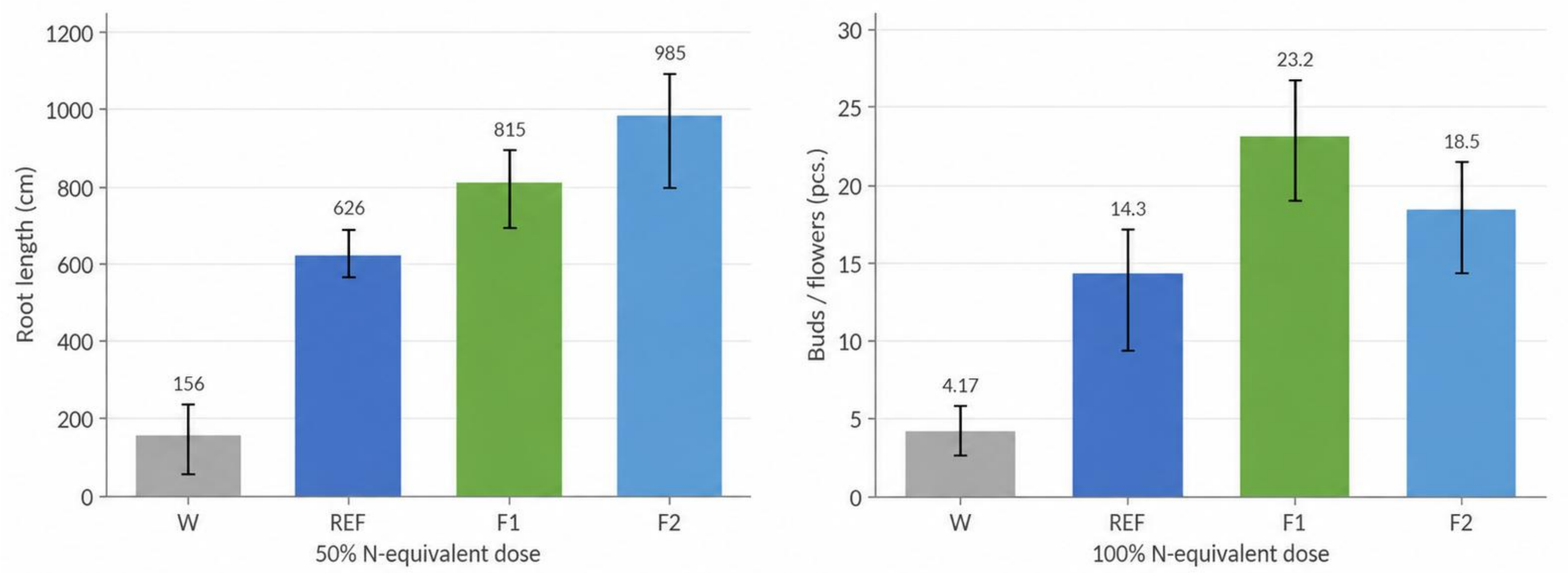


Figure 6. *Effect of MBM-derived fertilizers on cucumber root development and flowering*

985±188 cm
F2/50 ROOT LENGTH
6.3× THE CONTROL

3.02±0.82 g
F2/100 ROOT FRESH MASS

9.25±2.19 g
F2/100 SHOOT FRESH MASS

23.2±4.2
F1/100 BUDS AND FLOWERS

Leaf chlorophyll was statistically comparable to the control across doses, confirming the absence of phytotoxicity.

NUTRIENT ACCUMULATION IN CUCUMBER

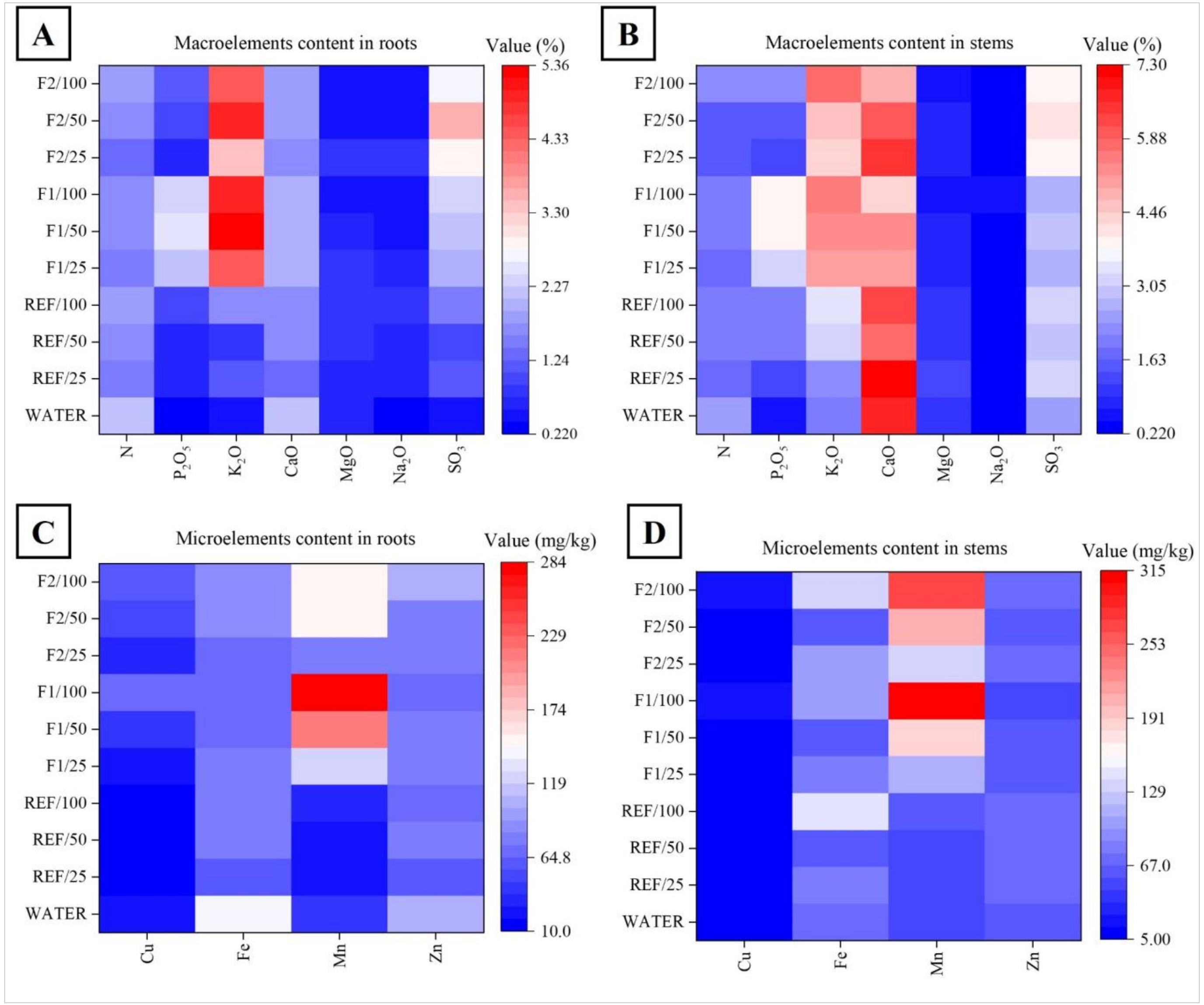


Figure 7. *Heatmaps of macro- and micronutrient contents in cucumber roots and stems*

F1: PHOSPHORUS UPTAKE

P_2O_5 reached 2.56±0.52% in roots (F1/50) and 3.97±0.80% in stems (F1/100).

F2: SULFUR UPTAKE

SO_3 reached 3.49±0.70% in roots and 4.20±0.84% in stems at F2/50.

MICRONUTRIENT ENRICHMENT

F1/100 increased root Cu by 237% and Mn by 616% relative to the water control.

COMPARATIVE PERFORMANCE

Nutrient levels were comparable to or higher than the reference fertilizer.

ECONOMIC POTENTIAL AND MAIN CONCLUSIONS

INITIAL COST ASSESSMENT

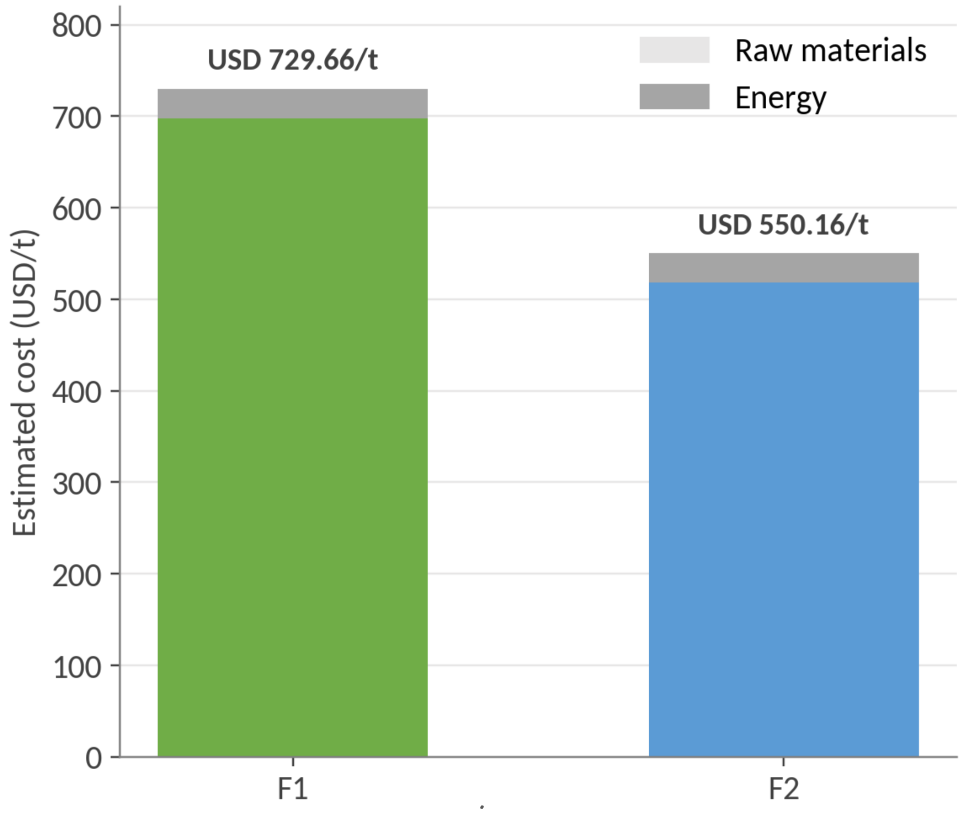


Figure 8. *Costs include raw materials and approximately 200 kWh/t of energy; labor and equipment depreciation were excluded*

MAIN CONCLUSIONS

- Optimised alkaline hydrolysis recovered up to 18,219 ± 427 mg/kg total amino acids from MBM.
- **F1 was P-rich, while F2 was S-rich and had the more balanced N:P:K profile.**
- Both granules were microbiologically safe and low in toxic elements.
- The formulations improved cucumber root architecture, biomass, flowering and nutrient accumulation without phytotoxicity.

ECONOMIC ADVANTAGE OF F2

Estimated total process cost: USD 550.16/t for F2 versus USD 729.66/t for F1. The USD 179.50/t difference (~25%) was driven mainly by the neutralising acid.

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