



Diversifying revenue in rural Africa through circular, sustainable and replicable biobased solutions and business models

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List of Abbreviations

°C	Celsius
CIRAD	Centre de Cooperation Internationale en Recherche Agronomique pour le Developpement - C.I.R.A.D. Epic
CH ₄	Methane
CO	Carbon Monoxide
CO ₂	Carbon Dioxide
D	Deliverable
DFF	Days to 50% Flowering
ENERGECO	Societe d'Economie d'Energie et d'Electro-Mecanique Energeco
EU	European Union
Fig.	Figure
FTIR	Fourier-Transform Infrared Spectroscopy
H	Hypothesis
H ₂ O	Water
H ₂ S	Hydrogen Sulphide
Ha	Hectare
HPLC	High-Pressure Liquid Chromatography
HTC	Hydrothermal Carbonisation
IHE	Stichting IHE Delft Institute for Water Education
INP-HB	Institut National Polytechnique Felix Houphouet-Boigny
IPAR	Intercepted Photosynthetically Activate Radiation
Kg	Kilogram
KRC	Kabarole Research and Resource Centre

L	Litre
LAI	Leaf Area Index
M	Metre
M	Month
MTU	Munster Technological University
N	Sample size
N ₂	Nitrogen gas
NIR	Near Infra-Red
No.	Number
NTU	Nephelometric Turbidity Units
PHA	Polyhydroxyalkanoate
PM	Particulate matter
QPLAN	Q-PLAN INTERNATIONAL
RAGT	RAGT ENERGIE SAS
Stylo	<i>Stylosanthes guianensis</i>
T	Tonne
TRH	Hydraulic Retention Time
TRL	Technological Readiness Level
UASZ	University Assane Seck of Ziguinchor
μS/cm	MicroSiemens per centimetre
UV	Ultra-violet
WP	Work package

1. Executive Summary

This document details the planned pilot trials in four African countries, **Uganda, Ghana, Côte d'Ivoire, and Senegal**, of the European Union Horizon 2020-funded **BIO4AFRICA** project. The document describes the trial timelines and parameters to be examined, and results of trials initiated up to and during the **interim trial reporting period (M25-34)**, where available. Three main categories of product are being piloted through a total of 22 different pilot trials: **animal feed** and **whey** from green biorefinery technology, **biochar** products from carbonisation technology, i.e. slow pyrolysis and hydrothermal carbonisation (HTC), and **pellets** and **briquettes** from densification technologies. These products have been adapted to local needs and contexts to develop specific use cases, which are also examined in the pilot trials.

This deliverable builds on the knowledge generated through **WP1** and **WP2** of the BIO4AFRICA project, namely the identification and adaptation of small-scale, bio—based technologies that can add greater value to agri-food residues and other low-value bio-based materials. In large part, the pilot trials follow directly from the technology testing and piloting being carried out in **WP3** and outlined in **D3.1-3.3**. Work on the pilot trials began in **M18** (November 2022), after completion of initial work in other work packages, and initial trial plans and results were described in **D4.1 (M24)**.

Nine pilot trials were underway during the interim trial reporting period, using both locally available and novel technologies, and low-value agri-food residues to create novel, bio-based products. These include:

- In Uganda (**section 4**): screening of biorefinery whey for high-value components.
- In Ghana (**section 5**): soil amendment field trials with biochar (continued from initial trial reporting period), screening of biorefinery whey for high-value components, fish feeding trials with pellets made of agri-food residues.
- In Côte d'Ivoire (**section 6**): soil amendment greenhouse trials with biochar (continued from initial trial reporting period), water filtration trials with biochar, rabbit feeding trials with pellets made of agri-food residues.
- In Senegal (**section 7**): solid fuel (cooking fuel) trials with biochar briquettes (continued from initial trial reporting period), biochar as a biogas production improvement additive.

Results for nine trials in Uganda, Ghana, Côte d'Ivoire and Senegal have been provided in this report (**section 8**). Product production and use case parameters have been explored through these trials, enabling the evaluation of biorefinery whey protein content (Uganda and Ghana), sheep feed pellets using local forages with anthelmintic properties (Côte d'Ivoire), biochar as a soil amendment (Ghana and Côte d'Ivoire), and biochar-based solid fuel (cooking fuel) and biochar as a biogas production improvement additive (Senegal). The outcomes have fed into the validation of the circular bio-based business models (Task 5.3) and will support the development of BIO4AFRICA business plans (Task 6.3).

The report also describes the next steps for the pilot trials in **WP4**. The trials initiated during the **interim reporting period** will continue into the **final trial reporting period**, including comparison of products from local technology with improved technologies, e.g. Brazilian kiln technology. The remaining pilot trials will begin during the **final trial reporting period**. Final results for all trials will be available during the **final trial reporting period**. These will be included in **D4.4: Final report on trials and results (M44)**.

2. Introduction

This document describes the pilot trial strategies and initial results of the four pilot cases of the BIO4AFRICA¹ project, in Uganda, Ghana, Côte d'Ivoire, Senegal and Kenya. The BIO4AFRICA project has the primary aim of supporting local bioeconomy development in rural African regions. The project, comprising Partners from five African countries (Uganda, Ghana, Côte d'Ivoire, Senegal, and Kenya) and six European countries (Denmark, France, Greece, Ireland, the Netherlands, and Spain), was initiated in June 2021 (**M1**), and has a duration of 48 months, finishing in May 2025 (**M48**).

2.1 Pilot Trial Report

The pilot trial period has been scheduled to take place between **M18-M44** of the Bio4Africa project. This report is the second of three to report pilot trial results at different phases of the pilot trial period: initial results, interim results, and final results. This report presents the pilot trial strategies, including methodologies for each of the pilot case countries, and results of pilot trials that have been completed. This report is presented at the end of the interim period of the implementation of the pilot trials (**M34**), with a number of trials underway, and results pending completion of the trials. The final results will be described in the **Final Report (D4.4, M44)**. The following sections describe the pilot trials methodology and products (**Section 3**), the pilot trials being carried out from each country: Uganda (**Section 4**), Ghana (**Section 5**), Côte d'Ivoire (**Section 6**), and Senegal (**Section 7**), and available pilot trial results (**section 8**), with a final section describing conclusions and the way forward for the pilot trials over the next 10 months during the final reporting period (**Section 9**).

2.2 BIO4AFRICA Project Strategy

BIO4AFRICA aims to support the bioeconomy in rural African regions through the development of circular, bio-based solutions and value chains to promote the cascading use of local resources and income diversification for agrarian communities. In order to achieve this, the project is supporting the implementation of small-scale, robust bio-based technologies with high replication potential and adapted to local needs, socio-economic and agri-environmental conditions, and biomass types. The technologies involved have been co-defined by the BIO4AFRICA partners (**WP1**) and adapted for local conditions, biomass types, and integrated in viable combinations (**WP2**), to support development of novel, bio-based business models (**D5.2: Inclusive and sustainable bio-based business models for rural Africa**). Three technology types in particular are being combined and transferred: small-scale green biorefineries, carbonisation, including slow pyrolysis and hydrothermal carbonisation (HTC), and densification technologies, e.g. briquetting and pelletizing. Bio-composite and bioplastic production are also being evaluated at laboratory-scale, while

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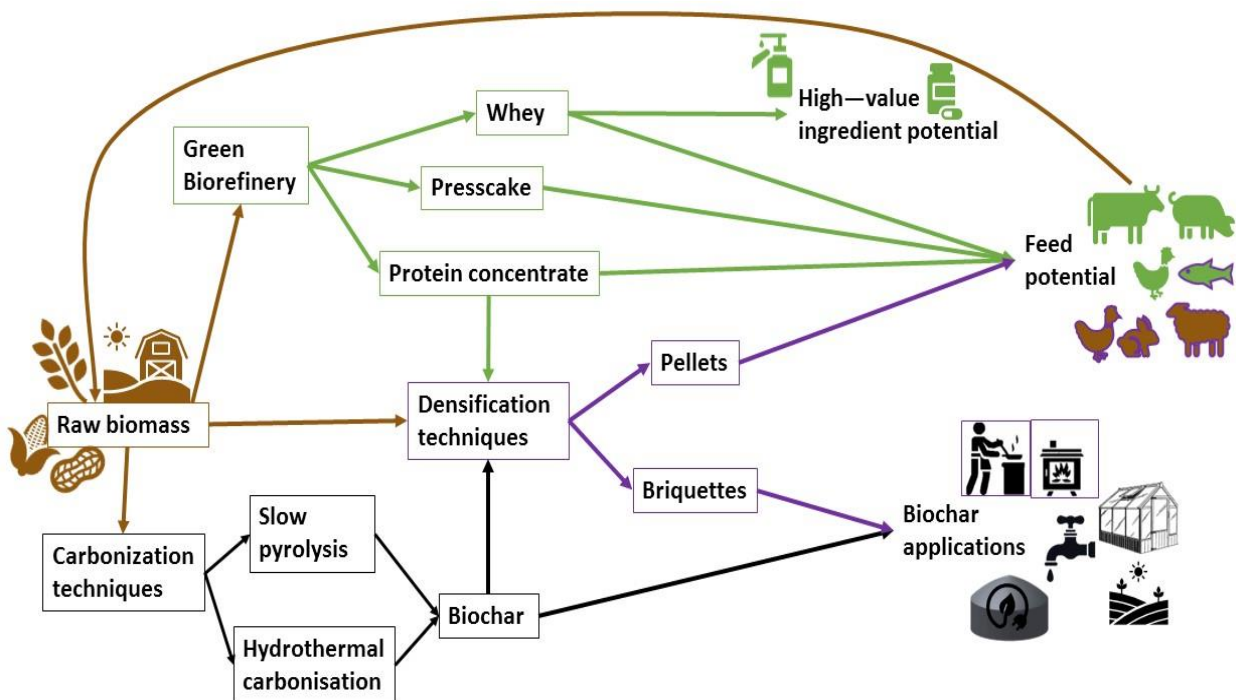
screening of bio-based products for further value addition opportunities is taking place, e.g. high-value components of bio-based side-streams.

A total of four pilot cases in Uganda, Ghana, Côte d’Ivoire, and Senegal, with 18 technology and product testing sites across the cases, is allowing farmers and farmer groups to test these products in their local context. The use of novel biomass types in existing local technologies, e.g. local pyrolysis technology, and in the novel, adapted technologies, e.g. green biorefinery, can enable farmers to add value to local biomass and produce diverse bio-based products, including:

- biomass pellets as animal feed
- biochar as a soil amendment product
- biochar as a solid biofuel product
- biochar as an additive to enhance biogas production
- biochar for H₂S treatment in biogas production
- biochar powder for water filtration
- green biorefinery press cake as ruminant feeds (e.g. cattle)
- green biorefinery protein concentrate as a feed supplement for pigs and poultry
- whey as animal feed for piglets
- fish feed pellets, including pellets that include green biorefinery protein concentrate

In addition, the potential for further value addition through side-stream valorisation, e.g. biorefinery whey extracts, is being explored through screening trials of whey applications. These products and applications are described in **Fig. 1**.

Figure 1: Diagram of bio-based technologies and products to be transferred and piloted during the BIO4AFRICA project



The implementation of pilot trials using existing, local technologies and novel, adapted technologies allows farmers and other local, bio-based value chain actors to compare the benefits of adapted technologies with local technologies, under their own agro-ecological and socio-economic conditions, e.g. soil, climate, agricultural practices, prevailing ownership models, market access, etc. This process acknowledges the influence of local and regional “Agricultural Knowledge and Information Systems” for sustainable agri-food system development (Klerkx *et al.*, 2012). **Table 1** summarises the technologies, biomass types (inputs), and products (outputs) that are being evaluated, including those being evaluated at pilot-scale.

Table 1: Testing and validation activities in BIO4AFRICA project (l = laboratory-scale validation tests, p = pilot-scale validation tests; s = product application screening tests)

Country	Technologies / processes	Inputs	Outputs	Validation tests
Uganda	<ul style="list-style-type: none"> Green biorefinery Carbonisation (hydrothermal carbonisation) Densification (briquetting) 	<ul style="list-style-type: none"> Protein-rich leguminous plants, cassava leaves Napier (elephant) grasses Manure from cattle/dairy cows Green biorefinery whey Biochar for briquetting 	<ul style="list-style-type: none"> Animal feed: <ol style="list-style-type: none"> Press cake for ruminants, Protein concentrate for pigs & poultry, Whey as animal feed for pigs and for high-value ingredients screening Biochar briquettes for cooking fuel Biochar with struvite & manure for soil improvement 	<ul style="list-style-type: none"> Animal feed trials (dairy cows, pigs, piglets, poultry) (p) High value whey ingredients screening (s) Field trials of soil amendments (p) Biochar briquettes for use as cooking fuel (l)
Ghana	<ul style="list-style-type: none"> Green biorefinery Carbonisation (slow pyrolysis) Densification (pelletizing) 	<ul style="list-style-type: none"> Various local forage species Green biorefinery whey Green biorefinery protein concentrate for pelletizing Crop residues (corn cobs, soybean husk, cowpea husk, rice bran, cassava peels, groundnut husk, maize stalks, cocoa husk) 	<ul style="list-style-type: none"> Animal feed: <ol style="list-style-type: none"> Press cake for ruminants, Protein concentrate for fish & pigs, Whey as animal feed for pigs Protein concentrate pellets as fish feed Biochar for soil improvement 	<ul style="list-style-type: none"> Animal feed trials (dairy cows, bulls, pigs, piglets) (p) Aquaculture feed trials (Tilapia and catfish) (p) High value whey ingredients screening (s) Field trials of soil amendments using biochar (tomatoes, okra, chilli pepper) (p)

Côte d'Ivoire	<ul style="list-style-type: none"> • Carbonisation (slow pyrolysis) • Densification (pelletizing) • Bioplastics & bio-composites 	<ul style="list-style-type: none"> • Cocoa pod shells • Cashew nut • Cashew shells • Cashew apple juice & molasses • Millet husks/stems • Leafy green biomass: <i>Cajanus cajan</i> (pigeon pea), <i>Leucaena leucophela</i> leaves, <i>Stylosanthes guianensis</i> (Stylo) leaves • Rubber seed • Coconut fibre • Palm tree branch fibre 	<ul style="list-style-type: none"> • Biomass pellets for animal feed • Biochar granules for adsorption of water pollutants • Biochar for soil improvement • Bio-composites/bio-plastics 	<ul style="list-style-type: none"> • Animal feed trials (sheep, rabbits, poultry) (p) • Tests of water filters using biochar (l, p) • Bioplastics/bio-composites tests (l) • Greenhouse and field trials of soil amendments (tomato and maize crops) (p)
Senegal	<ul style="list-style-type: none"> • Carbonisation (hydrothermal carbonisation & slow pyrolysis) • Densification (briquetting) • Bio-composites 	<ul style="list-style-type: none"> • Peanut shells • Cashew hulls/apples • Rice husk • Typha • Corn cob 	<ul style="list-style-type: none"> • Biochar briquettes for solid fuel (cooking fuel) • Biochar as biogas production additive & biogas pollutant adsorbent • Bio-composites 	<ul style="list-style-type: none"> • Solid fuel (cooking fuel) tests (l, p) • Anaerobic digestion tests: biogas production with biochar additives and pollutant adsorption (p)

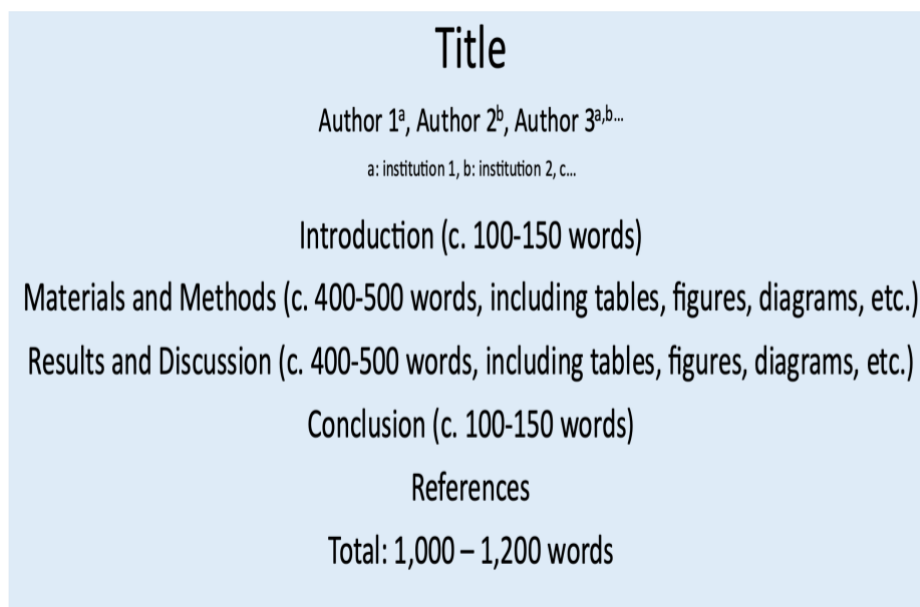
At least 300 farmers, farmer groups and other local bio-based value chain actors are expected to benefit from the pilot case trials, including pastoralists, small dairy farmers, low-income farmers, and female farmers. The pilot cases are embedded in a multi-actor, collaborative, and evidence-based value chain development strategy that engages communities, extension services, policy development, business supports and science and technology specialists, in the development of 10 sustainable business models, including life cycle analysis of the products developed. This approach should result in performance improvements for the triple bottom-line of local agri-food systems in Uganda, Ghana, Côte d'Ivoire and Senegal, i.e. environmental, economic, and social performance.

3. Pilot Trials: Methodology and Products

3.1 Methodology

Trial results are reported using a standardised reporting template (**Fig. 2**). This has been modelled on scientific abstracts, such as those used for conference submissions. The reporting template therefore supports those implementing trials to communicate the trial outcomes more broadly, e.g. in conferences and peer-reviewed journals. Trial reports for completed trials are provided in **section 8**.

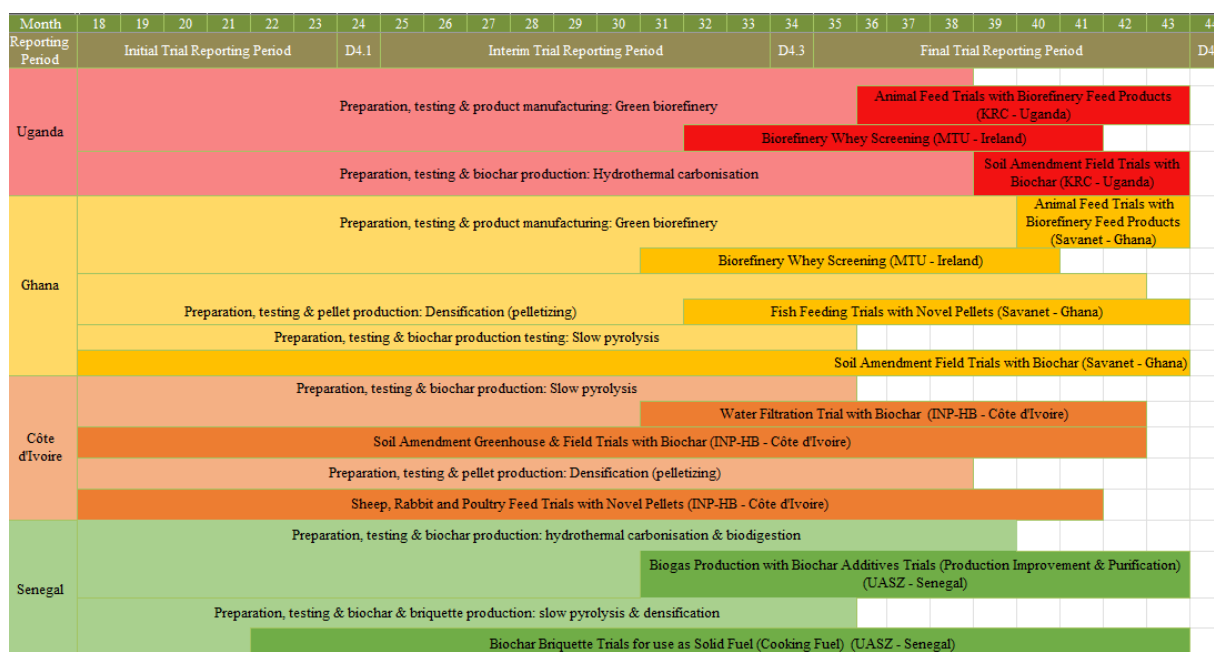
Figure 2: BIO4AFRICA Standardised Trial Reporting Template



3.2 Technology Types and Products

Fourteen types of novel bio-based product are being tested at pilot trial scale or screened for novel applications (biorefinery whey), from three of the technology types to be implemented in the BIO4AFRICA project, **green biorefinery**, **carbonisation**, and **densification**, and combinations of those technologies. Those products that are being evaluated during the pilot trials, and a short description of the technologies involved in their production, are described below. An overview of the pilot trial plan is provided in **Fig. 3**. Due to variation in local technology availability and technology adaptation requirements, trials in some countries have been able to start earlier than in others. In the **initial trial reporting period (M18-24)**, trials in two main trial categories began: biochar trials (Ghana, Côte d’Ivoire, Senegal), and raw biomass pellet feeding trials (Côte d’Ivoire). A number of other trials began during the **interim trial reporting period (M25—34)**, including green biorefinery whey screening trials in Uganda and Ghana, and pellet feeding trials in Ghana. All other trials will begin during the **final trial reporting period (M35-44)**. The technologies and associated product testing plans are described in greater detail in **D3.1**, the **Initial Testing, Monitoring and Assessment Plan** and its updates, included in **D3.2** and **D3.3**.

Figure 3: Overview of pilot trials taking place between M18-M44 of the BIO4AFRICA project



3.2.1 Green Biorefinery

Green biorefinery involves mechanical refining of leafy biomass to generate multiple bio-based value streams, including a silage “press cake” that can be fed to ruminants, a protein concentrate that can be fed to monogastric animals, e.g. poultry, pigs and fish, and concentrated whey that can be used as animal feed (piglets), silage preservative, fertiliser, and can contain high-value components with potential applications as nutraceuticals and cosmeceuticals. Suppliers of the leafy feedstock thus gain additional revenue opportunities compared with grazing alone, while also generating a local supply of high-protein animal feed (protein concentrate) and storable ruminant fodder that contributes to animal productivity and does not compete with human food uses, and fertiliser that can be sold or returned to feedstock-producing fields. The BIO4AFRICA project also examines the potential of “synergy forages”, e.g. green, leafy residue from sweet potato and banana, to be used in the green biorefinery, which would add value to these feedstocks that might not otherwise be exploited.

The small-scale systems being implemented in the BIO4AFRICA project enable co—location near feedstock producers, reducing environmental footprint and economic costs associated with transport and ensuring good feedstock quality due to transport distances being relatively short. The local provision of high-protein animal feed also has the potential to increase the economic efficiency and reduce the environmental impact of animal rearing, due to less dependence on high-cost, imported feedstuffs, e.g. soy.

The green biorefinery technology being utilised in the BIO4AFRICA project, corresponding to **no. 18** in **D1.3 (Catalogue of small-scale bio-based technologies suitable for rural Africa)**, is close to commercialisation in the EU, but is considered to have a Technology Readiness Level (TRL) of 5-7 in the African context, due to the very different type of feedstocks available. This technology is described in **D2.3 Small-scale green biorefinery**

units - initial version and results of adaptation to the African context will be described in **D2.10 Small-scale green biorefinery units – final version (M42)**

Press Cake

Ruminant fodder in the form of fibre-rich press cake from green biorefinery offers some advantages over other livestock feed types. These advantages derive from its suitability for storage and transport, good nutrient conversion by livestock, and the yield of useful co-products from the press cake production process. The co-products add more value to the feedstock than if it were grazed or ensiled in a traditional way, e.g. protein concentrate that can be fed to monogastric animals, and whey (residual juice) that is high in soluble sugars and minerals (Jørgensen *et al.*, 2022; Serra *et al.*, 2023).

The press cake is storable and transportable when baled and ensiled. This enables farmers to have greater access to appropriate livestock fodder on a year-round basis. Greater fodder access can give more security for urban and peri-urban farmers, as well as rural farmers, and improve resilience to challenging climatic conditions, e.g. as experienced in the Tamale region of Ghana where the biorefinery is being implemented. In this way, the green biorefinery can support climate change adaptation.

Figure 4: Biorefinery press cake produced in Uganda



Protein Concentrate

Protein concentrate is one of the main products of green biorefinery, comprising precipitated proteins from the juice fraction of leafy biomass (**Fig. 5**). This is dried and powdered, and fed to monogastric animals such as pigs, or it can undergo **densification**, e.g. using pelletizing equipment, to produce protein-rich **feed pellets** that are more easily consumed by smaller monogastric animals such as chickens, and fish (**see section 3.2.3**). Extracting a substantial portion of the protein content of grass from the fibrous portion increases the value of the feedstock material, generating appropriate feedstuffs for a broader range of livestock, increasing local feed availability across different production systems, and contributing to greater overall efficiency of regional agri-food systems (Jørgensen *et al.*, 2022).

Figure 5: Biorefinery protein concentrate drying in Uganda



Whey

The whey, which is the residual juice fraction following protein precipitation (see **Fig. 6**) typically contains soluble carbohydrates, minerals, and proteins, especially non-protein nitrogen compounds, with the specific composition depending on the feedstock and precipitation process used (Jørgensen *et al.*, 2022). This whey has multiple uses, e.g. production of amino acid concentrates and other valuable biochemical “building blocks” and metabolites relevant for biotechnological applications, use as a sugar—rich animal feed, fertilizer, and a silage preservative, and co-digestion in anaerobic digestion systems, resulting in energy production and digestate that can be applied as fertilizer (Jørgensen *et al.*, 2022; Ravindran *et al.*, 2022). These uses can also be exploited in a cascading fashion, e.g. metabolite extraction followed by anaerobic digestion to produce energy and fertilizer (Ravindran *et al.*, 2022). This project is examining application as animal feed (piglets) and the biochemical composition and potential high value uses, e.g. as nutraceuticals and cosmeceuticals.

Figure 6: Protein and whey collection tanks at small-scale green biorefinery in Uganda



Design considerations

Adaptation of the small-scale green biorefinery technology for use in the BIO4AFRICA test sites in Uganda and Ghana has involved careful consideration of biorefinery unit design and each stage of the biorefining process. These are summarised below and are described in detail in **D2.3 (Design of green biorefinery)** and **D3.1 (Initial version of testing, monitoring and assessment plan)** and its updates (D3.2, D3.3).

Design

- Structure
 - Five tonne platform capacity, roofed and easy to clean.
 - Soundproofing required if noise-sensitive structures are in the vicinity.
 - One-way system at biorefinery site, feedstock entry point and transport vehicles co-located.
 - Bulky products (whey and press cake) exit on the same side as feedstock entry and transport vehicle location.
 - Dried protein concentrate exits on the opposite side of feedstock entry.
 - Other facilities include an office, laboratory, and dressing room for operators.
- Transport and storage
 - Refrigerated trucks advisable when transport time from field to biorefinery is greater than one hour.
 - Refining should take place within 4 hours of fresh leaf harvest¹, or longer if cold transport and storage are available.
- Testing
 - The following data collection and analyses is taking place:
 - Registration of feedstock and products;
 - visual inspection of feedstock and products;
 - registration of energy consumption.
 - mass balance for refinery performance (wet and dry);
 - quality testing of products;
 - dry matter determination (small oven with temperature as low as 60° C);
 - protein content (N-Kjeldahl) (in future also NIR-analysis);

Biorefinery process

The various steps in the biorefinery process are described below, and a schematic diagram of the biorefinery system is presented in **Fig. 7**.

Feedstock preparation

- Weighing

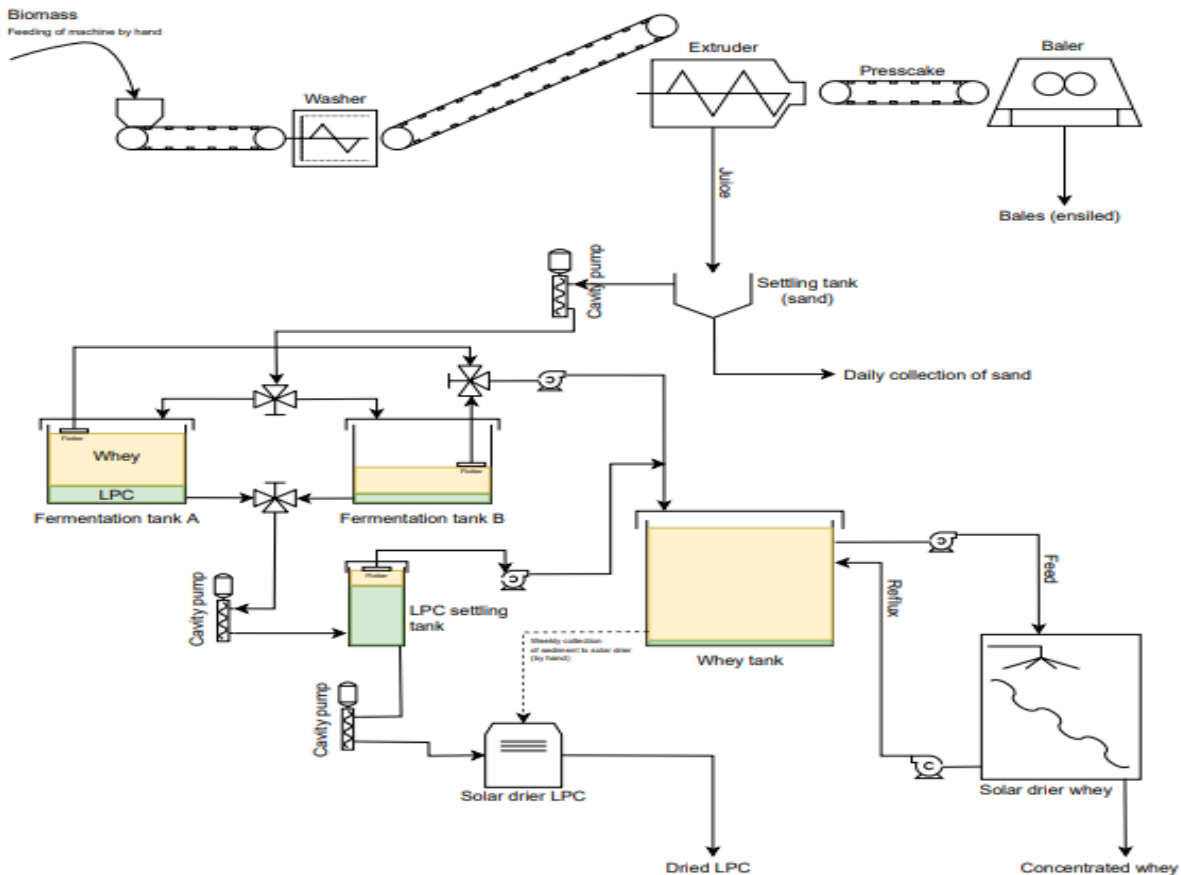
¹ Depending on type of leaves, temperature, and humidity.

- The feedstock is weighed as the first step of the intake process.
- Washing
 - The leaves are washed to remove dirt and other impurities, and are brought into the biorefinery unit by a 5m conveyor belt.

Feedstock refining

- Extrusion
 - The leaves are pressed and squeezed, creating two initial products: **press cake** (ruminant feed) and **juice**.
- Press cake ensiling
 - Press cake enters a baler, via another conveyor belt, where it is pressed and ensiled in bales of 50—60kg for easier transport and storage.
- Sedimentation
 - Juice is collected and enters a sedimentation container to remove any further solid materials, e.g. sand.
 - Juice is pumped to coagulation vessels.
- Protein coagulation and precipitation
 - Juice undergoes a primary precipitation process, resulting in two separate fractions: **whey** and **protein precipitate**. Whey is collected at this stage.
 - The protein precipitate goes through a secondary precipitation process, resulting in further separation of whey, which is again collected, and protein concentration. In the green biorefineries adapted for use in Uganda and Ghana, protein precipitation is *Lactobacilli*-mediated rather than achieved through heating, reducing energy requirements.
- Protein drying
 - The concentrated protein “slurry” is pumped to a paved drying house, with passive solar dryer and a solar-powered ventilator for low wind/high humidity days, where it is spread out and turned until dry.
- Protein concentrate powder
 - The **dried protein** is finally powdered and packed, and can be used as feed directly or condensed into pellets for feeding using **densification techniques (2.2.3)**.
- Whey collection
 - The whey fraction from juice refining is collected in 4m³ steel vessels, that can be treated with UV light to prevent contamination.
- Whey concentration
 - The **whey** is concentrated in a passive solar whey concentrator, to a concentration of approximately 10 times the original concentration.

Figure 7: Biorefinery process



3.2.2 Carbonisation

At least three different approaches for transforming bio-based waste, e.g. rice husk and cashew apple, to novel value-added products is being examined during the pilot phase. These include local slow pyrolysis technologies in Ghana, Côte d'Ivoire, and Senegal (Fig. 8a and b) and pollution-reducing slow pyrolysis technologies (e.g. Brazilian wood-burning kiln) in Ghana, Côte d'Ivoire, and Senegal (Fig. 8c), both of which utilise dry materials. Hydrothermal carbonisation technology (Fig. 8d), which utilises materials with higher moisture content, e.g. animal manure, Typha, and cashew apple, is also being adapted for use in Uganda and Senegal. In addition to adaptation of the technologies to the local context, the project is assessing the viability of different waste feedstocks, and generate specific biochar products to meet specific needs, e.g. cooking fuel, pollutant adsorption, anaerobic digestion additives, and soil amendment.

The Brazilian kiln technology has been designed to combust woody material. The kiln comprises four circular ovens, where the feedstock is carbonized to create biochar, in a process lasting 6-7 days. The ovens are connected to a brick furnace with a 3.5m chimney to collect gases released during pyrolysis. Brazilian kiln pyrolysis has been evaluated as having a TRL of 5-7, with an aim to improve the TRL through adaptation to local feedstocks derived from agri-food sidestreams. The slow pyrolysis technologies implemented in the

BIO4AFRICA project are described in greater detail in **D2.4 (Pyrolysis units – initial version)**, and **D3.1 (Initial version of testing, monitoring and assessment plan)** and its updates (D3.2, D3.3).

Figure 8: Carbonisation technologies in the BIO4AFRICA project using traditional kilns, e.g. kilns from a) Ghana and b) Côte d'Ivoire; adapted, pollution-reducing kilns, e.g. wood-burning kilns from Brazil (c); and hydrothermal carbonisation technology, e.g. as adapted for use in Senegal (d)



a)



b)



c)



d)

The HTC technology to be used in the BIO4AFRICA project is based on the design of Robbiani (2013) and is described in **D2.7 Small-scale hydrothermal carbonization units - initial version** and **D2.8 Small-scale hydrothermal carbonization units - interim version**. This is a highly prospective technology in the African context and is considered to have a TRL of 3-5. The suitability of the technology to wet biomass, e.g. cashew apple and livestock manure, and affordability of implementation make this technology particularly suitable

for pilot locations with wet agri-food sidestreams, e.g. cashew production in Senegal and livestock-rearing in Uganda. Final results of the adaptation of this technology through the Bio4Africa project will be described in **D2.10 Small-scale green biorefinery units – final version (M42)**.

Biochar

Biochar produced through the carbonisation technologies described above is being applied for a number of purposes during the regional pilots. The characteristics of biochar are influenced by pyrolysis temperature and feedstock type, e.g., biochar from slow pyrolysis technologies using dry feedstocks is termed “pyrochar” while biochar from HTC technologies using wet feedstocks is termed “hydrochar”, and there are differences in their characteristics. Hydrochar is rich in nutrients but with low surface area and porosity, while pyrochar is rich in carbon and has less nutrients than hydrochar but higher surface areas and porosity. This gives rise to differences in subsequent applications. For example, in the case of soil amendment using hydrochar or pyrochar, they react differently in soil and each has its own advantages and disadvantages. However, given the uncertainty of the pilot unit in Uganda, it might be more diplomatic to just mention biochar for now. The trials therefore present substantial knowledge and practice development potential, given that they use a variety of agri-food waste sidestreams, even where existing carbonisation technology is used to produce the biochar, e.g. local kilns. This is particularly true of the biochar generated through HTC, due to the relatively low TRL level of this technology compared with the other technologies being examined in the BIO4AFRICA project.

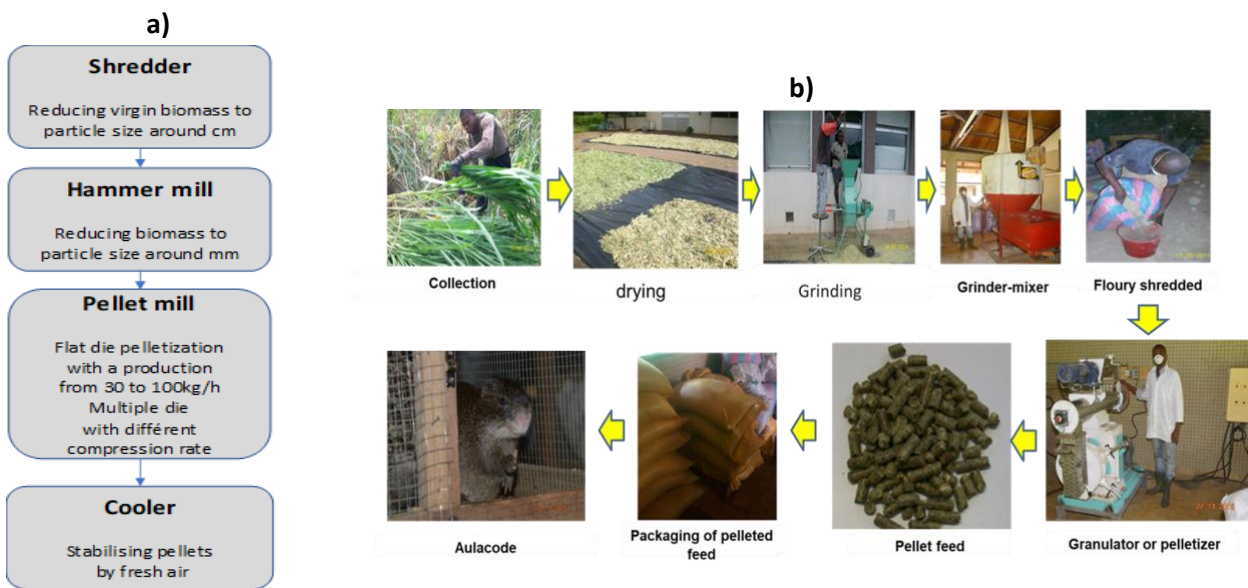
Biochar has diverse impacts on soil properties, including pH, cation exchange capacity, porosity, and soil organic carbon, with implications for fertility, water holding capacity, and nutrient retention, and consequently influencing crop production parameters, e.g. yield (Kamali *et al.*, 2022). In three of the pilot regions, Uganda, Ghana and Côte d’Ivoire, biochar is being applied as a soil amendment in a combination of pot, greenhouse, and field trials, depending on the region, with monitoring of both soil-specific and crop-specific parameters. In this project, biochar is also being further processed using **densification techniques (briquetting, see Section 3.2.3)** to improve its applicability to solid fuel uses, e.g. cooking fuel.

Biochar has the capacity to adsorb pollutants, a characteristic which has been applied for soil remediation (Kamali *et al.*, 2022; Brassard *et al.*, 2019). In the BIO4AFRICA project, this characteristic is being exploited for the purification of water in Côte d’Ivoire, with implications for human health. The pollutant adsorption capacity of biochar also has a beneficial role for anaerobic digestion. Zhao *et al.* (2021) describe the use of biochar for the purification of biogas through removal of other gases, e.g. CO₂ and H₂S, increasing the calorific value of the resulting biogas. The stability and efficiency of biogas production can also be enhanced through biochar addition, as biochar can mitigate the inhibitive effect of compounds arising in the anaerobic digestion feedstock, e.g. heavy metals, antibiotics, and compounds generated during the anaerobic digestion process, e.g. volatile fatty acids (Zhao *et al.*, 2021). The potential for biochar derived from carbonisation of local agri-food sidestreams to enhance anaerobic digestion efficiency and improve the purity of the resulting biogas is being examined in Senegal.

3.2.3 Densification

Densification techniques involve applying pressure to dry materials through different mechanical means, such as flat die or ring die pellet mills, in order to compact and compress the materials into a desired shape and size, e.g. pellets (small size), or briquettes (larger size). If biomass is entire or has large particle size prior to densification, it should pass through a shredding process and hammer mill to reduce particle size, before proceeding to densification and finally cooling (see Fig. 9). These techniques can add greater value to products by improving storability, transportability, and ultimately saleability (Zainuddin *et al.*, 2014). Densification can also adapt biomass and bio-based products, e.g. biochar and protein concentrate, to consumer needs, and especially in the case of livestock feed, making the target feed more ingestible and attractive to animals. The densification technologies implemented in the BIO4AFRICA project are described in greater detail in **D3.1 (Initial version of testing, monitoring and assessment plan)** and its updates (D3.2, D3.3).

Figure 9: a) Schematic description of densification stages (RAGT, 2022) and b) pelletising process for animal feed pellets in Côte d’Ivoire



In BIO4AFRICA, briquetting and pelletising processes are being enlarged to 150-200kg/h, to accommodate the local feedstocks with greater efficiency than that offered in existing systems, allowing better value chain development. Novel feedstocks, in both raw form and transformed through carbonisation or green biorefinery, are also being employed to explore value addition potential for these biomass types. Enlarged densification systems to accommodate raw biomass (Côte d’Ivoire), biorefinery protein concentrate (Ghana) and biochar (Uganda and Senegal), are perceived to have TRL of 5-7, with biochar briquetting in particular being less mature, with TRL of 3-6.

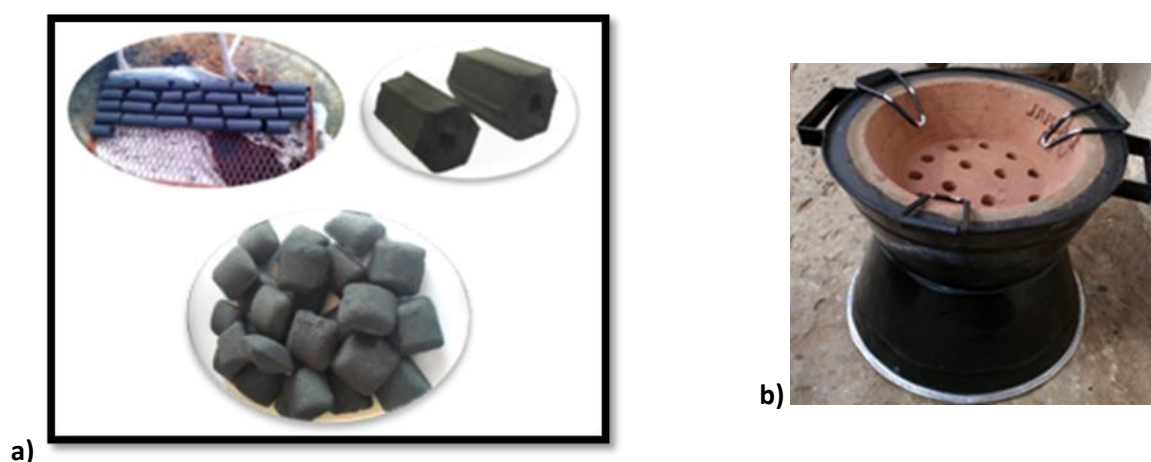
Pellets

Pellets are being produced in the BIO4AFRICA project for animal feed purposes. Protein concentrate from green biorefinery is being processed into pellets in Ghana, in order to make this feedstuff easier to feed to fish and piloted for aquaculture—based fish production. Combinations of novel raw biomass types, e.g. agri—food residues such as rubber seeds, and forage species, are being processed into pellets in Côte d’Ivoire (**Fig. 9b**) and piloted among poultry, sheep and rabbit farmers for feeding and additional outcomes, e.g. anthelmintic effects on intestinal parasites of sheep.

Briquettes

Biochar briquettes from agri—food residues are being produced in Senegal and Uganda for solid and cooking fuel purposes (see **Fig. 10a** for examples). Firewood and charcoal are used as domestic cooking fuel in many countries in sub—Saharan Africa and can result in indoor air pollution and negative health impacts, especially for women and children, in addition to forest degradation and deforestation (Sow, 2022; Chidumayo & Gumbo, 2013). Biochar from agri—food residue has the potential to provide a more sustainable alternative to wood-derived charcoal, while also generating value for feedstock producers. These briquettes are being evaluated and piloted in Senegal, including using improved “Jambar” stoves (**Fig. 10b**) that produce less smoke and are more efficient in fuel use than traditional stoves, when using traditional fuels (wood and charcoal) (Sow, 2022). The results will thus illustrate the viability of biochar briquettes compared with traditional fuels, but also using improved cookstove technology.

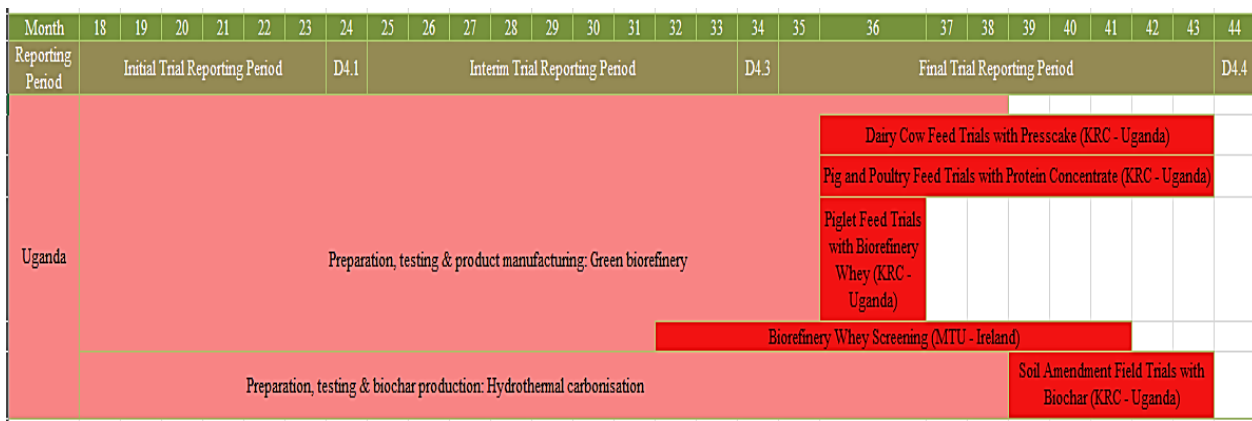
Figure 10: a) Various types of biochar “briquettes” and b) improved “Jambar” stove, used in Senegal



4. Pilot Trials in Uganda

In Uganda pilot trials of green biorefinery products (press cake, protein concentrate, and whey) and HTC products (manure-derived biochar as soil amendment) are being evaluated, with trials led by Kabarole Research and Resource Centre (KRC). An overview of trial plans specific to Uganda is provided in **Fig. 11**. As indicated in this overview, biorefinery whey screening began in **M32** (January 2024) at Munster Technological University (MTU), using whey samples from biorefinery operation during the optimisation process in Uganda. Results from all trials during the final trial reporting period, **M35-M44**.

Figure 11: Overview of pilot trials taking place in Uganda



4.1 Green Biorefinery

Testing and optimisation of the small-scale green biorefinery and biorefinery products is currently underway in Uganda. This includes preparatory trials of feed products with four local farmers and the piggery of the local church on a voluntary, non-commercial basis, e.g. assessing livestock acceptance of press cake as fodder (**Fig. 12**), and informal trials of protein concentrate as a poultry feed. Biorefinery whey screening for high-value ingredients began in Jan 2024 (**M32**) with the delivery of two whey samples, and will continue as whey from different feedstocks becomes available. Pilot trials of green biorefinery products are planned to take place from May 2024 (**M36**).

Figure 12: Dairy cows consuming press cake during pilot trial preparation phase in Uganda



4.1.1 Press Cake

In Uganda, press cake will be trialled with dairy cows, in order to assess its performance as a feedstuff in comparison with a control diet (Elephant grass – *Pennisetum purpureum*). Two experimental treatment diets, each including a different press cake type, and the control diet will be trialled with pregnant dairy cows at six months into gestation. Three replicates will be carried out, with one cow per treatment (total no. dairy cows = nine). The parameters to be examined are described in **Table 2**. The trial is planned to take place in May 2024 (**M36**) and results should be available during the final reporting period (**M35-M44**).

Table 2: Parameters to be examined in press cake feeding trials in Uganda

Dairy cow intake/ performance	Dairy cow manure
Voluntary intake	Organic matter
Milk yield	Nitrogen
Milk composition	Phosphorus
	Potassium

4.1.2 Protein Concentrate

Protein concentrate will be examined in Uganda as a feedstuff for two different animal types: pigs and laying poultry. Pig feed trials will evaluate protein concentrate performance in terms of voluntary intake, growth performance, feed efficiency, and carcass composition/characteristics. Poultry trials will evaluate protein concentrate performance in terms of growth performance, feed efficiency, egg characteristics and production, and carcass composition/characteristics.

Pig feed

Three experimental treatment diets incorporating protein concentrate and a control diet will be trialled with three-month old pigs. Three replicates will be carried out, with two pigs per treatment, one male and one female (total no. pigs = 24). The parameters to be examined are described in **Table 3**. The trial is planned to take place in May 2024 (**M36**), and results should be available during the final reporting period (**M35-M44**).

Table 3: Parameters to be examined in protein concentrate pig feeding trials in Uganda

Pig intake/ performance	Pig slurry
Daily feed intake	Organic matter
Average daily weight gain	Nitrogen
Feed conversion efficiency	Phosphorus
Carcass characteristics/ composition	Potassium

Poultry feed

Preparatory trials of poultry feed began at KRC Uganda in January 2024 (**M32**). The focus is on assessing the effect of protein concentrate on growth of the birds, health of the birds, and quality of the eggs. These trials will supplement the formal trials that are scheduled to begin in May 2024 (**M36**).

For the formal trials, three experimental treatment diets incorporating protein concentrate and a control diet will be trialled with one day old laying hen chicks. Three replicates will be carried out, with 20 birds per treatment (total no. birds = 240). Carcass characteristics will be examined using a sub-sample of two birds from each treatment, at the end of the starting period (eight weeks) and the growing period (16 weeks), in each replicate (total no. of birds = 48). Eggs will be analysed weekly for each treatment, in each replicate. The parameters to be examined are described in **Table 4**. The trial is planned to take place in May 2024 (**M36**), and results should be available during the final reporting period (**M35-M44**).

Table 4: Parameters to be examined in protein concentrate poultry feeding trials in Uganda

Egg characteristics on a weekly basis	Bird performance @ 8 weeks/16 weeks	Chicken manure
Egg weight	Gastro-intestinal tract weight	Organic matter
Shell weight	Caecal weight	Nitrogen
Shell thickness	Dressed carcass weight	Phosphorus
Albumen weight	Carcass skin colour	Potassium
Albumen length & width		
Yolk weight		
Yolk colour		

4.1.3 Biorefinery Whey

The whey fraction of juice from the green biorefinery feedstocks will be evaluated as a feedstuff, by incorporation into weaner piglet diets and examining voluntary intake, growth performance, and feed efficiency. The whey is also being shipped for screening at Munster Technological University (MTU) for high-value ingredients, e.g. bio-active compounds.

Pig feed

Preparatory trials of pig feed using biorefinery whey will begin at KRC Uganda in January 2024 (**M35**). These trials will supplement the formal trials that are scheduled to begin in May 2024 (**M36**).

For the formal trials, three experimental treatment diets incorporating biorefinery whey and a control diet will be trialled with seven-week-old piglets (weaners), in three replicates. Two piglets will be involved in each treatment, one male and one female (total no. piglets = 24). The parameters to be examined are described in **Table 5**. The trial is planned to take place in May 2024 (**M36**), and results should be available during the final reporting period (**M35-M44**).

Table 5: Parameters to be examined in biorefinery whey pig feeding trials in Uganda

Piglet intake/ performance	Pig manure
Daily feed intake	Firmness
Average daily weight gain	Organic matter

Piglet intake/ performance	Pig manure
Feed conversion efficiency	Nitrogen
	Phosphorus
	Potassium

High-value ingredient screening

Samples of green biorefinery whey were transported to MTU in Ireland to evaluate the presence of high-value ingredients, especially bio—active compounds and those with applications for animal and human health, e.g. cosmeceuticals and pharmaceuticals. Screening includes chromatography techniques, including High-Pressure Liquid Chromatography (HPLC), Fourier-Transform Infrared Spectroscopy (FTIR), and biochemical assays. Screening is being carried out for bioactivities relating to digestive, immune, skin and hair health using established biomarkers. The results will provide insight into additional applications of biorefinery whey that could provide greater value addition opportunities compared with use as animal feed, silage preservative, or fertilizer alone, especially if derived through a cascading biorefinery approach, through which the aforementioned known applications could also be achieved. The initial steps for biorefinery whey screening, e.g. planning logistics, began in April 2023 (**M23**). The first whey samples, comprising two samples (Pakchong and Alfalfa) were collected by Grassa in January 2024 (**M32**) and subsequently shipped to MTU (Ireland) in February 2024 (**M33**). Analysis is underway, and results of protein content evaluation for the initial samples are reported in **section 8.1**. Further results will be available during the final reporting period (**M35-M44**).

4.2 Carbonisation

In Uganda, HTC will be used to transform animal manure, e.g. from the feeding trials, into biochar. This will be used as a soil amendment in field trials. The transfer of HTC technology to Uganda is expected to be completed between **M37-39** (June-August 2024), after which biochar production will begin.

4.2.1 Biochar soil amendment

The biochar (hydrochar) produced in Uganda will be used as a soil amendment, and compared with baseline, pre-application data, and manure and struvite applied as a control. Complete Randomized Block Design will be used for designing the pilot-scale field trial experiments, with biochar amount increasing in 20g increments from 0-100g. The pilot trial will examine the effect on crops produced under the experimental and control treatments (high-value short-term crops, e.g. green vegetables, and annual crops, e.g. maize and beans), over the course of two seasons.

Parameters and Timeline

The parameters to be examined are described in **Table 6**. The trial is planned to take place by **M39** (August 2024), after installation of the HTC technology. Results will become available during the final trial reporting period, **M35-M44**.

Table 6: Parameters to be examined in soil amendment trials in Uganda

Soil characteristics	Crop characteristics
Soil physical parameters	Crop health
Soil chemical characteristics	Crop growth
Soil fertility	Crop yield

5. Pilot Trials in Ghana

An overview of trial plans specific to Ghana is provided in **Fig. 13**. As described in this overview schedule, biochar soil amendment field trials began in Ghana in **M18** during the **initial reporting period**, using biochar produced using local pyrolysis technologies. Results of these trials are described in **section 8.2**. Further soil amendment trials using biochar from the adapted Brazilian kiln technology will take place during **final reporting period, M35-M44**. Fish feeding trials using commercially available protein concentrate and agri-food residues, e.g. palm kernel, cassava peels, began during the **interim reporting period (M25-M34)**, and will continue during the **final reporting period**. Screening of biorefinery whey produced during initial biorefinery operation also began during this period, and will continue during the **final reporting period**. Cow, bull, pig and piglet feeding trials using green biorefinery products will also take place during the **final reporting period, M35-M44**.

Figure 13: Overview of pilot trials taking place in Ghana

Month	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	
Reporting Period	Initial Trial Reporting Period						D4.1		Interim Trial Reporting Period								D4.3		Final Trial Reporting Period						D4.4			
Ghana	Preparation, testing & product manufacturing: Green biorefinery																							Dairy Cow and Bull Feeding Trials with Presscake (Savanet - Ghana)				
																								Pig Feeding Trials with Protein Concentrate (Savanet - Ghana)				
																								Piglet Feeding Trials with Biorefinery Whey (Savanet - Ghana)				
															Biorefinery Whey Screening (MTU - Ireland)													
	Preparation, testing & pellet production: Densification (pelletizing)																											
	Preparation, testing & biochar production testing: Slow pyrolysis																											
Soil Amendment Field Trials with Biochar (Savanet - Ghana)																												

5.1 Green Biorefinery

The small-scale green biorefinery has been installed and is being prepared for operation in Ghana, and should be operational later in 2024, and livestock facilities have been prepared, e.g. for pig feeding trials (**Fig. 14**). Biorefinery whey screening for high-value ingredients began in **M31** (December 2023), while further biorefinery product trials are due to start in **M40** (September 2024), following the optimisation of the biorefinery equipment and production of feedstuff during the wet season (May-September).

Figure 14: Pigs and piglets of type likely to be used in feeding trials in Ghana



5.1.1 Press Cake

In Ghana, press cake will be trialled with dairy cows and bull cattle in two separate experiments, in order to assess its performance as a supplementary feedstuff, in comparison with a control diet of pasture grazing only. The nutritional characteristics of the press cake will be examined before feeding.

The press cake trials will involve a randomized trial with two experimental treatment diets, and a control diet treatment (no press cake supplementation). The treatments will be replicated five times with dairy cows and bull cattle in separate trials, with one animal per treatment (dairy cows = 5; bulls = 5). Animals will be housed at night and will have free access to water, mineral lick and after consumption of the press cake, to natural pasture during the daytime. Animals will be of the same age and will be managed for optimal health during the trials.

Parameters and Timeline

The parameters to be examined in each trial are described in **Table 7**. The data collected will be analysed with appropriate statistical software. The trial is planned to take place from September 2024 (**M40**), and results will be available during the final reporting period (**M35-M44**).

Table 7: Parameters to be examined in press cake feeding trials in Ghana

Dairy cow performance	Bull performance
Feed efficiency	Feed efficiency
Feed conversion ratio	Feed conversion ratio
Milk response	Initial weight

Dairy cow performance	Bull performance
Milk composition	Daily weight gain
Faecal Organic matter	Final weight
Hematological parameters	Carcass yield and composition
	Faecal Organic matter
	Hematological parameters

5.1.2 Protein Concentrate

Protein concentrate will be examined in Uganda as a feedstuff for two different animal types: pigs and fish. Pig feed trials will evaluate protein concentrate performance in terms of voluntary intake, growth performance, feed efficiency, and carcass composition/characteristics. In fish feeding trials, protein concentrate will undergo densification treatment first, to convert the concentrate powder to pellets. This is described in greater detail in **section 5.2.1**, below.

Pigs

In Ghana, the protein concentrate feeding trial with pigs will involve a control treatment diet (Treatment 1), containing no protein concentrate but 11.8% fish meal. These will be compared with two experimental treatment diets, containing 10% protein concentrate (Treatment 2) and 8.5% protein concentrate (Treatment 3), respectively. The balance of other dietary components has been adjusted to account for the differences in protein concentrate and fish meal in these three treatment diets (see **Table 8** below).

Table 8: The formulated pig trial treatment diets. Treatment 1=control (0% BIO4AFRICA product); Treatment 2=10% inclusion of BIO4AFRICA concentrate; Treatment 3=8.5% inclusion of BIO4AFRICA concentrate

Diet composition	Treatment1	Treatment 2	Treatment 3
Maize (%)	45.91	47.93	49.73
Brewers spent grain (%)	21.26	23.00	24.73
Fishmeal (%)	11.81	0.00	0.00
Soya bean meal (%)	19.22	16.95	14.80
BIO4AFRICA protein concentrate (%)	0.00	10.12	8.54

Diet composition	Treatment1	Treatment 2	Treatment 3
Oyster shell (%)	1.10	1.30	1.50
Salt (%)	0.50	0.50	0.50
Premix (%)	0.20	0.20	0.20

The experiment is designed as a completely randomized, controlled trial, with five animals randomly allocated to randomly to each of the three dietary treatments (no. pigs = 15). The animals will be weaned piglets of the same weight and age, that have received worming and vaccination treatment. There will be a two-week period of adjustment to the diets, and piglets will be housed and fed individually. Feeding will take place twice a day, at 08:00 and 14:00, with free access to water.

Parameters and Timeline

The parameters to be examined are described in **Table 9**. The data will be analysed with appropriate statistical software. The trial is planned to take place from September 2024 (**M40**), and results will be available during the final reporting period (**M35-M44**).

Table 9: Parameters to be examined in protein concentrate pig feeding trials in Ghana

Pig intake/ performance	Pig slurry
Daily feed intake	Organic matter
Average daily weight gain	Nitrogen
Feed conversion efficiency	Phosphorus
Carcass characteristics/ composition	Potassium

5.1.3 Biorefinery Whey

The whey fraction of juice from the green biorefinery feedstocks will be evaluated as a feed for piglets, examining voluntary intake, growth performance, and feed conversion efficiency. The whey is also being shipped for screening at MTU for high-value ingredients, e.g. bio-active compounds.

Pig feed

The experimental design for feeding piglets with biorefinery whey concentrate will involve five treatments: four experimental weaner diets containing biorefinery whey, and one control treatment without whey. Three seven-week-old piglets will be randomly assigned to each of the treatments (no. piglets = 15). The parameters to be examined are described in **Table 10**. The trial is planned to take place from September 2024 (**M40**), and results will be available during the final reporting period (**M35-M44**).

Table 10: Parameters to be examined in biorefinery whey pig feeding trials in Ghana

Piglet intake/ performance	Pig manure
Daily feed intake	Firmness
Average daily weight gain	Organic matter
Feed conversion efficiency	Nitrogen
	Phosphorus
	Potassium

High-value ingredient screening

As in the case of biorefinery whey produced in Uganda, samples of green biorefinery whey is being transported to Ireland, where MTU is evaluating the presence of high-value ingredients, especially bio—active compounds and those with applications for animal and human health, e.g. cosmeceuticals and pharmaceuticals. Screening includes chromatography techniques, including HPLC, FTIR, and biochemical assays. Screening is being carried out for bioactivities relating to digestive, immune, skin and hair health using established biomarkers. The results will provide insight into additional applications of biorefinery whey that could provide greater value addition opportunities compared with use as animal feed, silage preservative, or fertilizer alone, especially if derived through a cascading biorefinery approach, through which the aforementioned known applications could also be achieved. The first whey sample, (Pigeon pea – *Cajanus cajan*) was collected by Grassa in December 2024 (**M31**) and subsequently shipped to MTU (Ireland) in January 2024 (**M32**). Analysis is underway, and results of protein content evaluation of the first sample are described in **section 8.2**. Results of further analysis with more samples will be available during the final reporting period (**M35-M44**).

5.2 Densification

Protein concentrate from the small-scale green biorefinery will be incorporated into fish feed pellets in Ghana using densification, specifically pelletizing. The pelletizing equipment transforms the dried pellet ingredients

into a compressed, transportable, and storable feed that can be easily handled and fed to animals, including fish.

5.2.1 Fish feed pellets

The fish feed pellets being developed in Ghana use diverse biomass types to create a balanced food for the fish species involved, the Nile Tilapia (*Oreochromis niloticus*) and Catfish fingerlings, with a separate trial for each species. The control treatment diet includes protein sources (fish meal, palm kernel meal, cowpea husk, soybean husk), carbohydrates (fermented corn cob, rice bran, cassava meal/peels), lipids (palm kernel oil, palm oil), vitamins and minerals (premix), salt, and starch or other binders. Four experimental treatment diets are being examined, in which some of the protein content in the “control” pellet ingredients is being substituted with biorefinery protein concentrate.

The trials are being replicated twice, with each species divided into five groups of five (no. tilapia = 30, no. catfish = 30). During the experiment, water quality (acidity, alkalinity, salinity, water temperature, and the rate of water circulation) are also being analysed, and the system of production and feeding schedule is consistent across treatments.

Parameters and Timeline

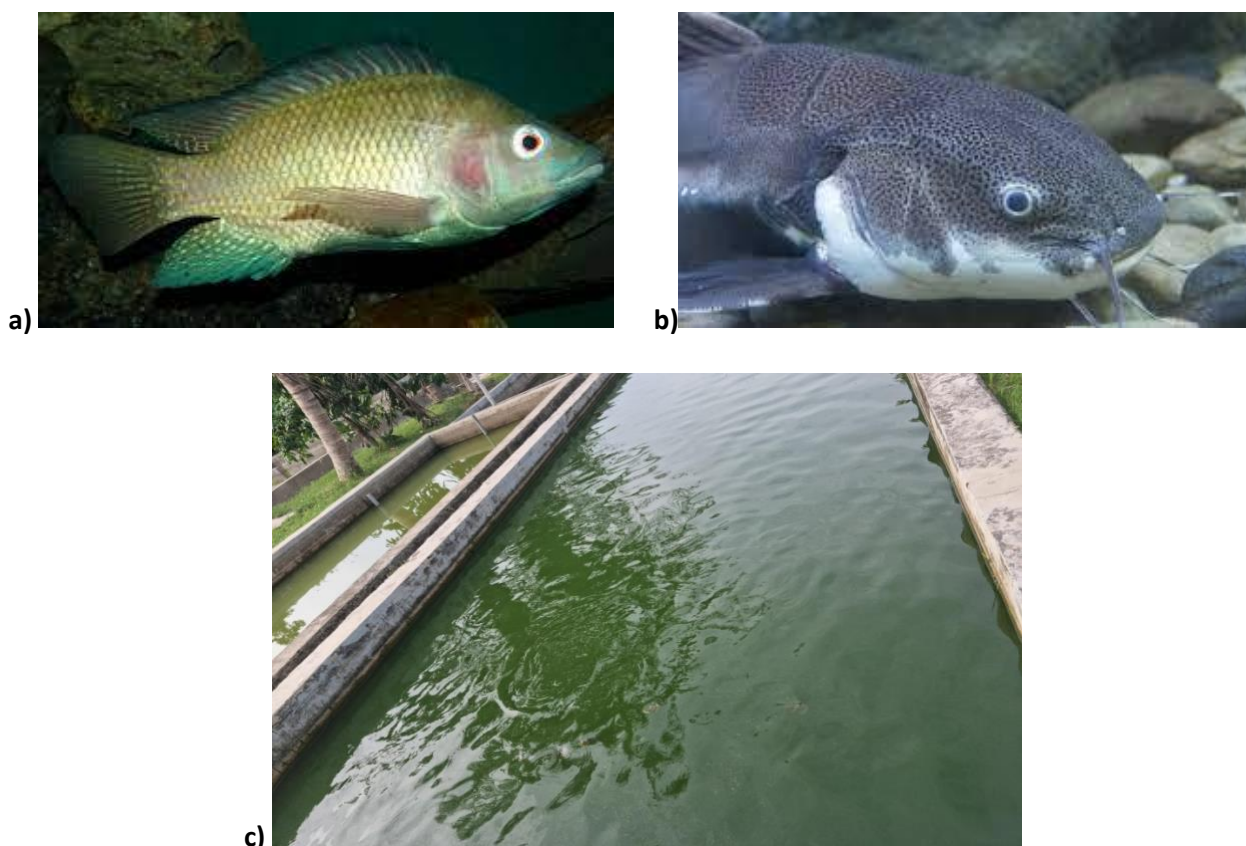
The parameters to be examined are described in **Table 11**, and include growth, health, and nutritional value parameters. The trial began in December 2023 (**M31**) and will continue through the final reporting period (**M35-M44**) using adapted pelletizing technology and protein concentrate from the green biorefinery. Results will be available in the final reporting period (**M35-M44**).

Table 11: Parameters being examined in protein pellet fish feeding trials in Ghana

Fish performance	Biochemical parameters	Hematological parameters	Other
Growth	Total cholesterol	Red blood cells	Digestive enzyme activity in the gastrointestinal system (protease, amylase, lipase)
Feed utilisation	High-density lipoprotein in cholesterol	Haemoglobin	Gene expression of growth-related genes (n = 5)
Digestibility	Low-density lipoprotein in cholesterol	Packed cell volume	

Fish performance	Biochemical parameters	Hematological parameters	Other
Liver histology	Total protein	Mean corpuscular volume	
Proximate composition (Protein, lipid, ash, moisture)	Albumin	Mean corpuscular haemoglobin & haemoglobin concentration	
Fatty acids	Globulin	White blood cells	
Amino acids	Alanine		
	Aminotransferase		
	Aspartate aminotransferase		

Figure 15: Fish species: tilapia (a) and catfish (b), and c) aquaculture pools to be used in fish feeding trials in Ghana



5.3 Carbonisation

In Ghana, local pyrolysis technology has been used to generate biochar (pyrochar) from agricultural residues. This biochar has been used as a soil amendment in field trials, and trial results from those trials are described in **section 8.2**. Adapted Brazilian kiln technology was implemented in Ghana in **M28** (September 2023), after which further biochar (pyrochar) was produced. Additional field trials will be conducted using that biochar as a soil amendment (**M34 – March**).

5.3.1 Biochar soil amendment

In Ghana, biochar has been produced from groundnut husk, rice husk/hulls, and corn stover. This was applied as a soil amendment in three different experimental treatments: alone (5t/ha application rate) and in combination with compost¹ (5t/ha application rate). Two different control treatments were used: 0 fertilizer application, and compost (5t/ha application) (see **Table 12**). Three different types of crops are being examined: tomato, okra, and chili pepper. These crops are of agronomic interest in Ghana, and important for

¹Comprised of blended compost components, rice husk, and cow dung.

food and nutritional security. A Complete Randomized Block Design was chosen, with half-acre plots for each crop in different parts of the North-East region of Ghana - Zangum, Nabari, and Gbeligu. Three replications will be carried out, and the plots will be irrigated during the dry season, and rainfed otherwise. This approach will also be implemented for replicate trials using biochar made from the same biomass but using the adapted Brazilian kiln technology.

Table 12: Soil amendment trials in Ghana: Experimental design

Treatment Name	Treatment Type	Amendment application rate
T1	Control	Control: No fertilizer
T2	Compost with biochar	5t/ha Compost with biochar added
T3	Biochar	5t/ha biochar
T4	Compost	5t/ha compost

Parameters and Timeline

The parameters being examined are described in **Table 13**. Partial budget analysis is also being applied to conduct an economic analysis of biochar-based soil amendment. The trial started in **M18** (November 2022), using biochar created from local pyrolysis technologies. Trials using biochar produced from the same feedstocks but using Brazilian kiln technology are planned to begin in **M34**. Final results for soil amendment trials using biochar from adapted Brazilian kiln technology, and comparative results between both trials, will be available during the **final trial reporting period, M35-M44**.

Table 13: Parameters being examined in soil amendment trials in Ghana

Soil Characteristics	Compost Characteristics	Crop characteristics
Soil Structure	Moisture	Plant Height
Soil pH	Volatile Matter	Branches/Plant
Water Retention Rate	Fixed Carbon	No. Leaves
Nutrient content: Nitrogen, Phosphorus and Potassium	Ash Content	Stem Diameter
	Water Retention	Chlorophyll Content
	Permeability	Days to 50% Flowering (DFF)

Soil Characteristics	Compost Characteristics	Crop characteristics
	Water Infiltration	Leaf Area Index (LAI)
	Aeration	Intercepted Photosynthetically Activate Radiation (IPAR)
	Structure	No. Fruit/Plant
		Average Fruit Weight
		Fruit Yield/Plot
		Ash Content
		Protein Content
		Moisture Content
		Fibre
		Vitamins

6. Pilot Trials in Côte d’Ivoire

Three main types of trial are being undertaken in Côte d’Ivoire: biochar applications as a soil amendment and a water filtration medium, and raw biomass pellet feeding trials. An overview of trial plans specific to Côte d’Ivoire is provided in **Fig. 17**. As described in this overview schedule, a number of pilot trials began in Côte d’Ivoire during the initial reporting period, in **M18**:

- Greenhouse trials (biochar as soil amendment);
- Sheep feed trials (raw biomass pellets)

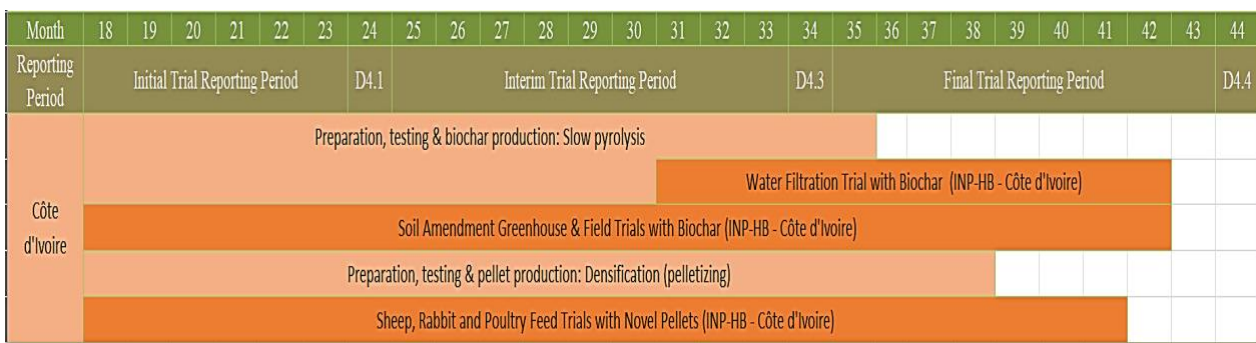
The biochar and sheep feed trials have used locally available technologies for biochar production and pellet production. Results of soil amendment greenhouse trials and sheep feeding trials are available and are described in **section 8.3**.

Further adaptation and improvement of production technology took place during the **interim reporting period (M25-M34)**, with the instalment of adapted Brazilian kiln technology (**M28, June 2023**) for biochar production. Further trials began during this period, including:

- Water filtration trials (biochar for water filtration);
- Rabbit feed trials (pellets from agri-food residues).

Initial rabbit feeding results are described in **section 8.3**. A larger, more efficient pelletizing machine, with greater productivity and quality will be installed during the **final reporting period**. Results from further biochar and animal feed trials using the new technology adapted to conditions in Côte d’Ivoire will become available during the **final trial reporting period, M35-M44**.

Figure 16: Overview of pilot trials taking place in Côte d’Ivoire



6.1 Carbonisation



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6.1.1 Water filtration

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Laboratory scale

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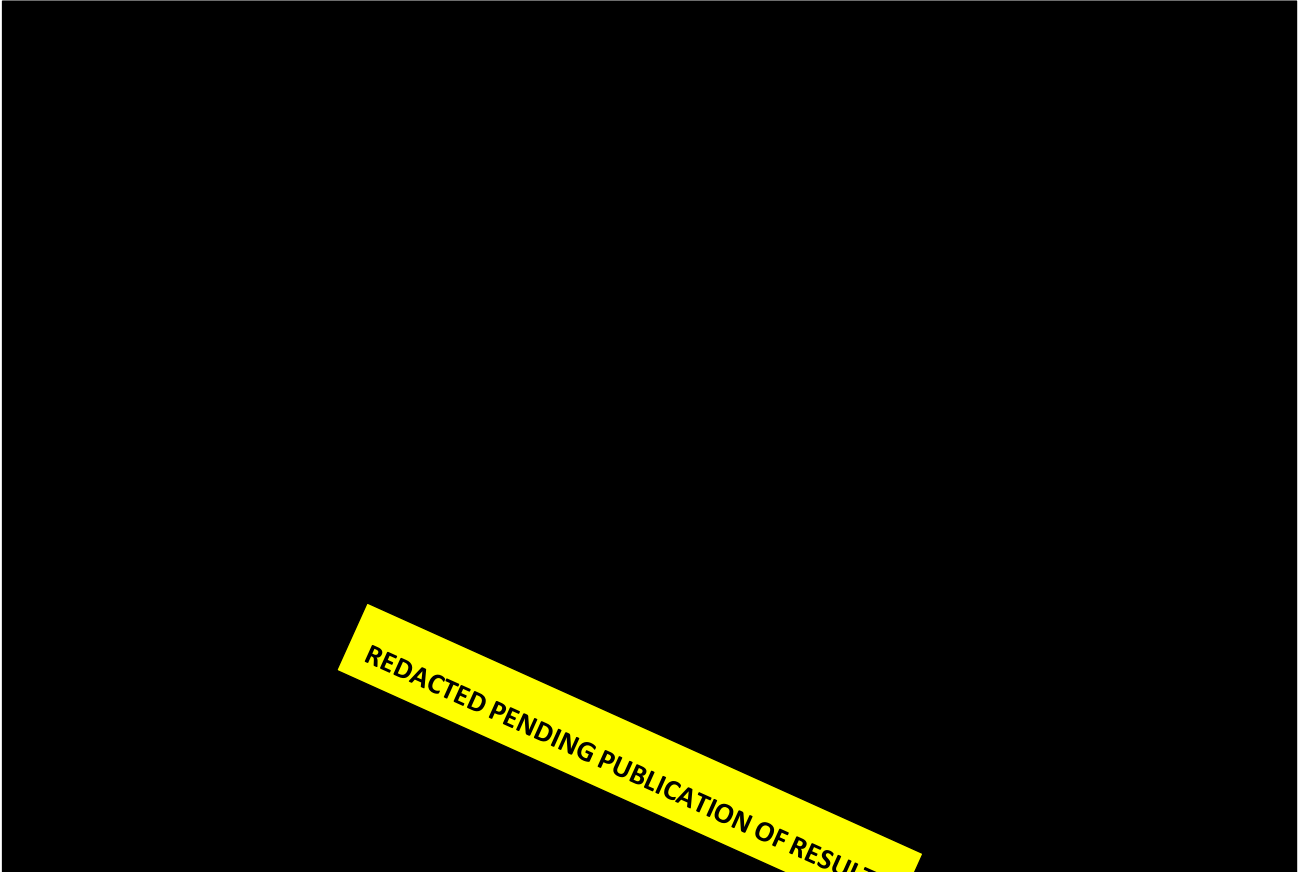
Pilot trials

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Parameters

The parameters to be examined in the pilot trials are described in **Table 14**.

Table 14: Parameters being examined in water filtration trials in Côte d'Ivoire



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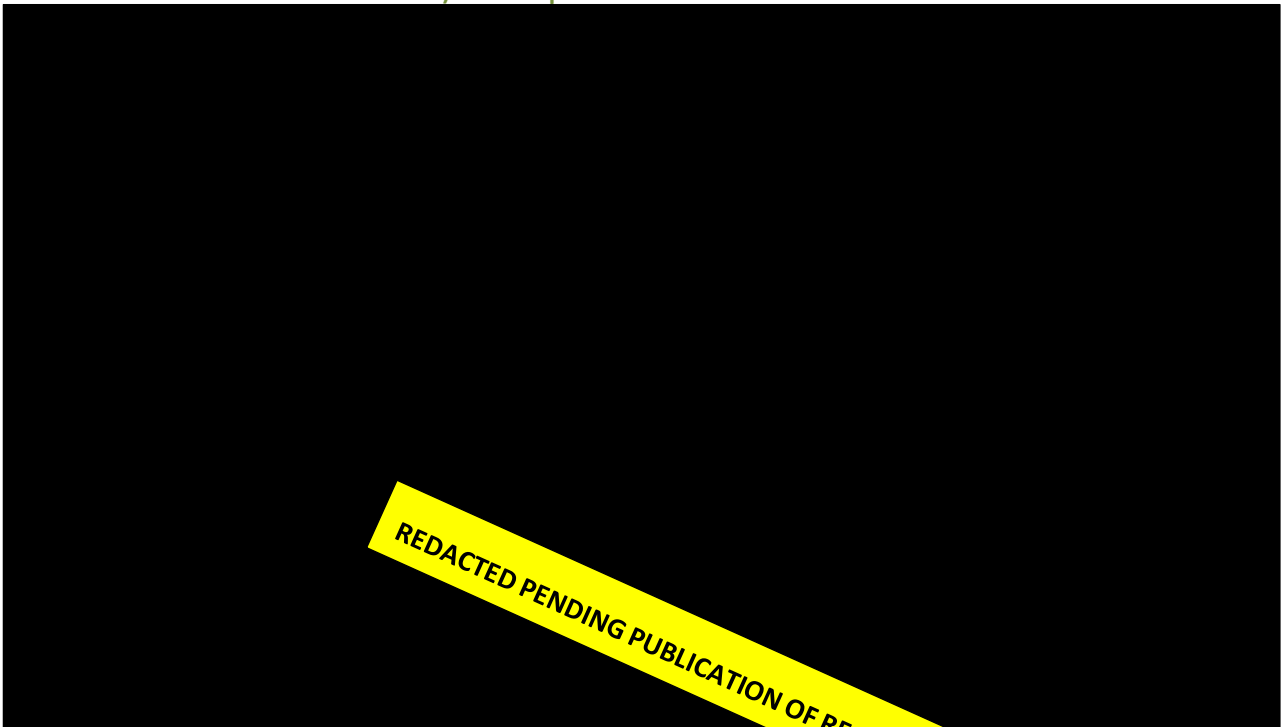
6.1.2 Biochar as a soil amendment



Greenhouse trials



Figure 17: Greenhouse trials using biochar-based soil amendments for a) tomato production and b) maize production in Côte d'Ivoire



Field trials

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Parameters and Timeline

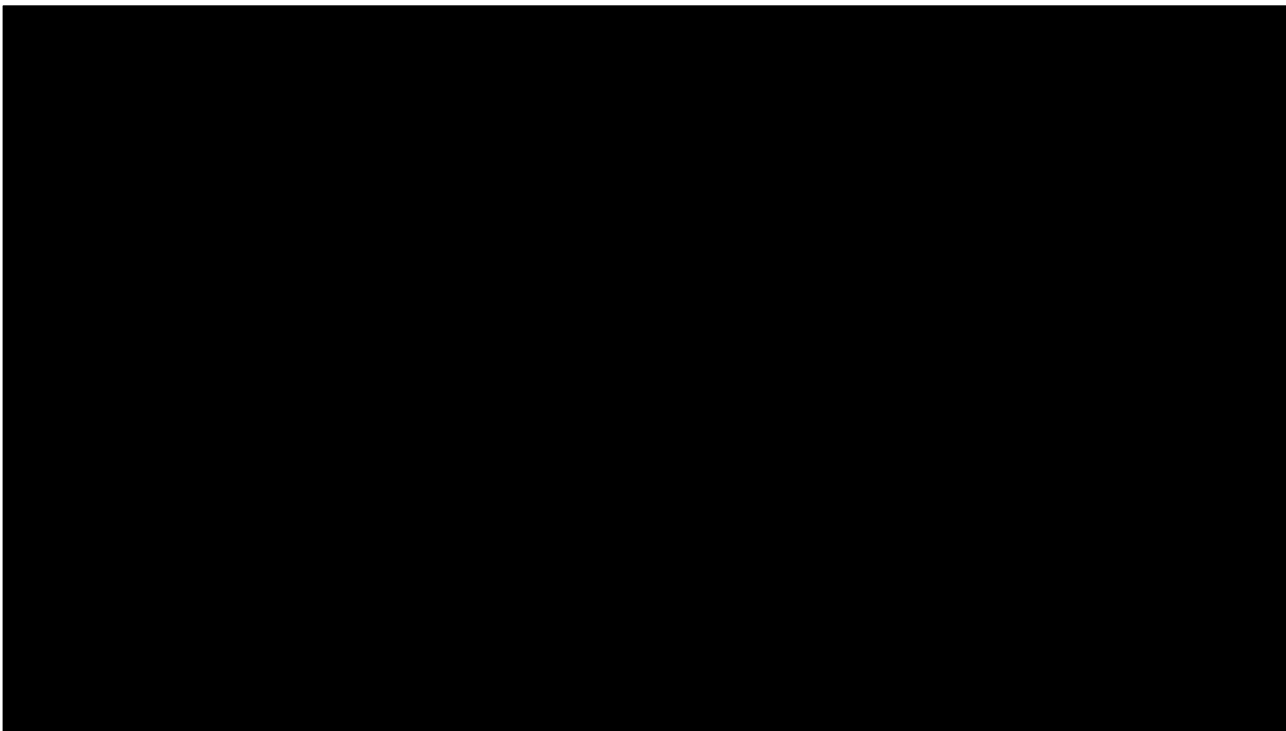
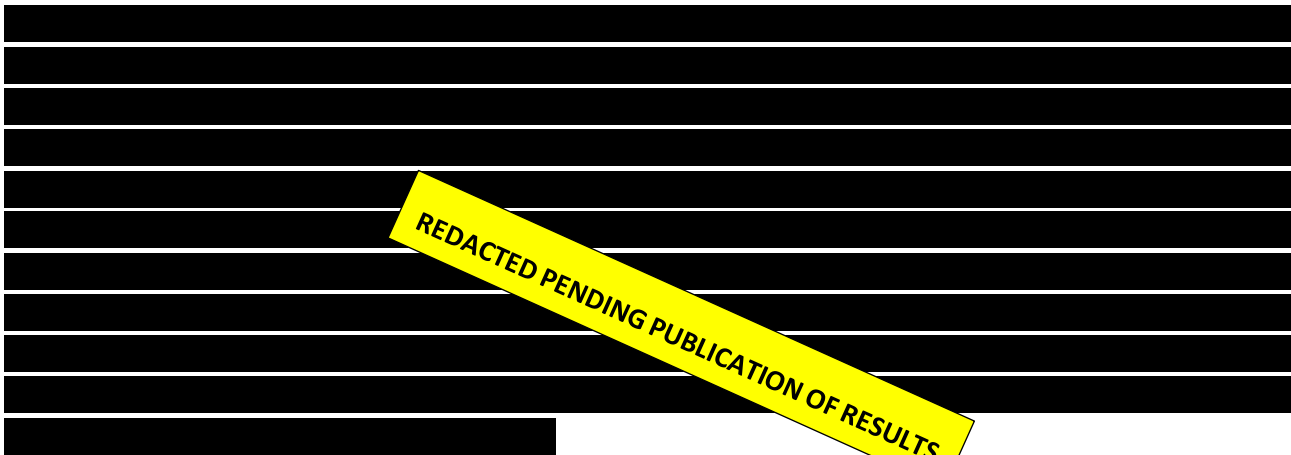
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Table 15: Parameters being examined in soil amendment trials in Côte d'Ivoire

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6.2 Densification



6.2.1 *Sheep feed pellets*

[REDACTED]

Parameters

[REDACTED]

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Table 16: Parameters to be examined in sheep feeding trials in Côte d'Ivoire

[REDACTED TABLE]

6.2.2 *Rabbit feeding trials*

[REDACTED]

6.2.3 *Chicken feeding trials*

[REDACTED]

[REDACTED]

Parameters and Timeline

[REDACTED]

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Table 17: Parameters to be examined in raw biomass pellet chicken feeding trials in Côte d'Ivoire

[REDACTED]

¹ To reduce cyanide content.

Pellet composition	Chicken intake/ performance
Total ash	

7. Pilot Trials in Senegal

A number of trials of biochar-based products began in the Senegal in the period from **M22-M34** (initial and interim trial reporting periods), as described in **Fig. 19**. These include:

- biochar as an additive to optimise biogas production
- biochar as a pollution adsorbent during biogas production
- biochar briquettes for use as solid fuel, specifically for cooking.

Initial results are described in **section 8.4**. These trials will continue during the final trial reporting period (**M35-M44**), and final results will be provided in **D4.4: Final report on trials and results**.

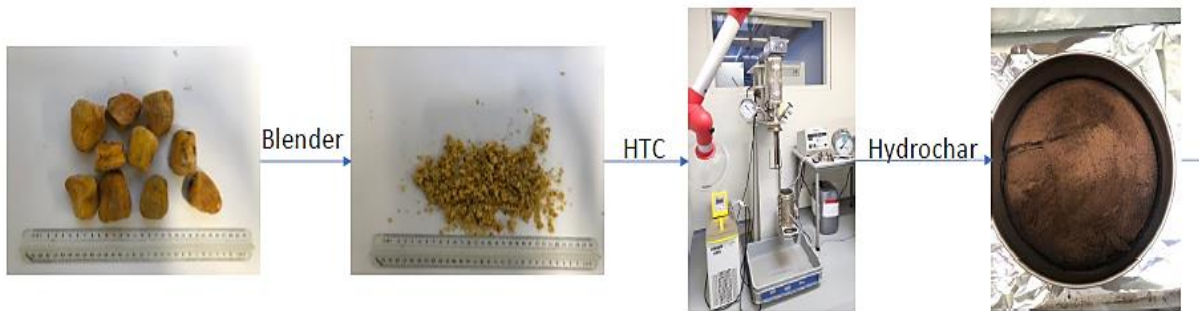
Figure 19: Overview of pilot trials taking place in Senegal

Month	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44						
Reporting Period	Initial Trial Reporting Period						D4.1	Interim Trial Reporting Period						D4.3	Final Trial Reporting Period						D4.4												
Senegal	Preparation, testing & biochar production: hydrothermal carbonisation & biodigestion																																
Senegal													Biogas Production with Biochar Additives Trials (Production Improvement & Purification) (UASZ - Senegal)																				
Senegal	Preparation, testing & biochar & briquette production: slow pyrolysis & densification																																
Senegal							Biochar Briquette Trials for use as Solid Fuel (Cooking Fuel) (UASZ - Senegal)																										

7.1 Carbonisation

In Senegal, local dry pyrolysis technology (barrel reactor), Brazilian kiln and HTC technology have been used to transform agri-food residues, e.g. cashew apple pulp (**Fig. 20**), into biochar. Biochar from dry feedstocks and wet feedstocks is being produced with each technology, respectively. This is being used as a pollution adsorbent and performance improver in biogas production (**section 7.1.1**). Biochar will be further treated using **densification technology (briquetting, section 7.2)**, to be evaluated for use as solid fuel (cooking fuel) (**section 7.2.1**). The pyrolysis technologies were implemented in Senegal in **M30** (November 2023). Results from trials so far are described in **section 8.4**.

Figure 20: Schematic diagram illustrating biochar production from cashew apple pulp (hydrochar) using HTC at laboratory scale in IHE, to be used as a biogas additive



7.1.1 Biogas additive

Biochar produced using cashew apple pulp and Typha (HTC technology, described in D2.8, WP2) and rice husk, corn cob and peanut shells (dry pyrolysis technology) are being used as a biogas additive with two different purposes. The first is to use the biochar during anaerobic digestion to enhance biogas production (performance improver). The second is to use biochar as pollution adsorbent to purify the biogas post-production (purification). At the moment, biochar of peanut shells and corn cobs from local kiln and biochar of rice husks from a muffle furnace are used as an additive in anaerobic digestion of cow dung.

Performance improver

The biochar is being added as an additive to an agri-food residue substrate generated by a 10m³ digester on the UASZ campus which includes cow dung, rice husk, and cashew apple. The rate of biochar addition is controlled, ranging from 0% biochar addition to a 1:1 biochar and substrate mixture (dry basis), in increments of 5% increase in biochar and a corresponding 5% decrease in substrate concentration, per trial. A continuously stirred tank reactor of 20L effective volume will be used, working under mesophilic conditions. At the moment, the reactors used for experiments have volume capacities of 500ml and 1,000ml. The composition of the mixture in anaerobic digestion is shown in **section 8.4**.

Purification

Biochar is also being tested as an adsorbent for the removal of H₂S present in biogas generated using the pilot digestors. Trials are being conducted by UASZ using a 6L capacity tubular filter containing a fixed bed of biochar. Laboratory scale analysis in preparation for these trials was carried out in cooperation with IHE, who applied biochar as a biogas production catalyst. The trial is evaluating different Empty Bed Contact Times, and the regeneration potential of the adsorbent by repeating the adsorption-desorption cycle using hot and

cold water and evaluating the performance of the resulting product. The H₂S adsorption capacity is being evaluated against international standards on a daily basis, and a control trial using a bed of commercial activated carbon is also being run for comparison. At UASZ, biochars produced by the HTC reactor will be tested as filter media on a 10m³ biodigester to measure the H₂S processing capacity

Parameters and Timeline

The parameters being examined in both biogas additive trials are described in **Table 18**. The trial began in **M31** (December 2023), using biochar produced in the local pyrolysis reactor and oven, and later adapted pyrolysis kiln technology designed to take dry materials for rice husk, corn cob and peanut shell biochar, and HTC technology for cashew apple pulp and Typha biochar. Initial trial results are described in **section 8.4**, while final trial results will be available during the final trial reporting period, **M35-M44**.

Table 18: Parameters being examined in biochar biogas additive trials in Senegal

Biodigester operating parameters	Biogas characteristics	Pollution adsorption parameters
Temperature	Gas composition (CH ₄ , H ₂ S, CO ₂ , N ₂ , H ₂ O, trace gases) & proximate analysis	H ₂ S removal efficiency
Waste feedstock (volume of feeding)	Cumulative biogas and methane yield (total and per organic dry matter)	Breakthrough time
TRH	pH (alkalinity)	Regeneration potential
pH of substrate (alkalinity)	Methane production rate	
Kinetics of daily & cumulative biogas production (%N, % organic matter, % dry matter, %C, C/N ration)	Biogas quality analysis	
	Heating value	

7.2 Densification

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7.2.1 *Solid fuel for cooking*

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Table 19: Parameters to be examined in trials of solid fuel for cooking in Senegal

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8. Pilot Trial Results: Interim Reporting Period

8.1 Pilot Trials in Uganda

8.1.1 Residual green biorefinery whey screening to assess the presence of high-value ingredients

Protein content determination – initial samples

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Introduction

The process of biorefining fresh leafy biomass results in two main outputs, a solid fraction - press cake, and a liquid fraction – press juice. These two fractions are further processed using secondary processing (Keijsers and Mandl, 2010; Xiu and Shahbazi, 2015). In the Bio4Africa project, press juice from fresh green biomass was subjected to the coagulation and precipitation to separate proteins. The process results in generation of two separate fractions: whey and protein precipitate. The whey typically contains soluble carbohydrates, minerals, and proteins, especially non-protein nitrogen compounds, with the specific composition depending on the feedstock and precipitation process used (Jørgensen et al., 2022). The whey has a range of applications including production of amino acid concentrates, sugar-rich animal feed, fertilizer, silage preservative, and co-digestion in anaerobic digestion systems (Jørgensen et al., 2022; Ravindran et al., 2022).

Residual biorefinery whey generated in Task 4.1 biorefinery activities (Validation of products, solutions and integrated value chains in Uganda) was collected after the protein precipitation and transported to MTU, Ireland for screening for high-value components with potential application in nutraceuticals, pharmaceuticals and cosmetics.

Materials and Methods

Whey samples

The liquid whey samples were generated in Uganda on January 16th and 23rd, 2024. The biomass used for the biorefinery process derived from Alfalfa (*Medicago sativa*) and Pakchong (*Pennisetum purpureum*) (Fig. 20).

Figure 20: Biorefinery residual whey samples generated in Uganda and analysed by MTU, Ireland



Protein content determination

Determination of protein concentration in the analysed whey samples was performed using the Pierce™ BCA Protein Assay (Thermo Scientific™; product code A55864). The assay combines the well-known reduction of Cu^{2+} to Cu^{1+} by protein in an alkaline medium with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu^{1+}) by bicinchoninic acid (BCA). Working solutions of BCA were prepared by mixing 50 parts of reagent A (BCA, sodium carbonate, sodium bicarbonate, bicinchoninic acid and sodium tartrate in 0.1 M sodium hydroxide) with 1 part of reagent B (CuSO_4 , 4%), as indicated by the manufacturer. 200 μL of BCA working solutions were pipetted onto the wells of a 96-well plate, and 25 μL of samples (diluted 1:100 in ddH₂O) were added, giving a BCA working solution: sample ratio of 8:1. Bovine serum albumin at concentrations from 2 mg/ml to 0.025 mg/ml (in ddH₂O) was used as a standard. Solvent used for the dilution of standard and samples (ddH₂O) was used as a blank. The plate with samples and BCA working solution was covered and at 37°C for 30 minutes. Cool plate to RT. The absorbance at 562 nm was measured on the plate reader (Thermo Scientific™ Varioskan™ LUX spectrophotometer) after cooling the plate to RT. Subtract The average 562 nm absorbance measurement of the blank standard replicates was subtracted from the 562 nm measurements of all other individual standard and unknown sample replicates. The concentration of protein in each sample was extrapolated from the standard curve. The experiment was performed in triplicate (N=3, n=6).

Results

The results obtained from the BCA assay are presented in Figures 21 and 22 and Table 20.

Figure 21: The results of the BCA protein assay. The more intense the purple colour in the well, the higher concentration of protein in the sample

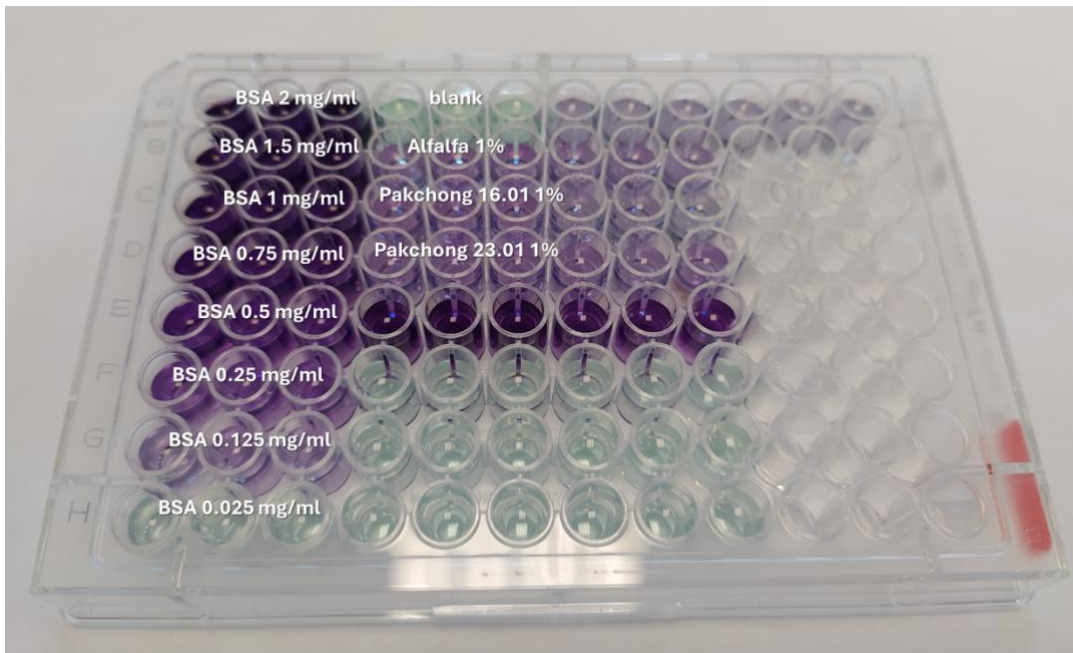


Figure 22: Standard curve for bovine serum albumin (BSA)

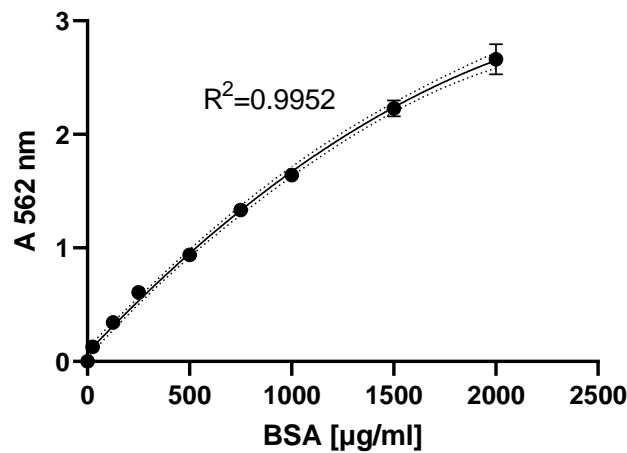


Table 20: Protein concentration of three whey biorefinery samples measured using BCA assay

Sample	Protein concentration [$\mu\text{g/ml}$]	SD
Alfalfa 1%	117.710	29.722
Pakchong 16.01.24 1%	118.600	21.992
Pakchong 23.01.24 1%	132.630	11.414

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8.2 Pilot Trials in Ghana

8.2.1 Soil amendment field trials: first trial results using biochar from local pyrolysis technology Impact of Biochar As Soil Amendment for Selected Vegetables Production in Ghana

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Introduction

Vegetables, such as tomato, okra, and chili pepper, are important food and nutrition security crops in Ghana. However, declining soil quality is negatively affecting vegetable production in the Savannas of Ghana because the soils are inherently nutrient-poor (low SOC, N, P, K) and degraded with low pH (< 5). Amending such nutrient-degraded soils with organic resources like biochar and compost can help restore their fertility. Organic resources particularly, biochar help improve soil biophysicochemical properties and sequester SOC (Lehmann et al., 2011). Although studies exist on impact of biochar on crop production and soil biophysicochemical properties (Brunetti et al., 2019; Duan et al., 2022), vegetable producers in Ghana are still reluctant to adopt the biochar technology because of inconclusive results on crop yield and benefits to the soil/environments. This study therefore, aimed; to determine the effect of soil amendments specifically, sole and co-applications of biochar, compost, and mineral fertilizer on growth and yield of okra, chilli pepper and tomato, and to evaluate their economic benefits.

Materials and Methods

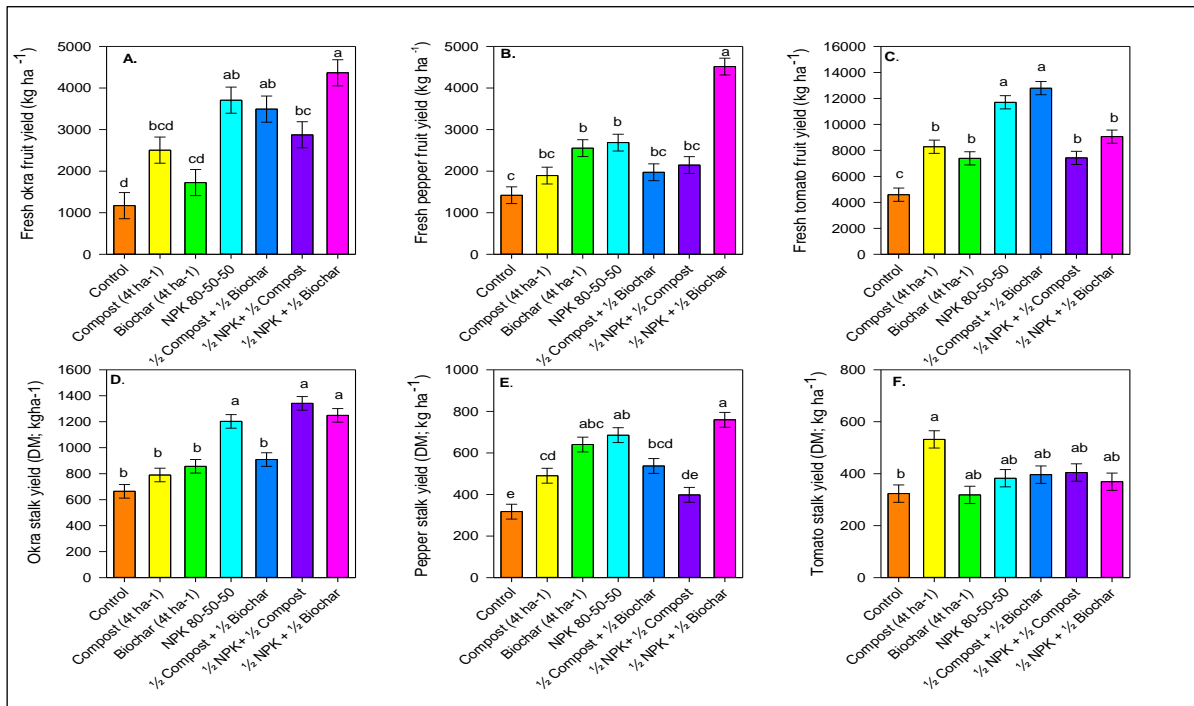
A rain-fed field study was conducted in Nyankpala (lat. 9.393395; long.-1.002223), Ghana during the 2023 cropping season. The experiment was designed in randomized complete block with seven treatments; a control (no amendment); full mineral fertilizer rate (NPK; 80 N-50 P₂O₅-50 K₂O kg ha⁻¹); Compost applied at 4t ha⁻¹; Biochar applied at 4t ha⁻¹; 2 t ha⁻¹ Compost and 2 t ha⁻¹ Biochar (½ Compost + ½ Biochar); half NPK rate; 40 N-25 P₂O₅-25 K₂O kg ha⁻¹ + 2 t ha⁻¹ Compost (½ NPK + ½ Compost); half NPK rate; 40 N-25 P₂O₅-25 K₂O kg ha⁻¹ + 2 t ha⁻¹ Biochar (½ NPK + ½ Biochar). The treatments were replicated four times for okra and pepper trials and three times for the tomatoes trial. Each experimental plot measured 12 m². The biochar was prepared by pyrolysis of groundnut shell and rice husk using fabricated metal klin, and was provided by SavaNet. The compost was manufactured by Accra compost recycling plant (ACARP). The vegetables planted included okra [*Abelmoschus caillei* L. cv HIRE], chilli pepper [*Capsicum annum* cv Shito Adope] and tomato [*Solanum lycopersicum* Mill cv Cobra 34]. All the vegetables were planted at 75 cm x 40 cm. The okra was directly seeded while the pepper and the tomato were nursed and later transplanted. Data were collected on fresh fruit weight (kg ha⁻¹) and stalk yield (dry matter; kg ha⁻¹). Data were analysed using a Proc-mixed model in SAS 9.4 at 5% probability level, and means separated using Tukey-Kramer post hoc test. Economic analysis was done using Benefit-cost ratio (BCR) based on partial budgeting approach. The BCR threshold was; BCR < 1 is considered not profitable ; BCR =1 ; considered as break even points; BCR > 1 considered profitable.

Figure 23: Plants in soil amendment trials in Ghana at various stages of growth: a) growing okra plant, b) fruiting okra plants, c) growing pepper plant, d) fruiting pepper plant, e) fruiting tomato plant, f) tomato plant with matured fruits



Results and Discussion

Figure 24: Effect of soil amendments of fruit yield and stalk yield (dry matter) of okra cv Hire (a & d), pepper cv Shito Adope (b & e) tomato cv Cobra 34 (c & f) in Nyankpala, Ghana in 2023. Error bars represent standard error of means. Different lowercase letter(s) on top of error bars indicate(s) significant different ($p < 0.05$) using Tukey-Kramer post hoc test. Control = No amendment, Compost = 4t ha⁻¹; Biochar = 4t ha⁻¹; NPK 80-50-50 kg ha⁻¹; ½ Compost + ½ Biochar = 2 t ha⁻¹ Compost + 2 t ha⁻¹Biochar; ½NPK+½Compost = NPK 40-25-25 kg ha⁻¹+ 2 t ha⁻¹Compost; ½ NPK +½ Biochar = NPK 40-25-25 kg ha⁻¹+ 2 t ha⁻¹ Biochar.



The ½ NPK +½ Biochar produced an enhanced okra fresh fruit yield compared to the other amendments except NPK 80-50-50 kg ha⁻¹ and ½ Compost + ½ Biochar. Similarly, the NPK 80-50-50 kg ha⁻¹ and ½ Compost + ½ Biochar yielded greater okra fresh fruit yield over the control and sole Biochar. The ½NPK+ ½Compost boosted okra fresh fruit yield more than the control. Additionally, NPK 80-50-50 kg ha⁻¹, ½ NPK +½ Compost,

and $\frac{1}{2}$ NPK + $\frac{1}{2}$ Biochar yielded increased okra stalk yield compared to the other amendments. Adding soil amendment would potentially improve okra fresh fruit and stalk yields. Nonetheless, co-applicating mineral fertilizer + biochar produced the most outstanding okra fresh fruit yield.

With pepper, except for NPK 80-50-50 kg ha⁻¹ and sole Biochar, the $\frac{1}{2}$ NPK + $\frac{1}{2}$ Biochar had the highest pepper fruit yield and stalk yield (DM) compared to the other amendments. Likewise, the NPK 80-50-50 kg ha⁻¹ and sole Biochar yielded higher fruit yield and stalk yield over the control. Remarkably, pepper yield achieved with sole Biochar was comparable to those obtained with NPK 80-50-50 kg ha⁻¹, implying that producers who use either of the amendments will likely achieve the same results. Applying amendments to pepper, particularly $\frac{1}{2}$ NPK + $\frac{1}{2}$ Biochar would greatly enhance yield (fruit and stalk biomass).

For tomato, improved fresh fruit yield was achieved with NPK 80-50-50 kg ha⁻¹ and $\frac{1}{2}$ Compost + $\frac{1}{2}$ Biochar compared to the other soil amendments. In addition, the Compost, Biochar, and $\frac{1}{2}$ NPK + $\frac{1}{2}$ Compost yielded greater fresh fruit yield than the control. Results revealed $\frac{1}{2}$ Compost + $\frac{1}{2}$ Biochar produced fresh tomatoes fruit yields similar to the NPK 80-50-50 kg ha⁻¹. It was also observed that Biochar produced fresh fruit yield comparable to the Compost and the $\frac{1}{2}$ NPK + $\frac{1}{2}$ Compost. Applying sole compost improved tomato stalk yield relative to the control. Producers who used either mineral fertilizer or co-compost + Biochar would achieve greater fresh fruit yield of tomato. Besides the aforementioned treatments, the other amendments equally enhanced tomato's fresh fruit yields more than no input production systems.

Economically, order for increased BCR follows; (i) Okra; NPK 80-50-50 (2.4) > $\frac{1}{2}$ NPK + $\frac{1}{2}$ Biochar (2.0) > $\frac{1}{2}$ NPK + $\frac{1}{2}$ Compost (1.3) > $\frac{1}{2}$ Compost + $\frac{1}{2}$ Biochar (1.1); (ii) tomato; NPK 80-50-50 (7.5) > $\frac{1}{2}$ Compost + $\frac{1}{2}$ Biochar (5.9) > $\frac{1}{2}$ NPK + $\frac{1}{2}$ Biochar (4.3) > Compost (3.9) = $\frac{1}{2}$ NPK + $\frac{1}{2}$ Compost (3.9) > Control (3.2) > Biochar (2.6); and (iii) for pepper, the $\frac{1}{2}$ NPK + $\frac{1}{2}$ Biochar (1.5) was the most profitable. Results also indicated all the technologies tested on okra and tomato were profitable.

Conclusion

The various vegetables responded differently to the amendments applied. For okra and pepper, integrated mineral fertilizer and biochar application produced outstanding yields. Remarkably, the co-application of mineral fertilizer and biochar outperformed the popular integrated soil fertility management (ISFM) recommendation of mineral fertilizer and compost. This indicates that the biochar technology could replace the compost in ISFM technologies. With tomato production, combined compost + biochar or mineral fertilizer appeared as the most promising technology. However, the recent concern about the negative impacts of mineral fertilizer, especially N, on the environment via greenhouse gas and N leaching makes combined compost + biochar look favourable as an alternative to using mineral fertilizer for tomato production. We recommend a second year evaluation to validate findings at both on-station and on-farm since the results of this study could be used for policy formulation on food security and soil health.

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8.2.2 Residual green biorefinery whey screening to assess the presence of high-value ingredients

Protein content determination – initial sample

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Introduction

The process of biorefining fresh leafy biomass results in two main outputs, a solid fraction - press cake, and a liquid fraction – press juice. These two fractions are further processed using secondary processing (Keijsers and Mandl, 2010; Xiu and Shahbazi, 2015). In the Bio4Africa project, press juice from fresh green biomass was subjected to the coagulation and precipitation to separate proteins. The process results in generation of two separate fractions: whey and protein precipitate. The whey typically contains soluble carbohydrates, minerals, and proteins, especially non-protein nitrogen compounds, with the specific composition depending on the feedstock and precipitation process used (Jørgensen et al., 2022). The whey has a range of applications including production of amino acid concentrates, sugar-rich animal feed, fertilizer, silage preservative, and co-digestion in anaerobic digestion systems (Jørgensen et al., 2022; Ravindran et al., 2022).

Residual biorefinery whey generated in the in the Task 4.2 (Validation of products, solutions and integrated value chains in Ghana) was collected after the protein precipitation and transported to MTU, Ireland for screening for high-value components with potential application in nutraceuticals, pharmaceuticals and cosmetics.

Materials and Methods

Whey samples

The liquid whey sample were generated in Ghana on November 21st, 2023. The biomass used for the biorefinery process derived from the pigeon pea (*Cajanus cajan*) (Fig. 25).

Figure 25: Biorefinery residual whey samples generated in Ghana and analysed by MTU, Ireland



Protein content determination

Determination of protein concentration in the analysed whey samples was performed using the Pierce™ BCA Protein Assay (Thermo Scientific™; product code A55864). The assay combines the well-known reduction of Cu^{2+} to Cu^{1+} by protein in an alkaline medium with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu^{1+}) by bicinchoninic acid (BCA). Working solutions of BCA were prepared by mixing 50 parts of reagent A (BCA, sodium carbonate, sodium bicarbonate, bicinchoninic acid and sodium tartrate in 0.1 M sodium hydroxide) with 1 part of reagent B (CuSO_4 , 4%), as indicated by the manufacturer. 200 μl of BCA working solutions were pipetted onto the wells of a 96-well plate, and 25 μL of samples (diluted 1:100 in ddH₂O) were added, giving a BCA working solution: sample ratio of 8:1. Bovine serum albumin at concentrations from 2 mg/ml to 0.025 mg/ml (in ddH₂O) was used as a standard. Solvent used for the dilution of standard and samples (ddH₂O) was used as a blank. The plate with samples and BCA working solution was covered and at 37°C for 30 minutes. Cool plate to RT. The absorbance at 562 nm was measured on the plate reader (Thermo Scientific™ Varioskan™ LUX spectrophotometer) after cooling the plate to RT. Subtract The average 562 nm absorbance measurement of the blank standard replicates was subtracted from the 562 nm measurements of all other individual standard and unknown sample replicates. The concentration of protein in each sample was extrapolated from the standard curve. The experiment was performed in triplicate (N=3, n=6).

Results

The results obtained from the BCA assay are presented in Figures 26 and 27 and Table 21.

Figure 26: The results of the BCA protein assay. The more intense the purple colour in the well, the higher concentration of protein in the sample

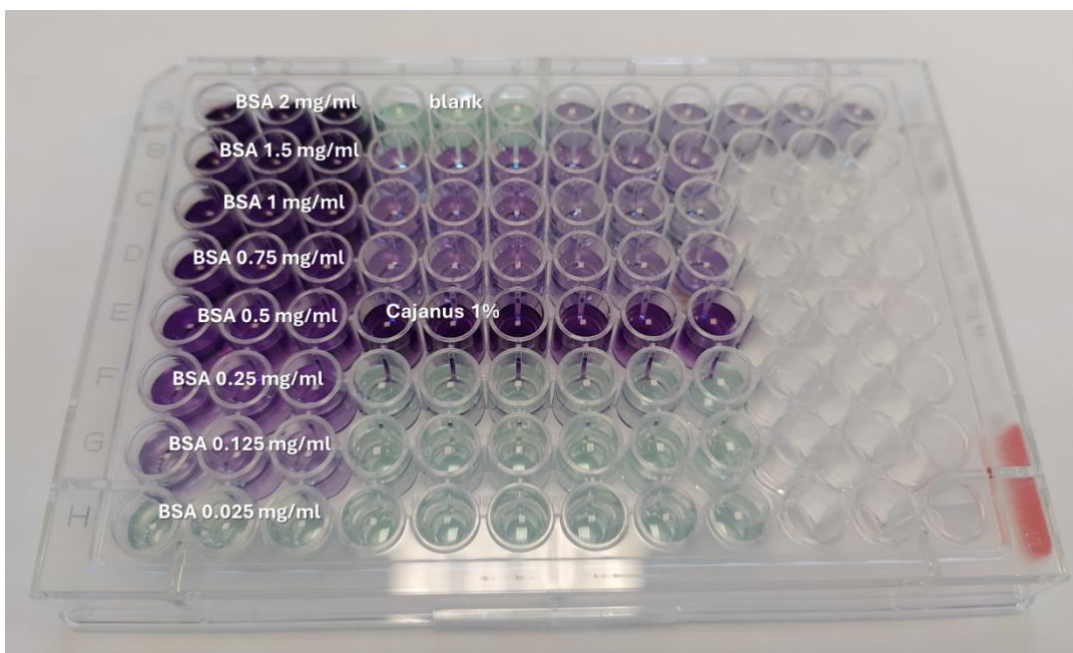


Figure 27: Standard curve for bovine serum albumin (BSA)

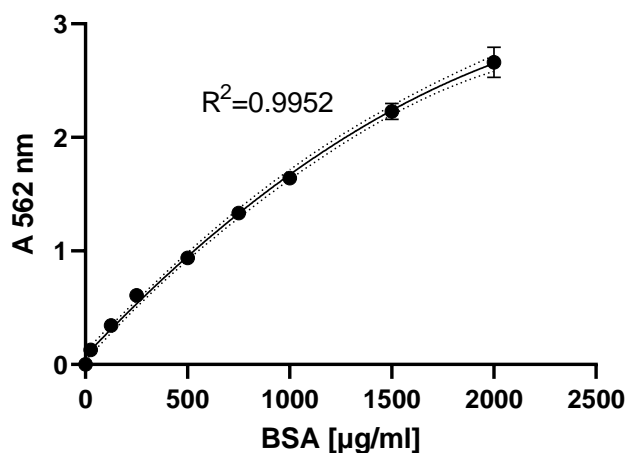


Table 21: Protein concentration of *Cajanus* whey biorefinery sample at concentration 1% measured using BCA assay

Sample	Protein concentration [µg/ml]	SD
Cajanus 1%	1064.253	94.541

References:

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8.3 Pilot Trials in Côte d'Ivoire

8.3.1 Biochar soil amendment: greenhouse trial results using local pyrolysis technology

Study of the agronomic efficiency of cassava peel-based biochar on vegetable and food crops: tomato and maize

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2: Laboratoire d'Agronomie, de Foresterie et de Défense des Cultures (LAFDC), UMRI Sciences Agronomiques et des Procédés de Transformation, Institut National Polytechnique Félix Houphouët-Boigny; BP 1093 Yamoussoukro.

Introduction

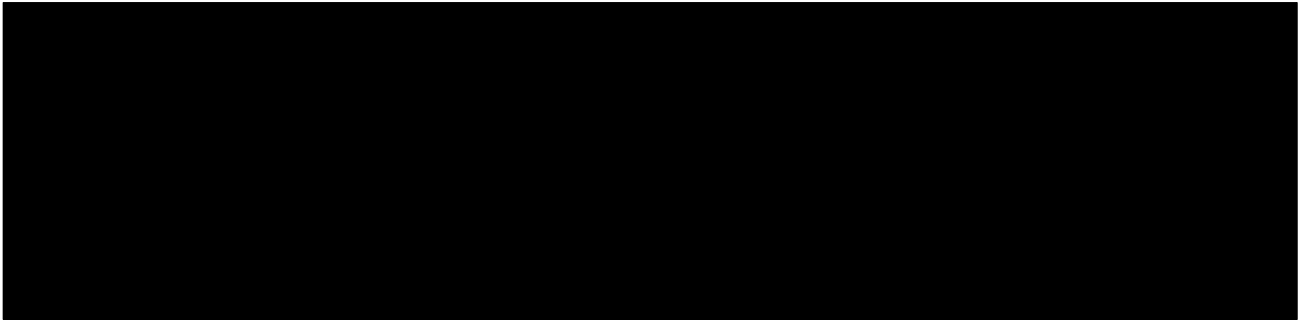
[REDACTED]

Materials and methods

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Figure 28: Photos of a) tomato and b) maize plants in the greenhouse, and c) tomato and d) maize in the field



Results and discussion

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Conclusion

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References

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8.3.2 Animal feed trials: first trial results with rabbits and sheep

Using *Cajanus cajan* fodder as pellets in rabbit feed to control coccidiosis

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b: Université Peleforo Gon Coulibaly, Korhogo, Côte d'Ivoire

Introduction

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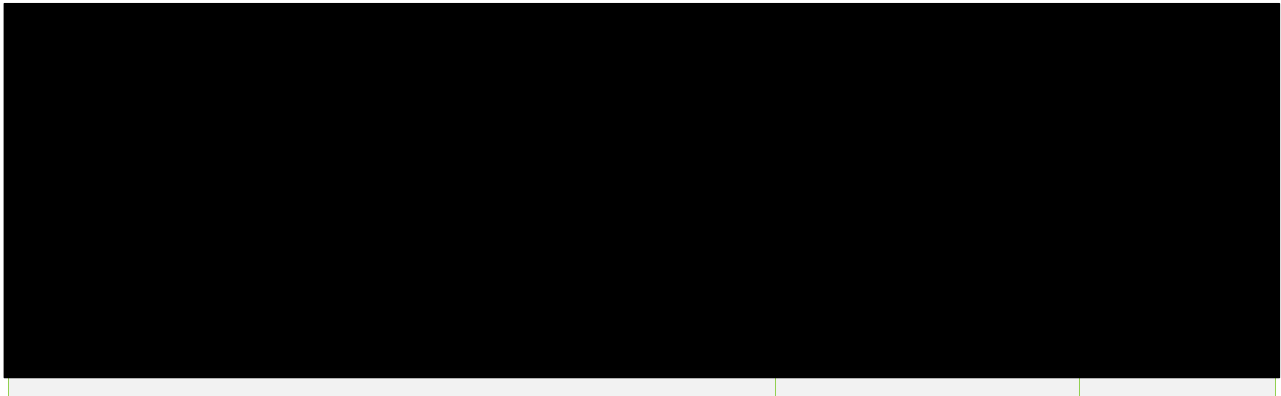
Materials and Methods

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Table 22. Chemical composition of experimental diets

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Results and Discussion

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Figure 29: Average daily gain of rabbits depending on diet

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Conclusion

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Leucaena leucocephala-based pellets use for the control of gastrointestinal parasitosis on sheep farms

Yapo Magloire Yapi^a, Casimir Kekou^a, Moussa Kimse^b, Faustin Parfait Koutouan^a

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b: Université Nangui Abrogoua, Abidjan, Côte d'Ivoire

Introduction

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Materials and Methods

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Results and Discussion

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Conclusion

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References

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8.4 Pilot Trials in Senegal

8.4.1 Solid biochar-based cooking fuel trials

Solid biochar-based cooking fuel trials: first results

Philippe Bernard Himbane^a, Diarra Diaby^a, Lat Grand Ndiaye^a

a: University Assane Seck of Ziguinchor, Senegal

Introduction

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Materials and Methods

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Results and Discussion

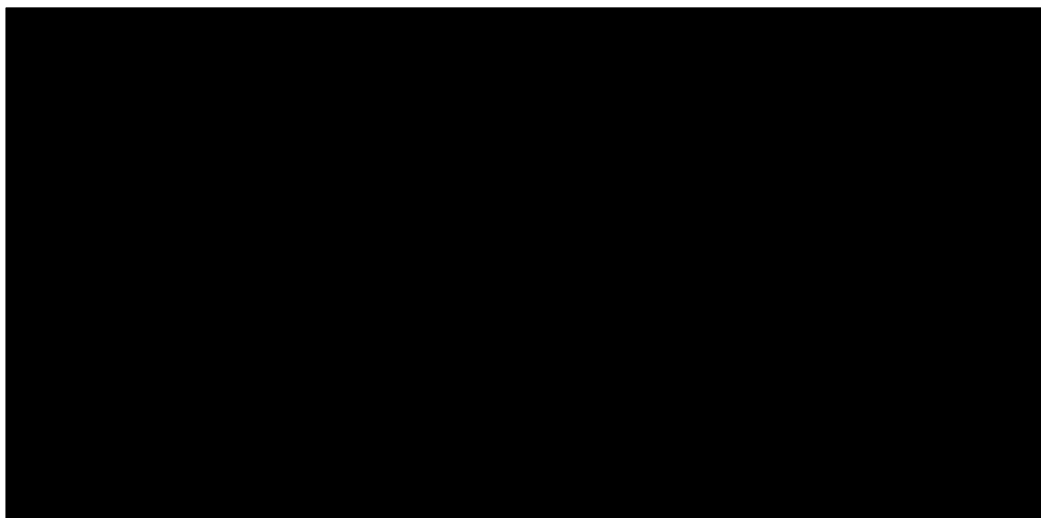
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Figure 30: Food used in the CCT (first trial)



The results of the controlled cooking tests are summarized in Figure 31.

Figure 31: Results of the CCT (first trial)



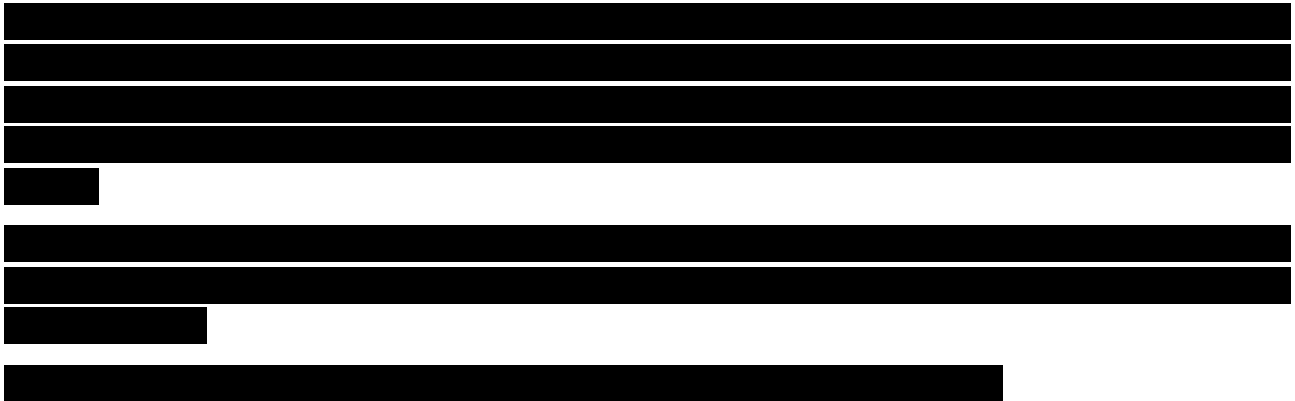


Figure 32: Images during the CCT tests (first trial)



Conclusion

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References

Bailis, R. (2004) *Controlled Cooking Test (CCT) protocol v2.0* Available at: <https://cleancooking.org/research-evidence-learning/standards-testing/protocols/>

Solid biochar-based cooking fuel trials: second results

Philippe Bernard Himbane^a, Omar Kata Faye^a, Lat Grand Ndiaye^a, Diarra Diaby^a

a: University Assane Seck of Ziguinchor

Introduction

[REDACTED]

Materials and Methods

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REDACTED PENDING PUBLICATION OF RESULTS

Results and Discussion

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Figure 33: Some images of the CCT (second trial)

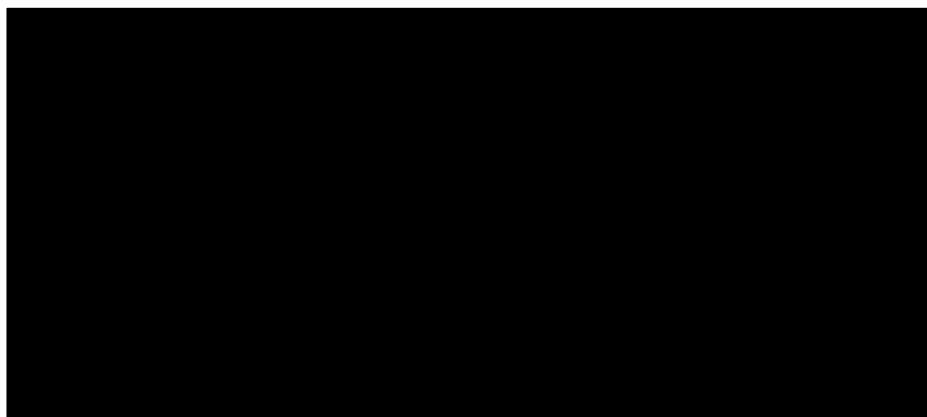
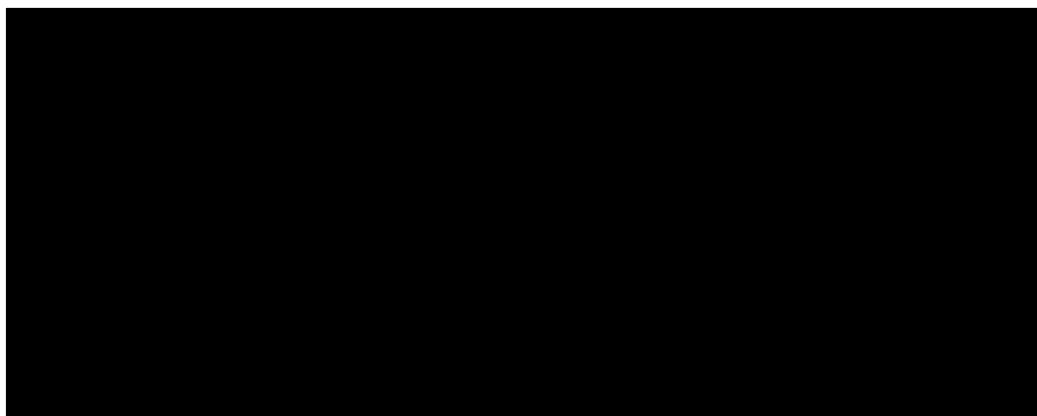


Figure 34: Food used in the CCT (second trial)



The results of the controlled cooking tests are summarized in Figure 35.

Figure 35: Results of the CCT (second trial)



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Conclusion

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References

Bailis, R. (2004) *Controlled Cooking Test (CCT) protocol v2.0* Available at: <https://cleancooking.org/research-evidence-learning/standards-testing/protocols/>

8.4.2 Biochar as a biogas additive

Biochar additive trials: first trial – initial results (from 12/12/2023 to 09/01/2024)

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b: Société de Commercialisation des Produits Locaux, SCP, SODEZI, Ziguinchor

Introduction

In the framework of the work package WP4, it was decided, in Senegal to produce biochar from different biomasses. The production of biochar will be conducted into local kiln, Brazilian kiln and HTC technology. The biochar obtained will be tested as additive in anaerobic digestion and as absorbent to remove the H₂S from biogas.

Materials and Methods

For these first tests, we chose to use two types of biochar (peanut shells and corn cobs) and a control. These two biochars were obtained during the pyrolysis in local kilns.

We created three biodigesters, each consisting of 3 bottles. The principle of water displacement is applied in these digesters (or reactors) to assess the volume of biogas produced. The first bottle receives the substrate to be methanized; the second bottle contains the water to which the biogas produced exerts pressure, while the third bottle is used to collect the volume of biogas produced.

Results and Discussion

The first trials on biogas using biochar as additive are conducted as indicated in Figure 36.

Figure 36: Formulations to conduct the first biogas experimentations (Biochar_1 is the biochar from peanut shells; Biochar_2 is the biochar from corn cobs)

Digester 1 (benchmark)	Digester 2	Digester 3
Cow dung: 30 g	Cow dung: 30 g	Cow dung: 30 g
Biochar: 0 g	Biochar_1: 2,26 g	Biochar_2: 2,26 g
Inoculum: 220 g	Inoculum: 220 g	Inoculum: 220 g
Water: 31 ml	Water: 31 ml	Water: 31 ml

At the 17th day of experimentation, the total volumes of biogas produced were:

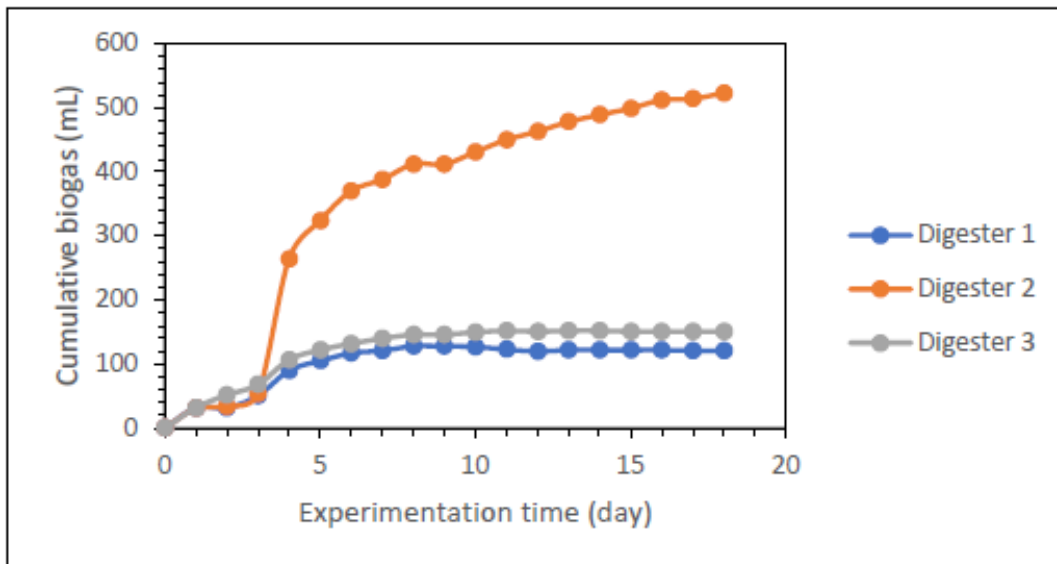
Digester 1: 121mL

Digester 2: 514mL, corresponding to an increase of 324,79%

Digester 3: 157mL, corresponding to an increase of 29,75%

At the 12th day, the total production of biogas remained constants in digesters 1 &3 while the production of biogas in digester 2 continue to increase. Figure 37 shows the cumulative biogas in each digester.

Figure 37: Cumulative biogas curves of the different biodigesters (in progress – first trial)



Below, in Fig. 38, some images of the biogas experimentations.

Figure 38: Some images of biogas trials (first trial)



Conclusion

Biogas trials show that the cumulative biogas can be increase in 17 days to 324,79% when biochar from peanut shells is used as additive in biogas production, or to 29.75% when biochar from corn cobs is used as additive in biogas production.

References

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Biochar additive trials: first trial - final results, second trial - initial results

Philippe Bernard Himbane^a, Omar Kata Faye^a, Lat Grand Ndiaye^a, Diarra Diaby^a

a: University Assane Seck of Ziguinchor

Introduction

In the framework of the work package WP4, it was decided, in Senegal to produce biochar from different biomasses. The production of biochar will be conducted into local kiln, Brazilian kiln and HTC technology. The biochar obtained will be tested as additive in anaerobic digestion and as absorbent to remove the H₂S from biogas.

Materials and Methods

We focused on characterization of the substrate used in the biogas experimentations. First experiments on biochar as additive for biogas production were ended at the 28th day. The second trials on biochar as additive for biogas production were begun on 21/01/2024. The principle of water displacement is applied in these digesters (or reactors) to assess the volume of biogas produced. The first bottle receives the substrate to be methanized; the second bottle contains the water to which the biogas produced exerts pressure, while the third bottle is used to collect the volume of biogas produced.

Results and Discussion

Biogas final results (first trials)

The first trials on biogas (from 12/12/2023 to 09/01/2024) using biochar as additive are conducted as indicated in Fig. 39. For these first tests, we chose to use two types of biochar (peanut shells and corn cobs) and a sample without additive for control. These two biochars were obtained during the pyrolysis in local kilns.

Figure 39: Formulations to conduct the first biogas experimentations (Biochar_1 is the biochar from peanut shells; Biochar_2 is the biochar from corn cobs)

Digester 1 (benchmark)	Digester 2	Digester 3
Cow dung: 30 g Biochar: 0 g Inoculum: 220 g Water: 31 ml	Cow dung: 30 g Biochar_1: 2,26 g Inoculum: 220 g Water: 31 ml	Cow dung: 30 g Biochar_2: 2,26 g Inoculum: 220 g Water: 31 ml

At the 28th day of experimentation, the total volumes of biogas produced were:

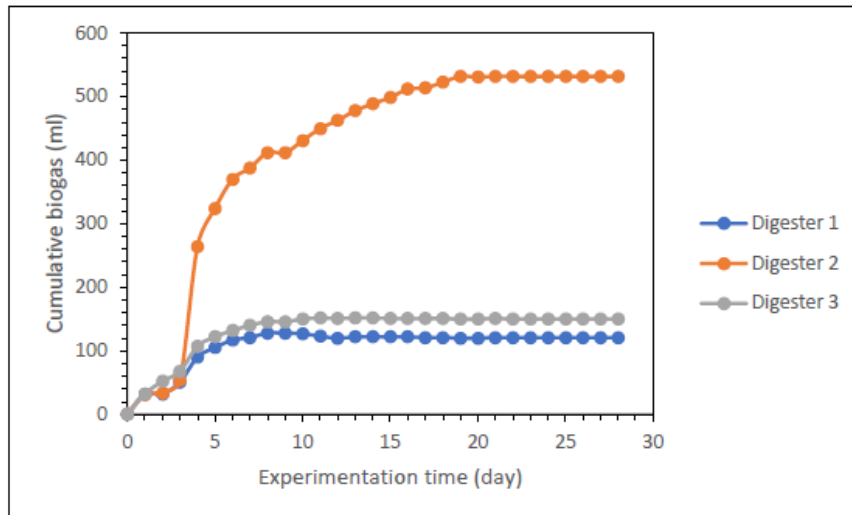
Digester 1: 121mL

Digester 2: 532mL, corresponding to an increase of 339,67%

Digester 3: 150mL, corresponding to an increase of 23,97%

Figure 40 shows the cumulative biogas of the different digesters.

Figure 40: Cumulative biogas curves of the different biodigesters (ended – first trial)



Below, in Figure 41, some images of the first biogas trials experimentations.

Figure 41: Some images of biogas trials (first trial)



Below in Figure 42, we show the balance of the biogas production on the first trials.

Figure 42: The balance of biogas production in the first trials

Benchmark (Digester 1)			Benchmark (Digester 1)			
	Quantity	Unit	Substrate	Quantity	Unit	Increasing (%)
Total TS	19,3410	g	Total biogas in 28 days	121,0000	ml	--
Total VS	9,8271	g	Total biogas in 28 days	6,2561	Nml/g TS	--
Total carbon	5,9642	g	Total biogas in 28 days	12,3129	Nml/g VS	--
Total nitrogen	0,4294	g				
C/N total ratio	13,8899	g				

Digester 2			Digester 2			
	Quantity	Unit	Substrate	Quantity	Unit	Increasing (%)
Total TS	21,4916	g	Total biogas in 28 days	532,0000	ml	339,67
Total VS	10,1577	g	Total biogas in 28 days	24,7538	Nml/g TS	295,67
Total carbon	7,2620	g	Total biogas in 28 days	52,3739	Nml/g VS	325,36
Total nitrogen	0,4664	g				
C/N total ratio	15,5691	g				

Digester 3			Digester 3			
	Quantity	Unit	Substrate	Quantity	Unit	Increasing (%)
Total TS	21,3924	g	Total biogas in 28 days	150,0000	ml	23,97
Total VS	9,9780	g	Total biogas in 28 days	7,0118	Nml/g TS	12,08
Total carbon	7,7272	g	Total biogas in 28 days	15,0331	Nml/g VS	22,09
Total nitrogen	0,4443	g				
C/N total ratio	17,3910	g				

Characterization of the different substrates and digestates

Figures 43 and 44 show the results of the characterization of the different substrates used in the first biogas trials.

Figure 43: Proximate analysis of the different substrates (first trial)

Substrates	M (%)	TS (%)	VS (%) ^s	Ashes (%) ^s	FC (%) ^s
Cow dung	81.73	18.27	62.03	21.69	16.28
Inoculum	93.70	6.30	46.37	43.72	9.91
Biochar 1 (Peanut shells)	4.84	95.16	15.37	27.73	56.89
Biochar 2 (Corn cobs)	9.23	90.77	7.36	6.64	86.00

Figure 44: Elemental analysis of the different substrates (first trial)

Substrates	Ashes (%) ^s	C (%) ^s	H (%) ^s	O (%) ^s	N (%) ^s	C/N
CWD	21.69	40.40	4.60	31.81	1.50	27.00
Inoculum	43.72	27.05	3.22	23.50	2.51	10.79
Biochar 1 (Peanut shells)	27.73	60.34	2.15	8.05	1.72	35.03
Biochar 2 (Corn cobs)	6.64	85.94	2.35	4.34	0.73	118.07

The different digestates were also characterized and the results are shown in Figures 45 & 46.

Figure 45: Proximate analysis of the different digestates (first trial)

Digestates	M (%)	TS (%)	VS (%) ^s	Ashes (%) ^s	FC (%) ^s
Digestate 1	94.28	5.72	50.40	36.46	13.15
Digestate 2	93.09	6.91	46.78	40.29	12.92
Digestate 3	93.86	6.14	44.71	38.19	17.11

Figure 46: Elemental analysis of the different digestates (first trial)

Digestates	ashes (%) ^s	C (%) ^s	H (%) ^s	O (%) ^s	N (%) ^s	C/N
Digestate 1	36.46	32.09	3.62	25.66	2.17	14.78
Digestate 2	40.29	30.26	3.33	23.77	2.35	12.89
Digestate 3	38.19	33.53	3.29	22.75	2.25	14.93

Biogas results (second trials in progress)

The second trials on biogas (23/02/2024 in progress) using biochar as additive are conducted as indicated in Fig. 47. For these second tests, we use biochar from rice husks and biochar from corn stalks as additives and cow dung with the addition of inoculum from cow dung digestion. The biochar of corn stalks come from the local kiln while that of rice husks was obtained by pyrolyzing rice husks in a muffle furnace.

Figure 47: Formulations to conduct the second biogas experimentations (Biochar_3 is the biochar from rice husks; Biochar_4 is the biochar from corn stalks)

Digester 1 (benchmark)	Digester 2	Digester 3
Cow dung: 30 g	Cow dung: 30 g	Cow dung: 30 g
Biochar: 0 g	Biochar_3: 2,26 g	Biochar_4: 2,26 g
Inoculum: 220 g	Inoculum: 220 g	Inoculum: 220 g
Water: 31 ml	Water: 31 ml	Water: 31 ml

At the 9th day of experimentation, the total volumes of biogas produced were:

Digester 1: 264ml,

Digester 2: 157ml,

Digester 3: 205ml.

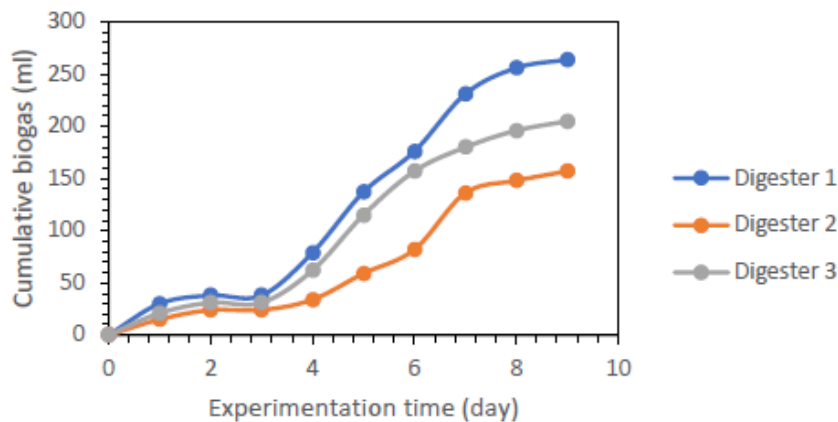
Below, in Fig. 48, some images of the second biogas trials experimentations.

Figure 48: Some images of the second biogas trials



The cumulative curves of the different digesters during the second biogas trials are shown in Fig. 49.

Figure 49: Cumulative biogas curves of the different digesters (in progress – second trial)



Conclusion

The addition of biochar of peanut shells and biochar of corn cobs in cow dung digestion can increase the biogas production. When the production of biogas is expressed in ml, the increases correspond respectively to 339.67% and 23.97%. When the production of biogas is expressed in ml/g TS, the increases correspond respectively to 295.67% and 12.08%. When the production of biogas is expressed in ml/g VS, the increases correspond respectively to 325.26% and 22.09%.

For the second trials, we note that the biogas productions during the first 9 days of experiments when using biochar of corn stalks and rice husks as additive are less important on digester 2 and digester 3 compare to digester 1. Trials for these protocols are ongoing.

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Biochar additive trials: second trial - final results, third trial – initial results, characterization of the substrates

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Introduction

In the framework of the work package WP4, it was decided, in Senegal to produce biochar from different biomasses. The production of biochar will be conducted in local kilns, Brazilian kilns, and HTC technology. The biochar obtained will be tested as an additive in anaerobic digestion and as an absorbent to remove the H₂S from biogas.

Materials and Methods

We focused on the characterization of the substrate used in the biogas experimentations, and the second trial on biochar as an additive for biogas production began on 21/01/2024. The principle of water displacement is applied in these digesters (or reactors) to assess the volume of biogas produced. The first bottle receives the substrate to be methanized; the second bottle contains the water to which the biogas produced exerts pressure, while the third bottle is used to collect the volume of biogas produced.

Results and Discussion

Biogas results (second trials ended)

The second trials on biogas (from 23/01/2024 to 21/02/2024) using biochar as an additive are conducted as indicated in Fig. 50. For these second tests, we use biochar from rice husks and biochar from corn stalks as additives and cow dung with the addition of inoculum from cow dung digestion. The biochar of corn stalks comes from the local kiln while that of rice husks was obtained by pyrolyzing rice husks in a muffle furnace.

Figure 50: Formulations to conduct the second biogas experimentations (Biochar_3 is the biochar from rice husks; Biochar_4 is the biochar from corn stalks)

Digester 1 (benchmark)	Digester 2	Digester 3
Cow dung: 30 g	Cow dung: 30 g	Cow dung: 30 g
Biochar: 0 g	Biochar_3: 2,26 g	Biochar_4: 2,26 g
Inoculum: 220 g	Inoculum: 220 g	Inoculum: 220 g
Water: 31 ml	Water: 31 ml	Water: 31 ml

On the 28th day of experimentation, the total volumes of biogas produced were:

Digester 1: 280ml,

Digester 2: 184ml,

Digester 3: 232ml.

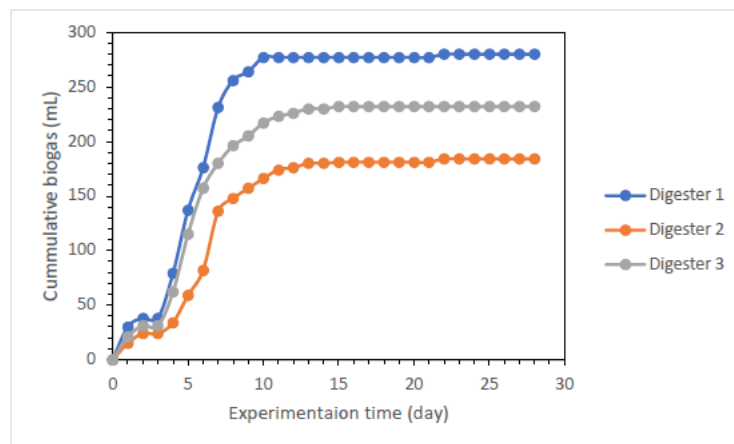
Below, in Fig. 51, are some images of the second biogas trial experimentations.

Figure 51: Some images of the second biogas trials



The cumulative curves of the different digesters during the second biogas trial are shown in Fig. 52.

Figure 52: Cumulative biogas curves of the different digesters (completed – second trial)



During the second trial, we remarked that the addition of biochar to rice husks (digester 2) and corn stalks (digester 3) decreased the volume of biogas compared to the benchmark (digester 1). The volume of biogas in digester 2 decreased to 34.28% compared to Digester 1 while the volume of biogas in Digester 3 decreased to 17.14% compared to Digester 1. This result led us to believe that these biochars have an inhibiting effect on the digestion process, or that gas leaks could occur at the tube connection points, contributing to the decrease of the biogas volume.

Third biogas trial using the new Bain Marie

The third trials on biogas (beginning on 26/02/2024) using biochar as an additive are conducted as indicated in Fig. 53. For these second tests, we use biochar from peanut shells, biochar from rice husks, and biochar from corn cobs as additives and cow dung with the addition of inoculum from cow dung digestion. The biochar of corn cobs and peanut shells comes from the local kiln while that of rice husks was obtained by pyrolyzing rice husks in a muffle furnace.

Figure 53: Formulations to conduct the third biogas experimentations (Biochar_1 is the biochar from peanut shells; Biochar_2 is the biochar from corn cobs; Biochar_3 is the biochar from rice husks)

Digester 1 (benchmark)	Digesters 2 &3	Digesters 4 & 5	Digesters 6 & 7
Cow dung: 60 g	Cow dung: 60 g	Cow dung: 60 g	Cow dung: 60 g
Biochar: 0 g	Biochar_1: 4,62 g	Biochar_2: 4,52 g	Biochar_3: 4,52 g
Inoculum: 440 g	Inoculum: 440 g	Inoculum: 440 g	Inoculum: 440 g
Water: 62 ml	Water: 62 ml	Water: 62 ml	Water: 62 ml

On 29/02/2024, the total volumes of biogas produced were:

Digester 1: 560ml,

Digester 2: 303ml; **Digester 3:** 242ml

Digester 4: 247ml; **Digester 5:** 454ml

Digester 6: 137ml; **Digester 7:** 241ml

Unfortunately, the trials were stopped because there were some leaks at the tube connection points. We will improve these issues and launch other trials.

Below, in Fig. 54, are some images of the third biogas trial experimentations.

Figure 54: Some images of the third biogas trials



Characterization of the substrate used in the second biogas trials

Figures 55 and 56 show the results of the characterization of the different substrates used in the first biogas trials.

Figure 55: Proximate analysis of the different substrates (third trial)

Substrates	M (%)	TS (%)	VS (%) ^s	Ashes (%) ^s	FC (%) ^s
Cow dung 2	77,65	22,35	59,68	23,72	16,60
Inoculum 2	94,76	5,24	--	--	--
Biochar 1 (Peanut shells)	4,44	95,56	15,04	33,59	51,36
Biochar 2 (Corn cobs)	8,53	91,48	11,82	14,44	73,74

Figure 56: Elemental analysis of the different substrates (third trial)

Substrates	Ashes (%) ^s	C (%) ^s	H (%) ^s	O (%) ^s	N (%) ^s	C/N
Cow dung 2	23,72	39,68	4,43	30,58	1,59	24,98
Inoculum 2	--	--	--	--	--	--
Biochar 3 (rice husks)	33,59	54,65	1,97	7,79	2,00	27,39
Biochar 4 (Corn stalks)	14,44	75,64	2,35	6,47	1,10	68,92

The characterization for the inoculum and the digestates obtained during the second biogas trial are in progress.

Conclusion

The addition of biochar from rice husks and biochar from corn stalks in cow dung digestion has decreased biogas production. When the production of biogas is expressed in ml, the decreases correspond respectively to 34.28% and 17.14%. These decreases could be attributed to an inhibitor effect of the biochar or to the leaks at the tube connection points.

For the third biogas trials, from the third day of production, we noted a large difference in the volume of biogas produced between two identical digesters (especially for digesters 4 & 5, with a difference of 207ml). These large differences are due to the leaks at the tube connection points.

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9. Conclusion and Way Forward

This document details the pilot trials, including timelines and parameters to be examined during the trials, and preliminary and final results from the **initial trial reporting period (M18-M24)** and **interim trial reporting period (M25-34)** where available.

Twenty-two trial plans have been developed around the products from three main technology types: **green biorefinery**, **carbonisation** (slow pyrolysis/HTC), and **densification** (pelletizing/briquetting). Four of those trials were initiated between **M18-M24**, in Ghana, Côte d'Ivoire and Senegal. These were trials that could begin using pre—existing locally available technologies with the novel agri-food residues and uses identified through this project, i.e. **biochar** products in Ghana (**soil amendment field trials**) and Côte d'Ivoire (**soil amendment greenhouse trials**), and products of **densification** in Côte d'Ivoire (**sheep feed**) and Senegal (**solid fuel for cooking**).

During the **interim trial reporting period**, new densification equipment was installed in Ghana and Senegal for the production of products for use in pilot trials, and green biorefinery equipment was installed in Ghana, while adapted carbonisation technology, (Brazilian kiln and HTC), was implemented in Ghana, Côte d'Ivoire and Senegal. This has allowed a further six trials to begin, including biorefinery whey screening in Uganda and Ghana, and biochar as a biogas production improvement additive in Senegal. The implementation of new technologies has also enabled replication of trials with products from new technologies, e.g. biochar from adapted Brazilian kiln technology in Ghana, Côte d'Ivoire and Senegal.

Significant delays have been experienced in the implementation of some trials, namely **pig, chicken and piglet feeding trials** in **Uganda** and **rabbit and poultry biomass pellet feeding trials** in **Côte d'Ivoire**. These delays arose due to unexpected issues in feedstuff production in the case of biorefinery products, particularly protein concentrate, and delayed delivery of equipment in the case of the feeding trials. Solutions have been identified to enable these trials to proceed in the **final trial reporting period (M35-M44)**, and these risks will be closely monitored to mitigate any risks to trial completion within the timeframe of Work Package 4.

Trial results have been provided in this report for nine of the trials in progress, including biorefinery whey screening (Uganda and Ghana), soil amendment trials (Ghana and Côte d'Ivoire), animal feeding trials (Côte d'Ivoire), and biochar-based solid fuel (cooking fuel) tests and biochar as a biogas production improvement additive (Senegal). Final results for all trials will be available during the final trial reporting period. These will be included in **D4.4: Final report on trials and results**. The preliminary results will already feed into other work packages before the final trial reporting period is over, and close cooperation with collaborators on other work packages, e.g. **WP5, WP6 and WP7** will ensure that applicable results are shared when available to ensure good functioning of the project.

10. References

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