



# Fruit and vegetable waste used as bacterial growth media for the biocementation of two geomaterials

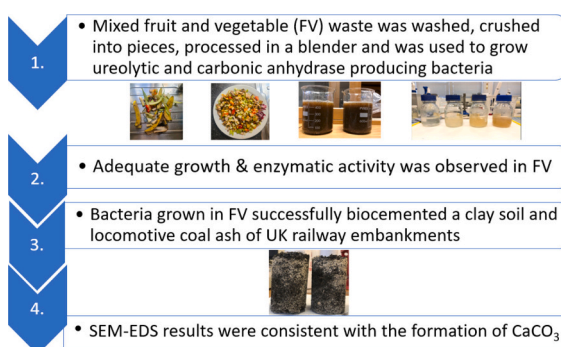
Wilson Mwandira, Maria Mavroulidou<sup>\*</sup>, Sumit Joshi, Michael J. Gunn

School of BEA, LSBU, London, UK

## HIGHLIGHTS

- To reduce biocementation costs, fruit and vegetable (FV) waste was used as bacterial growing media.
- Sugars and proteins of fermented, unfermented, autoclaved & non-autoclaved media were measured.
- Growth rate and activity of 2 ureolytic and 1 carbonic anhydrase producing strain in FV were good.
- UCS, CaCO<sub>3</sub> content and SEM-EDS confirmed biocementation in a fine-grained soil and locomotive ash.
- The environmental benefits of using FV were discussed.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

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## ABSTRACT

This paper investigates the feasibility of using randomly collected fruit and vegetable (FV) waste as a cheap growing medium of bacteria for biocementation applications. Biocementation has been proposed in the literature as an environmentally-friendly ground improvement method to increase the stability of geomaterials, prevent erosion and encapsulate waste, but currently suffers from the high costs involved, such as bacteria cultivation costs. After analysis of FV waste of varied composition in terms of sugar and protein content, diluted FV waste was used to grow ureolytic (*S. pasteurii*, and *B. licheniformis*) and also an autochthonous heterotrophic carbonic anhydrase (CA)-producing *B. licheniformis* strain, whose growth in FV media had not been attempted before. Bacterial growth and enzymatic activity in FV were of appropriate levels, although reduced compared to commercial media. Namely, the CA-producing *B. licheniformis* had a maximum OD<sub>600</sub> of 1.799 and a CA activity of 0.817 U/mL in FV media. For the ureolytic pathway, *B. licheniformis* reached a maximum OD<sub>600</sub> of 0.986 and a maximum urease activity of 0.675 mM urea/min, and *S. pasteurii* a maximum OD<sub>600</sub> = 0.999 and a maximum urease activity of 0.756 mM urea/min. Biocementation of a clay and locomotive ash, a geomaterial specific to UK railway embankments, using precultured bacteria in FV was then proven, based on recorded unconfined compressive strengths of 1–3 MPa and calcite content increases of up to 4.02 and 8.62 % for the clay and ash respectively. Scanning Electron Microscope (SEM) and energy dispersive X-ray spectroscopy (EDS), attested the formation of bioprecipitates with characteristic morphologies and elementary composition of calcite crystals. These findings suggest the potential of employing FV to biocement these problematic geomaterials and are of wider relevance for environmental and geoenvironmental applications involving bioaugmentation. Such

<sup>\*</sup> Corresponding author.

E-mail address: [mavroum@lsbu.ac.uk](mailto:mavroum@lsbu.ac.uk) (M. Mavroulidou).

applications that require substrates in very large quantities can help tackle the management of the very voluminous fruit and vegetable waste produced worldwide.

## 1. Introduction

Faced with the challenges of climate change and the contribution of CO<sub>2</sub> to global warming, civil engineers need to adopt transformative processes and materials to mitigate climate change, while ensuring infrastructure resilience and environmental protection and conservation. Traditionally, the civil engineering industry has been very taxing for the environment, due to the high demand for non-renewable natural resources (32 % of their global utilisation according to [Purchase et al., 2022](#)). Also, CO<sub>2</sub> emissions from the manufacture of cement, are responsible for up to 8 % of anthropogenic CO<sub>2</sub> emissions ([Mavroulidou et al., 2015](#)). An annual cement production of greater than 4 billion tons worldwide results in ca. 3 billion tons of CO<sub>2</sub> emissions ([Di Stefano, 2021](#)). The use of heavy machinery and fuel further contributes to greenhouse gas emissions and the overall environmental impact of the industry, in particular in large infrastructure projects (construction and maintenance). Such activities also generate vast amounts of waste (construction and demolition waste amounts to 30 % of total waste produced globally according to [Purchase et al., 2022](#)). Recent statistics showed for example that construction, demolition and excavation waste in the UK was 138 million tons in 2018, of which 58 million tons (42 %) was excavation waste (soils); 29 million tons of this excavation waste (50 %) ended to landfill ([Adams and Thornback, 2022](#)).

To reduce excavation waste and the demand for natural aggregates, ground improvement has been increasingly used by engineers when they encounter unsuitable ground (due to contamination or poor hydromechanical properties), thus avoiding the need to replace it. Still, most ground improvement processes rely on heavy machinery and are energy intensive, and may still require the use of cement, for example for rigid inclusions in soft soils, or when cement is used as a chemical stabiliser to improve the soil properties or to encapsulate waste. To overcome these drawbacks, biobased methods of ground improvement mimicking natural biological processes have recently gained popularity, as a potentially disruptive technology which can remediate unsuitable ground in an environmentally friendly and potentially sustainable way. In particular, the process of biocementation has drawn the interest of researchers internationally. Biocementation mimics the natural process of biomineralisation to produce biological cements (the so called “biocements”) through the metabolic action of microorganisms (or, alternatively, using bacterial or plant-derived crude enzymes -see e.g., [Cui et al., 2022](#)). This is done as a means of improving the ground by binding the soil particles together for a number of environmental or geotechnical applications. Namely, to stabilise soil by enhancing its mechanical properties ([Whiffin et al., 2007](#); [Keykha et al., 2017, 2018](#); [Cui et al., 2017, 2020](#); [Safdar et al., 2021a, 2021b, 2022](#)), to prevent granular soil liquefaction during earthquakes ([Montoya et al., 2013](#); [Xiao et al., 2018](#); [Sun et al., 2021](#); [Lee et al., 2022](#)), to suppress dust ([Fan et al., 2020](#); [Shi et al., 2021](#)) and protect against wind erosion and desertification ([Maleki et al., 2016](#); [Nikseresht et al., 2020](#); [Wang et al., 2023](#); [Dagliya et al., 2022a, 2022b, 2023](#)) or water erosion ([Wang et al., 2020](#); [Shahin et al., 2020](#); [Clarà Saracho et al., 2021](#); [Haouzi et al., 2023](#); [Sun et al., 2024](#)) including the formation of biomimetic beachrocks for natural coastal protection ([Danjo and Kawasaki, 2016](#); [Imran et al., 2019](#)), to enhance wellbore stability and seal cracks in geological formations, for energy applications or CO<sub>2</sub> storage ([Cuthbert et al., 2013](#); [Cunningham et al., 2014](#); [Minto et al., 2016](#); [Phillips et al., 2016, 2018](#)) and to encapsulate contaminants and various forms of waste (including hazardous waste) ([Liang et al., 2015](#); [Mwandira et al., 2019](#); [Tamayo-Figueroa et al., 2019](#); [Sharma et al., 2022](#)). Namely, the process involves the precipitation of various minerals, mostly carbonates (in particular calcite or aragonite and to a lesser degree, dolomite -see e.g., [Sun et al.,](#)

[2024](#)), but also, in fewer cases, phosphates ([Akiyama and Kawasaki, 2012](#); [Ivanov et al., 2019](#); [Avramenko et al., 2023](#)), which could act as binders of the soil particles (i.e., constituting a natural cementing agent, as an alternative to CO<sub>2</sub> emitting Portland cement). The precipitation is a result of different chemical reactions, which are catalysed by enzymes produced by bacteria or plants. Biocementation has usually been studied and proposed as an in situ treatment harnessing the metabolic action of microorganisms in the ground (whether stimulating existing bacteria in the ground or cultivating/ augmenting and introducing into the ground strains favourable for the biocementation process), although some ex situ processes have also been proposed but to a much lesser degree (see e.g., [Keykha et al., 2019](#)).

Whilst considerable advances have been made in the field of soil biocementation in the last 10 years ([Islam et al., 2020](#); [Montoya and DeJong, 2015](#); [Safdar et al., 2021a, 2021b](#); [Mwandira et al., 2022](#)), mostly in the laboratory but also, to a lesser extent, in pilot field applications ([Gomez et al., 2015, 2017](#); [Gowthaman et al., 2023](#)), the process is not widely adopted commercially. One of the major barriers preventing industry scale applications for geotechnical or geo-environmental engineering is the high cost of the techniques ([Kahani et al., 2020](#); [Dagliya et al., 2023](#)). In particular, the costs linked to cultivating bacteria producing the required enzymes were shown to account for 60 % of the total costs of biocementation ([Omoriegbe et al., 2019](#)) and for 20 % of materials production impacts ([Deng et al., 2021](#)). According to a number of recent Life Cycle Analyses (LCA) of the different biocementation metabolic pathways, the production of the chemicals used, including components of growing media, are likely to consume considerable energy for their production and can have a high carbon footprint, especially when purified laboratory grade chemicals are used (as is the case in the vast majority of studies). Thus, while a key driving factor behind the development of biocementation as a ground improvement technique has been its assumed environmental advantages, in the forms currently implemented by most researchers, biocementation can have its own environmental impacts that need to be addressed. Consequently, the LCA studies conclude that in order to improve the sustainability of the technique towards industry scale, the utilisation of naturally found nutrient sources or recycled wastes for the source of microbial nutrients would be required ([Deng et al., 2021](#); [Porter et al., 2021](#); [Faruqi et al., 2023](#)).

To address cultivation costs and media production impacts, some recent studies have attempted to use alternative media to cultivate the microorganisms, using a number of waste stream sources, as reviewed in [Mwandira et al. \(2023a\)](#). Such waste includes (but is not limited to) kitchen and dairy industry waste ([Achal et al., 2009](#); [Meng et al., 2021](#); [Kahani et al., 2020](#); [Kulanthaivel et al., 2022](#)), molasses and vinasses ([Nikseresht et al., 2020](#); [Dagliya et al., 2023](#)), effluents and wastewaters of different processes (e.g. effluent of palm oil mill in [Omoriegbe et al., 2023](#), corn steep liquor in [Achal et al., 2010](#); [Joshi et al., 2018](#) and [Amiri and Baqaran Bundur, 2018](#), chicken manure effluent in [Yoosathaporn et al., 2016](#) or tofu wastewater in [Fang et al., 2019](#)), as well as industrial sludges ([Yang et al., 2020](#); [Yu et al., 2023](#)). However, waste feedstock availability and characteristics can vary from one location to another, which can limit the applicability of a number of wastes for the industrial implementation of bacterial cultures.

It thus appears that a more viable source of bacteria growth media for biocementation would likely be fruit and vegetable (FV) waste, which is ubiquitous, can be widely obtained all year round in large quantities from various sources worldwide, including households through special municipal collection schemes, school or workplace canteens, the hospitality sector or food production factories. The feasibility of collecting FV waste in large quantities for bioaugmentation

purposes is guaranteed by regulations in force in a number of countries, including the UK through the Resources and Waste Strategy and the EU countries through European Union Waste Framework Directive 2018, which mandate that food waste must be sent from the relevant site as a clean, separate waste stream whose collection is done separately, both for households and businesses across the public and private sectors, while they prohibit its disposal to sewers, and its mixing with other refuse. Mixed FV is likely to contain high amounts of sugars, the most abundant organic substances in the biosphere, as they constitute structural components of most living biomass and are the most important carbon (C) and energy source for soil microorganisms, as well as building blocks for various cellular components. Consequently, in natural soils, one of the most important functions of sugars are to maintain and stimulate microbial activities in the rhizosphere and detritosphere (Gunina and Kuzyakov, 2015). Additionally, FV would contain proteins, essential for bacteria growth and viability, water, and several other elements, some in trace amounts (phosphorus, iron, calcium, magnesium, and sodium, as well as zinc, copper, potassium, and manganese) that are critical micronutrients for microbial development, for cellular inorganic cations, as co-factors in specific enzyme activities, and as endospore components (Anderson et al., 2015; Dong et al., 2022; Omoregie et al., 2023).

According to Lau et al. (2021) 45 % of fruit and vegetable produced worldwide is wasted, i.e. by far the largest portion of the one billion tonnes of food waste worldwide every year. In the UK this waste amounts to around 9.5 million tonnes of food waste per year, of which 6.6 million tonnes (70 %) coming from households, 1.5 million tonnes (16 %) from manufacturers, 1.1 million tonnes (12 %) from the hospitality and food service (HaFS) sector and 0.3 million tonnes (3 %) from the retail industry (WRAP, 2019). Costs to industry linked to food waste disposal are substantial. For example, UK food retailers spend on average £50,000 a year sending, organising and transporting wasted food to landfill sites (with ca £103.70 landfill tax per tonne of waste), whereas by recycling/reusing it, they could save up to £7000 per year (Keenan Recycling, Ltd, 2022). Importantly, landfilled food waste generates high amounts of greenhouse gases per year through decomposition, contributing to global warming and climate change: according to the USEPA (Krause, 2023) for every 907 tons of food waste landfilled, an estimated 34 tons of fugitive methane emissions are released. Similarly, food waste incineration contributes to climate change as it emits CO<sub>2</sub> into the atmosphere.

The UK as well as other countries have committed to halving the per capita food waste by 2030 as part of the United Nations' sustainable development goals (SDG), Target 12.3. Additionally, the Environment Act of 2021 in the UK aims to eliminate food waste in landfills by 2030. Despite this, it is likely that a large amount of FV waste will persist, due to the inedible parts of the fruits and vegetables, which guarantees the abundance of this waste for bacterial cultivation purposes. This unavoidable FV waste will keep arising in households, hospitality, as well as in food production chains, where vegetables and fruits may be consumed/processed to get the required parts of the fruit, discarding unwanted parts such as seeds, leaves, stalks/stems, peels, or pulp. Even surplus FV from primary producers which goes primarily to animal feed is not entirely consumed for this purpose, as soft fruit, leafy and other vegetables such as onions are typically not used as livestock feed (WRAP, 2019). Other recycling routes should therefore be found for this waste; the use for ground improvement projects involving biocementation could be an outlet for large amounts of this waste, considering the material quantities required for such projects.

This paper extends recent work by the authors' team presented at the CEST2023 conference (Mwandira et al., 2023b). Its scope is to prove FV waste as a bacterial growing medium towards soil biocementation by bioaugmentation. For this, FV waste is used to cultivate three different types of bacteria to biocement geomaterials, using two different metabolic routes. Namely, it studies two ureolytic bacterial strains, one from a commercial bank and another isolated by the authors' team from a UK

railway embankment foundation soil provided by Network Rail (the owners and operators of most of the UK railway network), and for the first time (to the Authors' knowledge) it also uses FV to grow carbonic anhydrase (CA) producing bacteria, namely a CA-producing bacterial strain isolated by the authors' team from another railway embankment site. Carbonic anhydrase (CA) producing bacteria are of major relevance for the carbon cycle in living organisms and the environment, as CA catalyses the reversible interconversion between CO<sub>2</sub> and bicarbonate, enabling a fast and efficient CO<sub>2</sub> conversion and utilisation. Thus, in the last two decades CA has been extensively researched for carbon capture, conversion and utilisation applications (Yadav et al., 2014; Migliardini et al., 2014; Zhan et al., 2021; Molina-Fernández and Luis, 2021; Gadikota, 2021; Talekar et al., 2022; Villa et al., 2023), as well as for biofuel production (Boone et al., 2013; Thakur et al., 2018; Jain et al., 2019). CA-producing bacteria can also be used to produce biocement by CO<sub>2</sub> capture and utilisation (Reddy and Joshi, 2018; Qian et al., 2023; Yu and Xu, 2023; Mwandira et al., 2023c, 2023d). By doing this, they provide a more sustainable way of biocementing the soil with increased environmental advantages, as they consume CO<sub>2</sub> for the production of biocement; industrially captured, waste CO<sub>2</sub> can thus be consumed to this effect (Mwandira et al., 2023c–f). Indeed, based on LCA (Porter et al., 2021) microbially induced calcium carbonate biocement produced using carbonic anhydrase producing bacteria was shown to be the most environmentally sustainable route for engineering applications, compared to all other metabolic pathways.

The research, therefore, addresses the weaknesses of current biocementation techniques in terms of costs and environmental impact identified in previous studies by a) using unsorted FV waste as nutrient source for bacteria, which can be prepared for large scale applications using existing food waste processing equipment (e.g. rotor choppers, scrapers, pumps and pipework used for anaerobic digestion/biogas generation applications) and b) by developing and studying the most promising metabolic pathway in terms of sustainability, i.e. the CA pathway.

After growing the strains in FV waste, the strains are implemented to biocement two problematic geomaterials of the UK railway network (both provided by Network Rail). Namely, a clay railway embankment foundation soil and for the first time, to the Authors' knowledge, a waste material, locomotive coal ash. In general, clays are only rarely considered for biocementation (sands are the main soil type researchers are commonly using for biocementation). As for the locomotive coal ash, this is a waste material particular to railway embankments in the UK, causing great challenges to railway asset management teams (see further details in the Materials and methods section). Biocementation is proposed here as a means of stabilising the ash and preventing erosion. To the Authors' knowledge, there is no published work proving biocementation for this material and this is done here using two different metabolic processes, including the little researched CA pathway.

The significance of this research is thus the contribution to enhancing the feasibility of the promising technique of biocementation by bioaugmentation for large scale ground improvement (stabilisation and bioremediation) projects, by reducing its costs, currently a significant barrier to its industrial adoption. This way it contributes to the replacement of the current environmentally costly conventional ground improvement methods (Safdar et al., 2021a). It also helps tackle the management of the very voluminous fruit and vegetable waste, considering the demand for substrates in very large quantities, when used for geoenvironmental and environmental applications of the bio-based processes. Indeed, while the paper studies the potential of employing FV to biocement two problematic geomaterials, it is of wider relevance for environmental applications (for example, biological methods of waste and wastewater treatment and environmental remediation of soil/groundwater or carbon capture, storage and utilisation) involving bioaugmentation, and has the added advantage of finding an additional outlet for this voluminous waste material.

## 2. Materials and methods

### 2.1. FV waste media preparation

The FV waste used to grow cultures for biocementation came from LSBU student canteens and consisted of 50 % by weight vegetable waste and 50 % by weight fruit waste. It was a varied mixture of FV that contained banana skins, apple skins and residues, strawberries, grapes, mango skins, carrot skins and residues, cabbage, white onions, red and green pepper, kale, celery and potatoes. Some additional FV waste of similar composition was also collected from household kitchen waste, to perform some further analyses on nutrient content to assess possible differences due to variability in the relative proportions of the different fruits and vegetables. The FV waste was washed with tap water, chopped into small pieces and mixed; it was then blended into a pulp using a kitchen blender; the pulp was then filtered to collect the liquor. The FV liquor was too thick to be used 100 % without any water; therefore, different FV concentrations were obtained by serial dilution. Namely 10 %, 30 %, 50 % and 80 % FV liquor were used (where, for example 80 % FV means that 80 % FV liquor was diluted with 20 % distilled water). The FV media were divided into different clean flasks. Part of the FV liquor was allowed to ferment at room temperature for four days before using it as culture media. The selected bacterial strains were then inoculated to the flasks with different FV media concentrations (i.e. one type of strain per flask). For comparison, both sterilised (autoclaved) and unsterilised (non-autoclaved) FV media were used to culture bacteria. The pH of the mixed FV waste (which is expected to affect bacterial growth and activity) was measured as 5.67; conversely, 100 % fruit waste (F) and 100 % vegetable waste (V) were measured to have a pH of 5.01 and 6.49 respectively.

The FV media thus prepared to be used for bacterial growth and biocementation studies were then tested for their soluble sugar content (Brix %) using a refractometer. For nitrogen and protein content the Kjeldahl method was used, involving digestion of FV with a strong acid to release nitrogen and quantify it using a titration technique; nitrogen content can then be correlated to the protein content. Additional FV mixes from household kitchen waste (which were not pursued for biocementation study) were prepared with different compositions of fruit and vegetables to investigate the variability in the contents of the ingredients and thus acquire an idea of typical range of nutritional values, as, unless the FV source is consistent (for example, originating from a food production factory or bakery factory) the composition of the ingredients is likely to vary widely.

### 2.2. Bacterial growth and enzymatic activity

A number of key factors affect the process of microbially induced carbonate precipitation. These include the type and amount of enzyme produced, the concentration of dissolved inorganic carbon, environmental factors such as temperature and pH which affect the growth and enzymatic activity of the bacteria and the precipitate form/type and solubility, as well as the availability of nucleation sites and the concentration of the cementation solutions (Mwandira et al., 2023e, 2024; Yi et al., 2021).

For this study, two metabolic routes that can lead to biocementation were used:

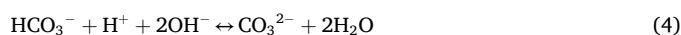
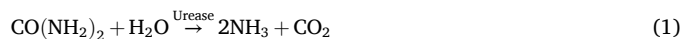
- (a) the ureolytic pathway, which has been commonly used by most researchers worldwide for soil biocementation; this pathway involves the hydrolysis of urea, generating carbon dioxide and ammonia (Eq. (1)). pH increases due to hydroxyl ions obtained from the conversion of ammonia to ammonium (Eq. (2)); The CO<sub>2</sub> reacts with H<sub>2</sub>O to produce bicarbonate ions (Eq. (3)); these react with hydroxyl ions, generating carbonate ions (Eq. (4)). If calcium ions are introduced, calcium carbonate precipitates (Eq. (5)):

**Table 1**

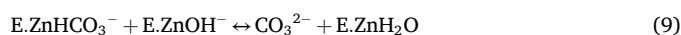
Composition of commercial nutrient media for the cultivation of bacteria.

Ingredient	NB-urea media (g/L)	CA media
Peptone	5	-
Yeast extract	1.5	10 g/L
Beef extract	1.5	-
Sodium chloride	5	-
Urea	2	-
Sodium bicarbonate	N/A	100 mM
Zinc sulphate	N/A	1 μM

NB-urea media: Nutrient broth – Urea media; CA media: Carbonic anhydrase media; mM: Millimolar; μM: Micromolar.



- (b) the biotic metabolic pathway of interconversion between CO<sub>2</sub> and the bicarbonate ion HCO<sub>3</sub><sup>-</sup> mediated by heterotrophic carbonic-anhydrase (CA) producing bacteria. In this process CA utilises gaseous CO<sub>2</sub> to give hydrated aqueous CO<sub>2</sub> (aq) (Eq. (6)); this reacts with H<sub>2</sub>O forming H<sub>2</sub>CO<sub>3</sub> (Eq. (7)). Once the H<sub>2</sub>CO<sub>3</sub> is formed it ionises in water to generate HCO<sub>3</sub><sup>-</sup> and H<sup>+</sup> (Eq. (8)); the HCO<sub>3</sub><sup>-</sup> further ionises in the alkaline environment, producing CO<sub>3</sub><sup>2-</sup> (Eq. (9)). To form a biomineral, metal ions (e.g., Ca<sup>2+</sup>, Mg<sup>2+</sup> or Fe<sup>2+</sup>) are provided so that precipitates are formed from the reaction. For example, Ca<sup>2+</sup> with CO<sub>3</sub><sup>2-</sup> (Eq. (10)) forms calcium carbonate. This reaction can occur either with CA-producing bacteria or purified CA enzyme (E) serving as nucleation sites.



CA-producing bacteria were isolated from the window samples originating from the foundation soil of a railway embankment, provided by the project partner, Network Rail. The method of isolation has been described in Mwandira et al. (2023e, f, 2024) and will not be repeated here as this is outside the scope of this paper. One of the best performing strains in terms of growth, enzymatic activity and bioprecipitate production at pH ranging 5–10 respectively were selected (Mwandira et al., 2023e). Based on DNA sequencing, they were subsequently identified as a *Bacillus licheniformis* species (Mwandira et al., 2024). Similarly, the ureolytic pathway strains previously isolated by the London South Bank University group (Safdar et al., 2022) from another nearby location in East Anglia had also been identified as another *Bacillus licheniformis* species based on MALDI-TOF biotyping (see Safdar et al., 2022 for further details). Additionally, *Sporosarcina pasteurii* purchased from NCIMB culture collection (NCIMB 8841), has been used for comparison and benchmarking. *Sporosarcina pasteurii* is a ureolytic strain that has been used by the vast majority of biocementation researchers worldwide.

**Table 2**

pH Values of raw extract from Fruit waste only (S1), Veg waste only (S2), 50 % Fruit- 50 % Veg mixed waste (S3).

Waste liquor concentration	S1	S2	S3
80 %	4.8	6.2	5.8
50 %	4.9	6.3	5.7
30 %	5.0	6.2	5.7
10 %	5.0	6.1	5.6

For bacterial growth and to induce urease enzyme in bacterial strains, autoclaved commercial nutrient broth supplemented with urea solution was used; the pH of these media was measured as 6.5. To grow and induce CA enzyme in bacterial strains, autoclaved yeast extract solution supplemented with sodium bicarbonate and zinc sulphate were used. The pH of these media was 7.5 (6.5 for yeast extract only). The composition of NB-urea commercial media and commercial media for the CA pathway is presented in Table 1. These media were then replaced by FV media at different concentrations, whose pH values are recorded in Table 2.

Microbial growth of the different strains in FV and commercial media was studied measuring optical density at 600 nm ( $OD_{600}$ ) using a spectrophotometer over a period of 4 days. In parallel, the enzymatic activity of the strains was measured over the same period. The microbial growth and enzymatic activity measurements were performed at room temperature.

A number of different methods have been established to determine CA activity (Henry, 1991). Of these, we selected a simple method proposed by Martin et al. (2009) that avoided costly activity kits, as described in Mwandira et al. (2023b, 2023e). The method correlates the CA activity to the quantity of *p*-nitrophenol generated per unit time as shown in Eq. (11). Namely, the activity for *p*-nitrophenyl acetate hydrolysis was assessed using 1.35 mL of reaction mixture with 3 mM *p*-nitrophenyl acetate in phosphate buffer (0.13 M and pH 7.2) at room temperature. The reaction continued for 5 min and the optical density

(OD) change at 348 nm was measured using UV-vis spectrophotometry. The quantity of *p*-nitrophenol generated per unit of time was then used to characterise the CA enzyme activity (Eq. (11)):

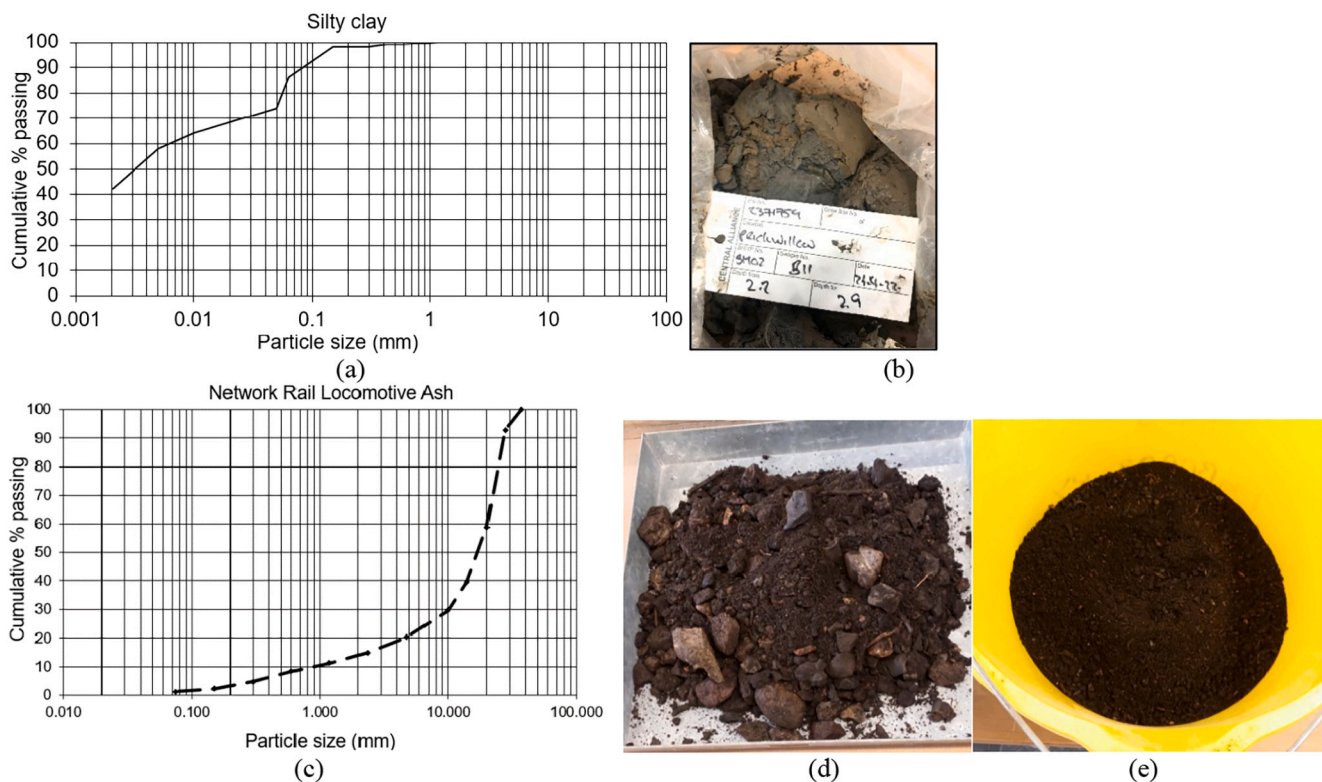
$$A \text{ activity } \left( \frac{U}{mL} \right) = \frac{(\Delta A_{348} T - \Delta A_{348} B) \times 1000}{5 \times V} \quad (11)$$

where  $\Delta A_{348} B$  is the initial (uncatalyzed) reaction at a 348 nm wavelength;  $\Delta A_{348} T$  is the final reading of absorbance; 1 U ( $\mu\text{mol}/\text{min}$ ) is the quantity of enzyme which catalyses the conversion of 1  $\mu\text{mol}$  of substrate per minute; and  $V$  is the volume of bacterial suspension added to the cell. Electrical conductivity (EC) measurements were used to attest urease activity, expressed as mM of urea hydrolysed per minute. It is calculated according to Eq. (12), where  $EC_1$  and  $EC_5$  are respectively EC measured at 1 and 5 min.

$$\text{Urease activity} \left( \text{mM} \frac{\text{Urea}}{\text{min}} \right) = \frac{EC_1 - EC_5}{5} \cdot 10 \cdot 11 \quad (12)$$

### 2.3. Geomaterial biocementation

The feasibility of biocementation with FV was tested for two different geomaterials encountered in the railway network in the UK: a silty clay foundation soil of intermediate plasticity from East Anglia railway network (see Fig. 1 and Table 2), and also waste locomotive coal ash. The latter geomaterial is specific to the UK railway network: in the late 19<sup>th</sup>/early 20<sup>th</sup> centuries, the construction of embankments generally comprised poorly compacted fill from adjacent cuttings. The ash, originating from the locomotives, was historically used as a regulatory layer to maintain the original height of the settling embankments, which were consolidating under their own weight in addition to any locomotive loads. The result is that the ash is currently exposed on the shoulders of embankments throughout the network (including the London Underground) and is almost in all cases unstable. In the dry state, erosion occurs under vibration, whereas precipitation promotes preferential



**Fig. 1.** Particle size distribution and photos of the geomaterials used: (a) Particle size distribution of silty clay foundation soil; (b) Photo of silty clay foundation soil; (c) Particle Size distribution of locomotive ash; (d) Locomotive ash photo (full sample); (e) Locomotive ash photo (part passing the 4.75 mm sieve, used in testing).

**Table 3**  
Properties of geomaterials tested for biocementation.

Parameter	Foundation Soil (silty clay)	Coal Ash
Organic matter (%)	4.0	0.52
Natural gravimetric moisture content (%)	34.3	30.4
Bulk density (g/cm <sup>3</sup> )	1.78	1.33
Plastic limit, w <sub>p</sub> (%)	26	N/A
Liquid limit, w <sub>L</sub> (%)	40	N/A
Plasticity index, I <sub>p</sub> (%)	14	N/A
pH	7.7	7.7–8.08
Natural calcium carbonate (%)	4.77	4.01

gully formation and surface sloughing, all of which frequently undermine the line side services (which are supported on posts) and the ballasted track. The problem is particularly severe at the interface with underbridges where embankments are narrow. A replacement scheme in some parts of the network, proved costly; it generated very extensive quantities of waste and a demand for natural, non-renewable, granite aggregate and was thus unfeasible for the whole extent of the network. UK railway owners and operators therefore look for ways of stabilising rather than replacing this ash (Mavroulidou et al., 2019). Biocementation is the proposed method in this study, which will be tried for the first time to cement this material, to the authors' knowledge. To prevent ash erosion, it is likely sufficient to apply a surface treatment to produce a biocemented ash crust, rather than attempting to cement the whole mass of the ash. The locomotive ash samples were a mix of ash, organic matter (plant remains), ballast, and other debris. The measured pH of the ash samples ranged from 7.7 to 8.08. Ash had a C<sub>u</sub> = 22 and was classified as multi-graded; its fines content was 1.3 %. The portion used for testing was prepared by sieving the ash sample through a 4.75 mm mesh (see Fig. 1) to discard large particles and debris, thus allowing the use of conventional laboratory apparatus. The natural moisture content of the retained portion was 30.1 %. At this water content the corresponding ordinary Proctor compaction bulk density was 1.33 g/cm<sup>3</sup>. Table 3 shows some salient physical characteristics of the two geomaterials; their respective Particle Size Distributions (PSD) according to British Standards 1377–2: 2022 (BSI, 2022) are shown in Fig. 1.

Unconfined compressive strength was assessed using specimens of a diameter of 50 mm and height of 100 mm. Clay specimens were statically compacted in three equal layers at a rate of 1 mm/min at a dry density of 1.78 g/cm<sup>3</sup> (which was the dry density of the soil in the field) and at the natural gravimetric soil water content. For the ash, dynamic compaction in 3 equal layers was used at the natural water content to prepare specimens at their natural dry density and water content. Treatments were introduced to the specimens by consecutive daily injections of bioaugmentation solution (1.5 per volume, L) with the commercial (Table 2) or FV media and a cell concentration of  $9 \times 10^8$  cells/L and biocementation solution (1 M urea & 1 M CaCl<sub>2</sub> for the ureolytic, and 0.25 M NaHCO<sub>3</sub> and 0.25 M CaCl<sub>2</sub> for the CA pathway) for 14 days following the protocol described in Danjo and Kawasaki, 2016 and Mwandira et al., 2017). Note that when attempting to use a compression frame to conduct unconfined compression tests, the untreated clay samples disintegrated upon loading; it was also difficult to test untreated locomotive ash, due to lack of cohesion. For this reason, the unconfined compressive strength was measured using a calibrated pocket penetrometer. At the end of the pocket penetrometer testing, samples across the treated specimens were collected and their oven-dried masses before and following acid wash (HCl 2 M) were measured. The difference in these two masses was then taken as the mass of CaCO<sub>3</sub>.

#### 2.4. Material analysis

Scanning electron microscopy (SEM) was used to observe the microstructure of the biocemented geomaterials and attest the precipitation of calcium carbonate within the soil. SEM was carried out using a

ThermoScientific Pharos FEG-SEM with 15KV acceleration voltage and high vacuum mode. Energy dispersive spectroscopy (EDS) analysis was carried out on the same SEM samples using a Silicon drift detector (SDD) and an integration time of 30s.

### 3. Results and discussion

#### 3.1. FV media nutritional content and bacterial growth and activity

Fig. 2 shows the mean and standard deviations of triplicate measurements of contents of sugars and proteins in the FV media used in the biocementation studies for three cases; namely, non-autoclaved media that were left to ferment versus autoclaved media that were respectively unfermented and fermented. It was found that in all cases FV had a 3 % total sugar. Proteins were found to slightly vary in the three cases with the fermented FV liquor having the highest protein content of 0.302 g/100 mL compared to the unfermented media. The difference in protein content in unfermented media when autoclaved and when non-autoclaved was small (although statistically significant according to *t*-test). Similar findings were obtained from the additional tests on different mixes of unfermented FV waste (of different compositions and from a different source, in order to address the impact of the variability in FV media composition on the reproducibility of the results) which were performed using a different method (Lowry et al., 1951) to determine directly the protein content and attest that proteins would not break down during autoclaving (Paula et al., 2022). Namely, protein content of autoclaved and non-autoclaved fruit (F) waste only (in this instance a different mixture than in set 1 discussed above, i.e., a mixture of banana, apple, and orange peels and some strawberry flesh) and vegetable (V) waste only (carrot skin, spinach stem leftovers, cucumber skin, potato peel and cauliflower leaf) were measured and they were found to be respectively 1.78 mg/mL (non-autoclaved) and 1.66 mg/mL (autoclaved) for the fruit waste mix only and 3.09 mg/mL (non-autoclaved) and 2.8 mg/mL (autoclaved) for the vegetable waste mix only. This confirmed a small protein content reduction after autoclaving. There was also a reduction in the total nitrogen content (attested by Kjeldahl's method) of the F and V after autoclaving; namely, 0.98 mg per gram of sample (F) and 1.54 mg per gram of sample (V) for autoclaved samples, versus 1.54 mg per gram of sample (F) and 2.2 mg per gram of sample (V) for non-autoclaved samples. Considering the above, the rest of the tests (bacterial growth and enzymatic activity) were performed on autoclaved media which would represent a worse case scenario in terms of nutrients but would offer a more rigorous control of the experimental conditions, although for engineering applications it would be important to use non-autoclaved media to reduce costs. Overall, although the content of micronutrients (elements in trace amount) was not tested, it was concluded that as the FV media were rich in sugar and proteins they should be able to support the growth and enzymatic activity of microorganisms.

This was confirmed by the growth curves and enzymatic activity of microorganisms shown in Fig. 3. There is a notable difference in the growth of *S. pasteurii* in commercial and 80 % FV medium (which gave the best growth), with the latter resulting in 2.5 lower maximum OD<sub>600</sub> value (OD<sub>600</sub> = 0.999) compared to that of the commercial medium (OD<sub>600</sub> = 2.546), which is potentially due to the relatively low pH of the FV media (see Table 2); however, the typical microbial growth phases (i.e., lag, exponential, stationary) are consistently shown also in the FV media at the same times as with the commercial medium. The enzymatic activity appears to be relatively less affected (although still lower than the commercial medium), namely a maximum urease activity of 0.976 mM urea/min for the commercial medium and 0.756 mM urea/min for the FV, at a maximum FV content of 80 %, however, the latter occurred at 96 h, whereas in the commercial medium maximum activity occurred at 24 h and was maintained until the end of the measurements at 96 h. For *B. licheniformis* -ureolytic route (Safdar et al., 2022)- the differences in bacterial growth and urease activity are smaller than for *S. pasteurii*.

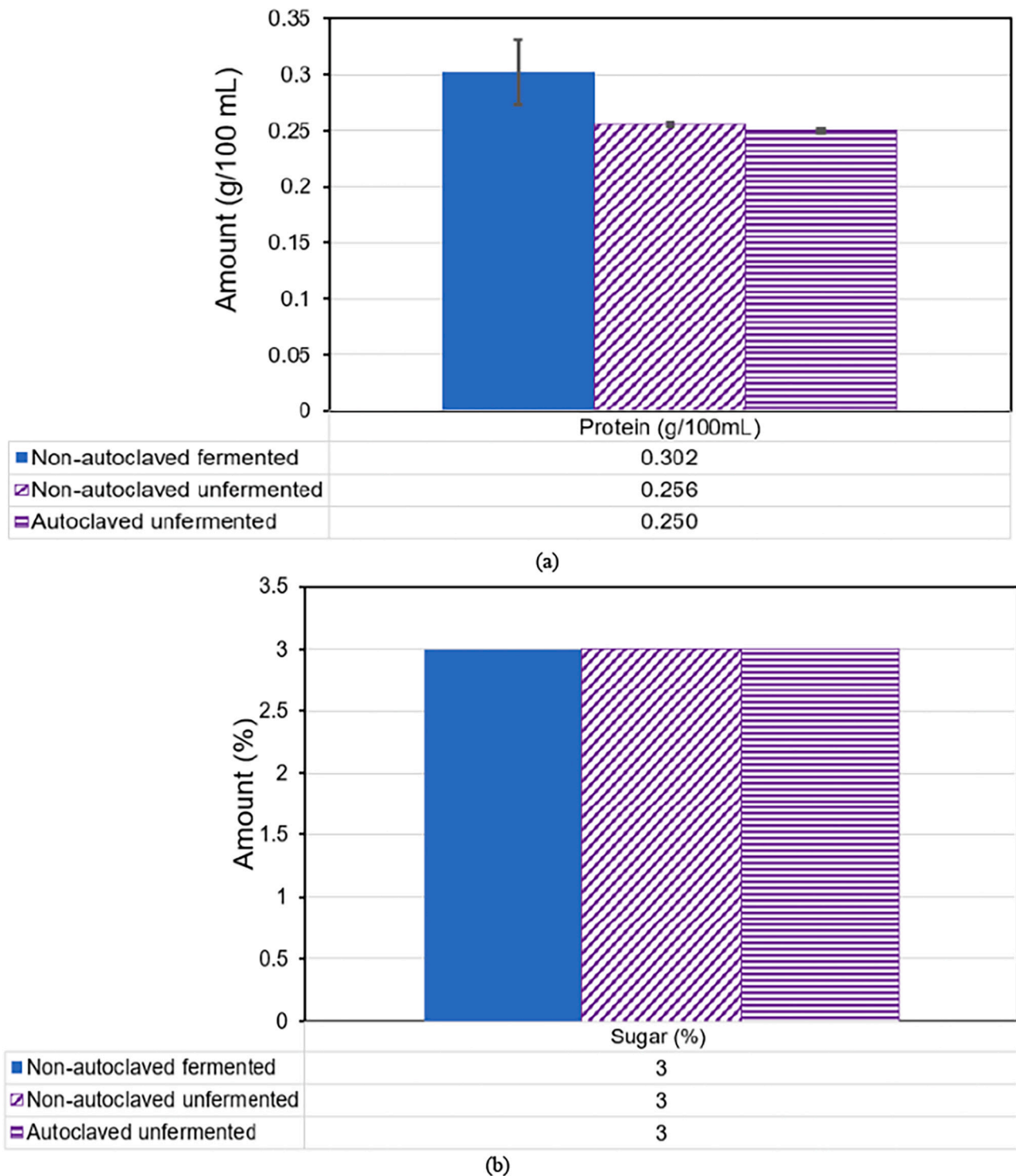
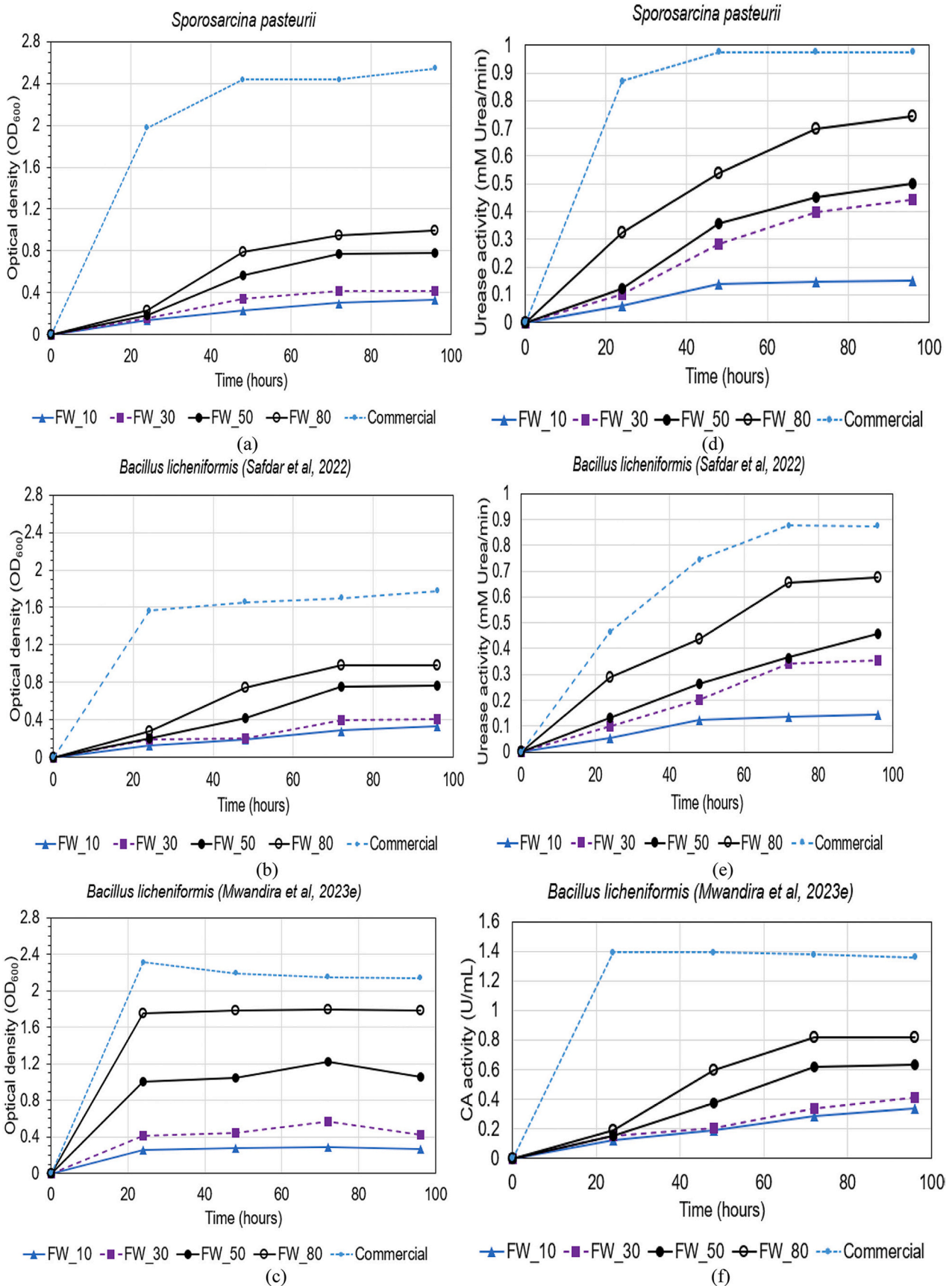


Fig. 2. Contents of FV used in the biocementation studies (a) protein and (b) sugars.

Namely in the commercial medium the strain reached a maximum OD<sub>600</sub> value of 1.776 and a maximum urease activity equal to 0.876 mM urea/min, whereas 80 % FV media, led to a maximum OD<sub>600</sub> of 0.986 and a maximum urease activity equal to 0.675 mM urea/min. As with *S. pasteurii* the lower FV mixing contents led to lower growth and activity. Looking at the literature, there are mixed findings regarding the urease activity in low-cost media from waste. For example, Meng et al. (2021) report a high urease activity of 4.19 mM urea/min for *S. pasteurii* cultured in kitchen food waste, whereas Achal et al. (2009) report 0.353 mM urea/min for lactose mother liquor media. The findings here are in

between these reported values in the literature.

For the CA pathway, the differences in the growth of *B. licheniformis* -CA route Mwandira et al. (2023e)- between the commercial medium and 80 % FV become even smaller with similar rates of growth and maximum OD<sub>600</sub> of 2.312 and 1.799 respectively for commercial and 80 % FV media. This was potentially because the strain was observed to grow well in pH ranging 6–10 (Mwandira et al., 2023e); however, it is difficult to explain why two *B. licheniformis* strains would grow so differently. With the growth in FV being closer to that in commercial media, it is also difficult to explain the differences in enzymatic activity

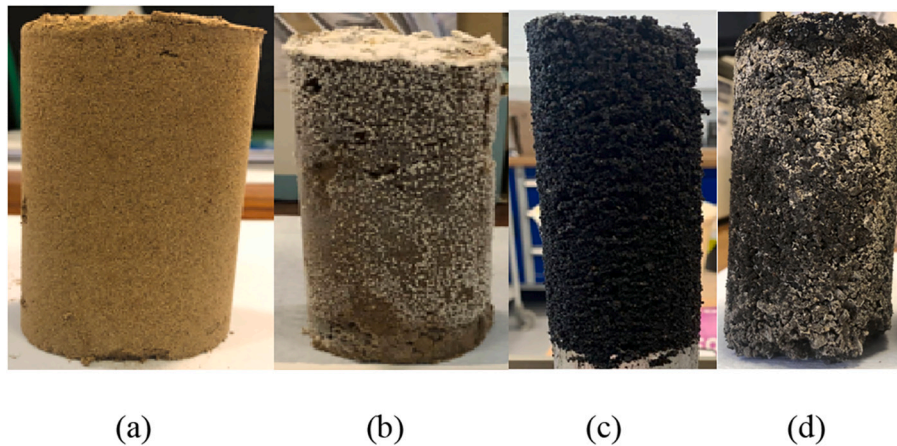


**Fig. 3.** Bacteria growth and enzymatic activity: (a) Growth curves of *S. pasteurii*; (b) Growth curves of *B. licheniformis* (Safdar et al., 2022) -ureolytic pathway (c) Growth curves of *B. licheniformis* (Mwandira et al., 2023e) -CA pathway (d)Enzymatic activity of *S. pasteurii*; (e) Enzymatic activity of *B. licheniformis* (Safdar et al., 2022) -ureolytic pathway (f) Enzymatic activity of *B. licheniformis* (Mwandira et al., 2023e) -CA pathway.



**Table 4**  
Unconfined compressive strength  $q_u$  and  $\text{CaCO}_3$  content of biocemented geomaterials.

Medium	Strain	Biocementation Pathway	Geomaterial	Maximum $q_u$ (MPa)	Maximum $\text{CaCO}_3$ (%)
80 % FV autoclaved	<i>Bacillus licheniformis</i> (Safdar et al., 2022)	Ureolytic	Silty clay	1.5	7.19
	<i>Bacillus licheniformis</i> (Mwandira et al., 2023e)	Carbonic anhydrase		1.0	8.54
	<i>Bacillus licheniformis</i> (Safdar et al., 2022)	Ureolytic	Locomotive coal Ash	3.0	6.7
	<i>Bacillus licheniformis</i> (Mwandira et al., 2023e)	Carbonic anhydrase		2.0	7.74
80 % FV non-autoclaved Medium	<i>Bacillus licheniformis</i> (Safdar et al., 2022)	Ureolytic	Silty clay	1.5	7.66
	<i>Bacillus licheniformis</i> (Mwandira et al., 2023e)	Carbonic anhydrase		1.0	8.79
	<i>Bacillus licheniformis</i> (Safdar et al., 2022)	Ureolytic	Locomotive coal Ash	3.0	12.63
	<i>Bacillus licheniformis</i> (Mwandira et al., 2023e)	Carbonic anhydrase		2.0	7.67



**Fig. 4.** Photos of (a) Untreated clay (b) biocemented clay using FV (c) untreated coal ash, (d) biocemented ash using FV.

which become more pronounced, namely 1.394 U/mL and 0.817 U/mL for the commercial and 80 % FV media respectively, and why the times when maximum activities are reached do not coincide (although growth rates are similar). It should be noted that the CA activity in FV media is higher than that reported for *B. schlegelii* isolated from garden soil (0.0453 U/mL) and grown in commercial media (Nathan and Ammini, 2019) as well as that of *B. altitudinis* isolated from mangrove sediments (0.695 U/mL) and grown in commercial media (Muley et al., 2014). Overall, it can therefore be concluded that despite the lower values when compared to commercial growth media, FV was able to promote bacterial growth and enzymatic activity at levels suitable for mineral bioprecipitation. This, despite the fact that the strains were not grown in optimal pH or temperature conditions, as determined in previous studies by the authors' team, using commercial media (Mwandira et al., 2024). Note that it is possible to adjust the pH of the media for better results, however this was avoided here to reduce costs of treatments. Equally, optimisation of the FV media composition or improvement by adding further ingredients included in commercial media as e.g. in Kahani et al. (2020) was not attempted, as the idea of this research was to assess whether randomly mixed FV waste, as collected and without any sorting, could achieve satisfactory bacterial growth and enzymatic activity (without trying to include all components of commercial growing media), and thus be used as a straightforward means of bacteria cultivation.

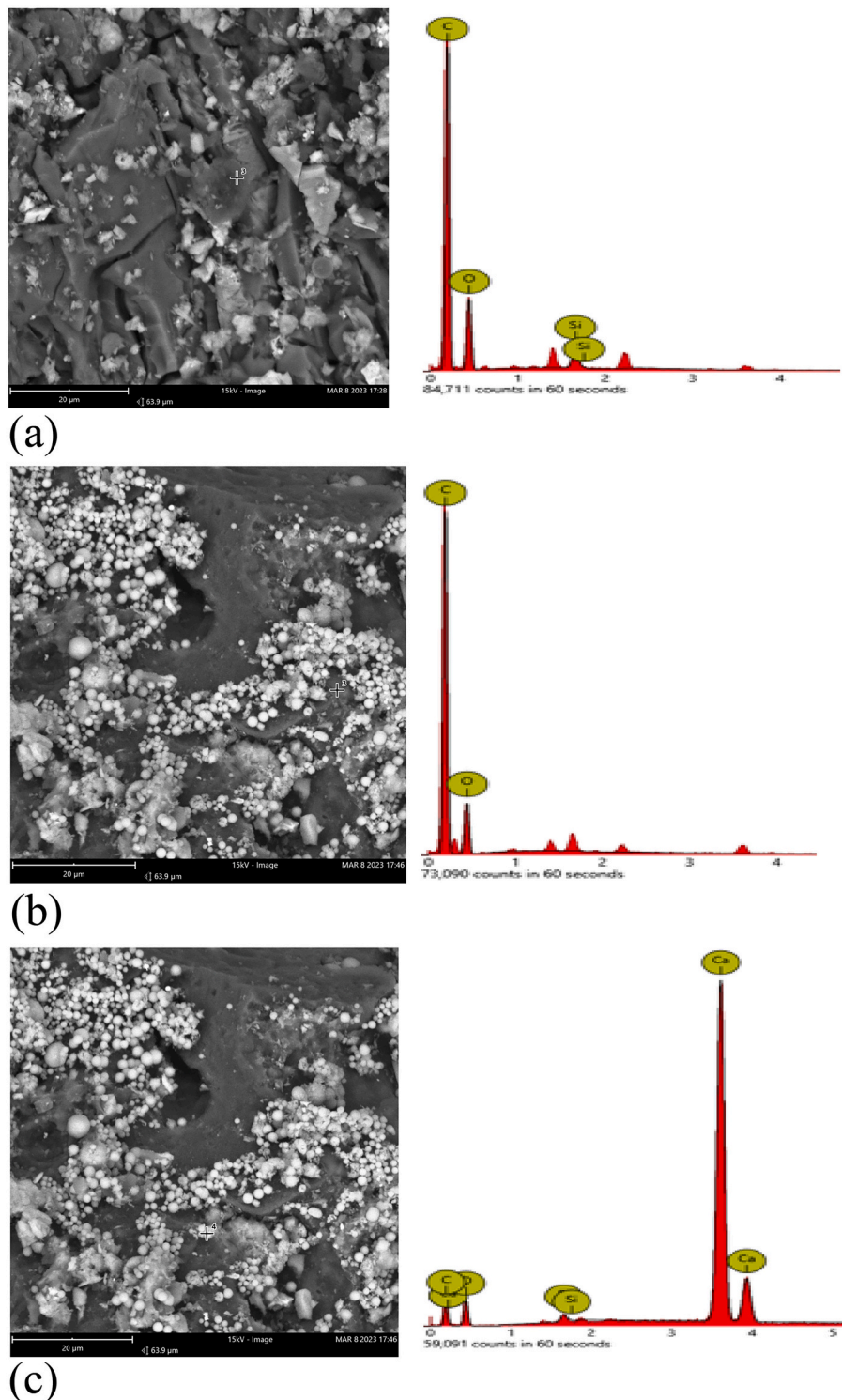
### 3.2. Unconfined compressive strength and calcite content

Table 4 shows treated geomaterial maximum unconfined compressive strength  $q_u$  and  $\text{CaCO}_3$  measurements based on the UCS testing specimens for the two native soil strains (only these were used, as for the field application the introduction of exogenous to the ecosystem strains will be avoided). It can be seen that biocementation using FV changed the clay and locomotive coal ash from materials without strength to

materials with UCS of 1–1.5 MPa and 2–3 MPa, respectively for the clay and locomotive coal ash. Although there are no similar treatment results for clays, and locomotive ash biocementation results have never been published before, the UCS results compare well with published UCS results (needle penetration) on biocemented sands (ureolytic pathway) subjected to the same injection protocol (Mwandira et al., 2017), namely 1.33 MPa for fine sand, 2.87 MPa for coarse sand and 2.80 MPa for mixed sand. Whilst it is possible that the FV waste could have some effect on the strength (e.g. starches), the  $\text{CaCO}_3$  results also compare well with those by Dagliya et al. (2023) using *S. pasteurii* (ureolytic pathway) grown in molasses, i.e. 12 % and 11 % for 1.0 M and 0.5 M cementation solution respectively with 1.00 PV treatment over 14 days. Indicative photos of biocemented specimens are presented in Fig. 4; these clearly show the formation of precipitates in the treated soil, consistent with the formation of  $\text{CaCO}_3$ , as measured by acid digestion. It is interesting that both autoclaved and non-autoclaved FV media performed equally well, which is of interest for practical applications, where the use of non-autoclaved media would further lower costs. Note that due to the implementation process (injection), parts of the samples closer to the injection point had higher  $\text{CaCO}_3$  contents and degree of cementation, hence the highest  $q_u$ , especially for the fine-grained soil. The optimisation of the implementation protocol to achieve a better uniformity of treatments is beyond the scope of this paper, whose focus is to prove the feasibility of biocementing these geomaterials with FV, which was proven based on the reported increase in  $q_u$  and calcite content.

### 3.3. SEM-EDS results

Fig. 5 shows SEM-EDS analysis results of untreated and biocemented geomaterials. The morphology of the geomaterials before and after treatment shows clear changes, with the particles coated with precipitates. The accompanying EDS analysis results confirm the difference



**Fig. 5.** SEM-EDS of: (a) locomotive coal ash (untreated), (b) Locomotive coal ash biocemented by *B. licheniformis* (Safdar et al., 2022) -ureolytic route-, using FV medium (background site, i.e. ash particle), (c) Locomotive coal ash biocemented by *B. licheniformis* (Safdar et al., 2022) -ureolytic route-, using FV medium (deposited precipitate site), (d) Untreated clay soil, (e) Clay soil biocemented by *B. licheniformis* (Mwandira et al., 2023e) -CA route- using FV medium (background site i.e., soil particle), (f) Clay soil biocemented by *B. licheniformis* (Mwandira et al., 2023e) -CA route- using FV medium (deposited precipitate site), (g) High magnification image of bioprecipitates -CA route- using FV medium.

in the chemical composition of the original geomaterial particle and that of the bioprecipitate. The original geomaterial particles are dominated by carbon and oxygen in the case of ash (Fig. 5a and b), and silica in the case of natural soil in addition to some potassium commonly found in

clays (Fig. 5d and e). For bioprecipitate on the other hand (Fig. 5c, f, and g), the prevalent chemical elements in all cases are calcium, carbon and oxygen, consistent with the composition of  $\text{CaCO}_3$ , providing further qualitative evidence of its formation.

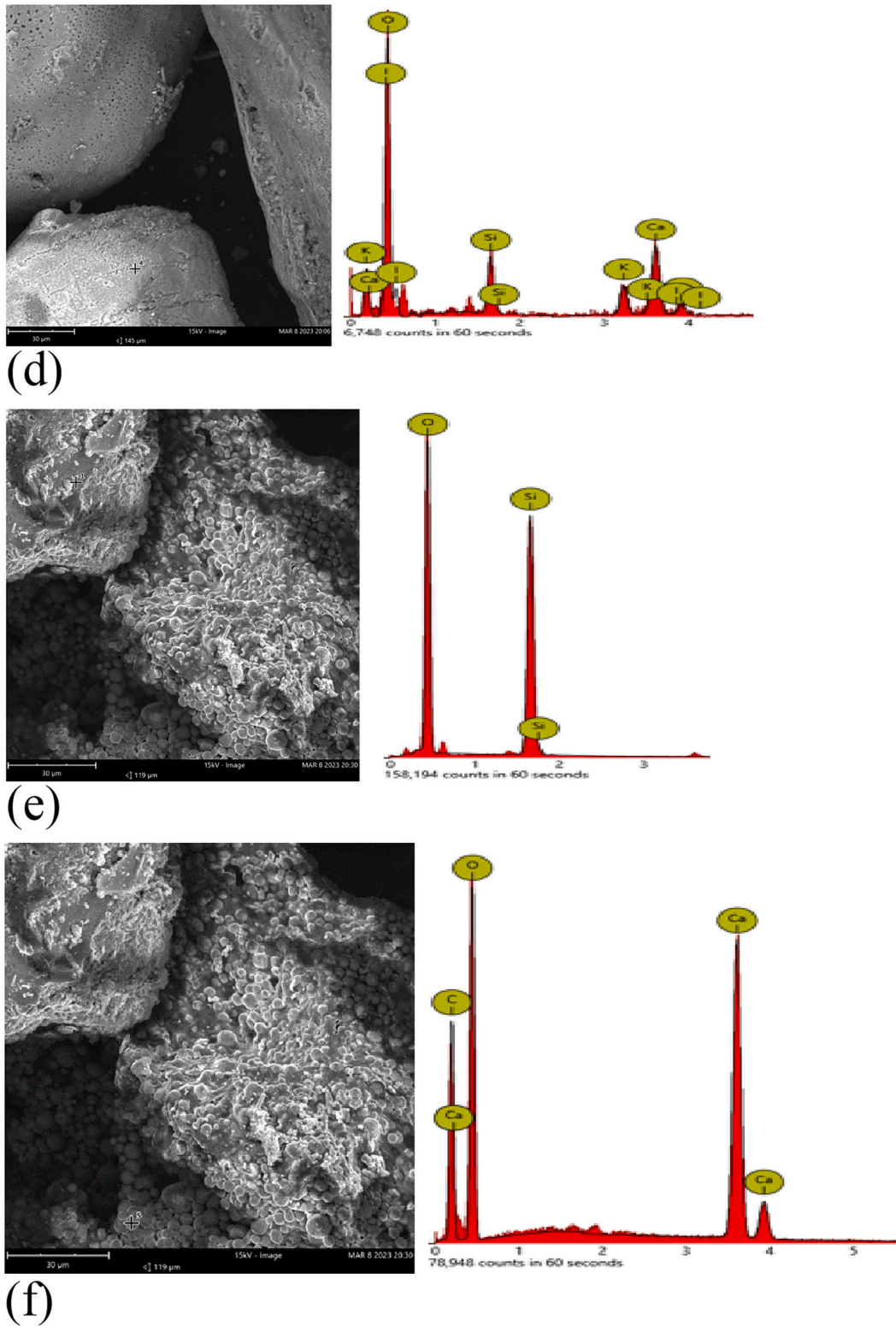


Fig. 5. (continued).

4. Conclusion

This paper presented a feasibility study of using FV waste as growth media for the bioaugmentation of bacterial cultures, of relevance for many environmental and geotechnical engineering applications involving microbial treatments; for example, bioremediation, waste and wastewater treatment, carbon sequestration and utilisation, and

biocementation for waste solidification and encapsulation, prevention of erosion, as well as soil stabilisation.

In this research, FV waste media were used to grow native strains of different types isolated from local soils of the UK railway network, cultivated for bioaugmentation applications. The strains cultivated in the FV waste media were then used to biocement two geomaterials from the UK railway network.

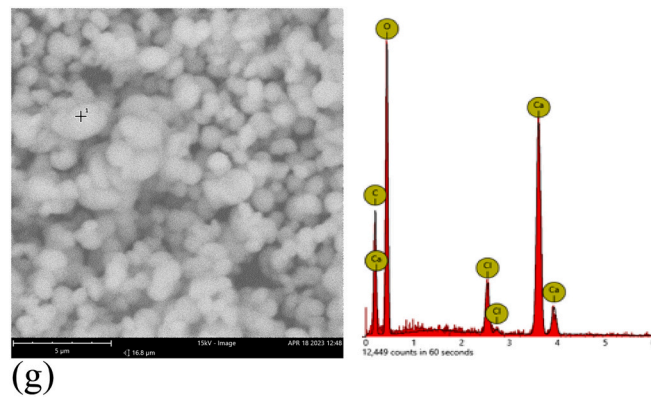


Fig. 5. (continued).

The main findings and conclusions are that:

- It is feasible to grow the required strains for biocementation (for both ureolytic and CA metabolic pathways) in mixed, diluted, raw FV waste media liquor without adjustments of pH or ingredient composition. This conclusion is based on the recorded growth rate and enzymatic activities, which, although lower than those of commercial growth media, were still adequate for the concerned applications. Namely, the CA-producing *B.licheniformis* had a maximum OD<sub>600</sub> of 1.799 and a CA activity of 0.817 U/mL in FV media, whereas for the ureolytic pathway, *B. licheniformis* reached a maximum OD<sub>600</sub> of 0.986 and a maximum urease activity of 0.675 mM urea/min, and *S. pasteurii* a maximum OD<sub>600</sub> = 0.999 and a maximum urease activity of 0.756 mM urea/min.
- Biocementation of a clay and locomotive ash (an erodible waste material) from railway embankments was achieved. This conclusion is based on the recorded unconfined compressive strengths of 1–3 MPa and calcite content increases of up to 4.02 and 8.62 % for the clay and ash respectively, as well as SEM-EDS analyses, which attested the formation of bioprecipitates with characteristic morphologies and elementary composition of calcite crystals.

These findings suggest the potential of employing FV waste to biocement problematic geomaterials and are of wider relevance for environmental and geoenvironmental applications involving bioaugmentation. The possibility of using raw FV waste, especially non-autoclaved FV waste, would be instrumental in lowering the costs of biological methods for large scale applications, thus overcoming one of the major barriers for their widespread industrial adoption. At the same time, these large scale geoenvironmental and environmental applications, which require large quantities of substrates, would constitute a useful recycling route for the very voluminous fruit and vegetable waste produced worldwide.

#### CRedit authorship contribution statement

**Wilson Mwandira:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Maria Mavroulidou:** Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Sumit Joshi:** Writing – review & editing, Validation, Methodology, Investigation. **Michael J. Gunn:** Writing – review & editing, Supervision, Conceptualization.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

#### Data availability

All data have been included in the manuscript

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