

ALTERNATIVE BINDERS TO IMPROVE HEMPCRETE MATERIAL PROPERTIES AND
MICROBIAL VIABILITY

by

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DEDICATION

For my dog, Sammy, thank you for your constant companionship and bright enthusiasm.
You are the best boy!

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ABSTRACT

Conventional insulation materials have high embodied energy and carbon. Hempcrete is a carbon-neutral to carbon-negative insulation material commonly made from hemp hurd and lime and offers a sustainable alternative to conventional insulation materials. Hempcrete achieves its low carbon footprint through carbon sequestration during hemp growth and binder carbonation over its material lifespan. However, hempcrete's low compressive strength limits its utilization primarily to wall infill insulation. Improving hempcrete's low compressive strength could expand its utility and durability. Efforts to improve the strength of hempcrete have included using mechanical compaction and a reduction in hurd content; however, while these methods can improve the compressive strength of hempcrete, they increase the thermal conductivity. Increased thermal conductivity increases the material requirements needed to achieve the same level of insulation.

Additionally, partial replacement of lime with ordinary portland cement (OPC) can increase the compressive strength of hempcrete. However, the inclusion of OPC reduces hempcrete sustainability due to the high embodied carbon of cement and the reduction in the binder carbonation potential. Pozzolanic materials like silica fume, metakaolin, fly ash, etc., could confer strength benefits to hempcrete. Additionally, it may be beneficial to leverage microbial functionalities such as biomineralization, crack healing, or environmental sensing in hempcrete by creating "living building materials."

This work investigated how hempcrete binders rich in silica fume, an industrial byproduct, and metakaolin affect hempcrete's pH, microbial viability, and strength. High silica fume ~39% and moderate metakaolin ~8% binder compositions generated hempcrete with strengths comparable to hempcrete with higher OPC content. These results signify that silica fume can strengthen hempcrete without diminishing its sustainability. Additionally, high silica fume content decreased the pH of both pastes and hempcrete. Reducing pH is relevant for microbial inclusion, as pH has been identified as an environmental stressor affecting microbial viability in cementitious materials. For very high silica fume mixes, which have the lowest pH, microorganisms had higher viability in paste samples immediately after mixing, compared to pastes with higher lime or OPC content. However, the viability at 24 hours was not maintained in paste samples compared to hempcrete, which maintained viability at 24 hours, demonstrating that other factors beyond pH continue to challenge microbial viability in these materials. Future work within this area should evaluate how these additives impact hempcrete's thermal conductivity and sustainability. It would also be beneficial to investigate other methods for incorporating microbes into this material and determine how microbes contribute to the observed strength differences, i.e., through biomineralization or other mechanisms.

INTRODUCTION

1.1 Overview

Current building operations, such as heating and cooling, have a significant contribution to anthropogenic carbon emissions [1]. Traditionally made from hemp hurd and a lime-based binder, hempcrete is a carbon-neutral to carbon-negative insulation material. Hempcrete's low operational and embodied carbon is achieved through carbon sequestration during hemp growth and carbonation of the lime binder over its lifespan [2]. However, traditional hempcrete has limitations that affect its broader adoption [3]. Hempcrete's relatively low compressive strength restricts its use to wall in-fill insulation, and its thermal resistance, while beneficial, is lower than some synthetic insulation materials [4].

Previous efforts to enhance hempcrete's strength, such as increased binder content and increased density achieved through mechanical compaction, have been practical but come with trade-offs. Mechanical compaction to create prefabricated blocks often requires specialized equipment and facilities [2]. Additionally, both increased binder content relative to hurd and mechanical compaction lower hempcrete's thermal resistivity; this increases the material required to achieve the same insulation levels [2], [5]. This is particularly relevant when ordinary portland cement (OPC) is used as a hydraulic binder to augment the early strength of hempcrete [6]. Cement's high contribution of approximately 8% to anthropogenic carbon emissions [7] may reduce the sustainability of hempcrete from a carbon-neutral to carbon-negative insulation material to a net carbon emitter [6].

One area of interest in expanding the utility of hempcrete through improving its compressive strength is living building materials. Living building materials integrate living

organisms into traditional materials to provide functionalities such as self-repair, environmental sensing, bioremediation, and biomineralization [8], [9], [10], [11]. These capabilities are advantageous in that they could expand traditional materials' utilities, functionalities, and lifespans. However, a critical challenge in using living building materials is maintaining microbial viability within the material over time [11], [12], [13], [14]. Within cementitious materials, stressors such as pH, high heat of hydration, nutrient limitations, low porosity, etc., limit long-term viability [13].

This research investigates the hypothesis that common cement additives can enhance the strength of hempcrete relative to mixes constructed with primarily lime-based binders. It additionally investigates silica fume's and metakaolin's ability to lower the pH of hempcrete for improved microbial viability. The significance of this research is that increased compressive strength could help expand hempcrete's applications as a low-load-bearing insulation material without including high concentrations of OPC. Additionally, improving microbial viability within hempcrete allows for the potential use of functionalities of microbes, including biomineralization and environmental sensing.

1.2 Thesis Aims

This thesis aims to design improved hempcrete mixes to enhance microbial viability and compressive strength while limiting cement binder content. Chapter One is an introduction and literature review. Chapter Two discusses how common binders, silica fume, and metakaolin can augment the strength of hempcrete and compares the strengths to traditional lime-based hempcrete. Chapter Two additionally discusses how silica fume and metakaolin affect the environment of hempcrete to improve immediate microbial viability. The results from Chapter

Two provide evidence that common cement additives, silica fume, and metakaolin can enhance the strength of hempcrete compared to traditional lime-based binders. The results also demonstrated that using these additives can improve the immediate viability of microbes within hempcrete, but other stressors still impact viability within these mixes. Chapter Three summarizes the key results of this investigation and provides recommendations for future work.

1.3 Literature Review

1.3.1 Traditional insulation materials

Building-related operations, such as heating and cooling, account for 28% of human-related carbon emissions, in addition to the carbon cost of building materials [1]. While environmental policy has focused primarily on improving energy efficiency and developing renewable energies [15], addressing the carbon cost of building materials is crucial for reducing emissions and meeting future infrastructure demands. Building and construction industry growth is expected to be driven by population increases and economic development [1], [16]. This is in addition to infrastructure and housing upgrades within highly urbanized areas [16]. Developing sustainable building materials that address building operations and the sustainability of the materials themselves is imperative for meeting guidelines set out by the Paris Climate Accord, which aims to limit global warming to 1.5°C to mitigate the severe impacts of climate change.

Traditional insulation materials like expanded polystyrene, polyisocyanurate, and inorganic plastics, like expanded perlite and aerogels, have high embodied carbon and energy [17]. Embodied carbon refers to the greenhouse gas emissions associated with the entire lifecycle of a product, from raw material extraction to manufacturing, transportation, use, and disposal. In contrast, embodied energy is the total energy consumed throughout its lifecycle [17]. For

example, the global warming potential, which is denoted as CO₂e and is the heat absorption relative to CO₂ for a 100-year horizon, is 4.52 kg CO₂e for expanded polystyrene and 10.4 kg CO₂e for polyisocyanurate [17]. The “cradle to gate” embodied energy, which is the energy required to produce one functional unit of material, is 147 MJ for both expanded polystyrene and polyisocyanurate [17], [18]. Additionally, common insulation materials like glass wool, stone wool, expanded polystyrene, and polyisocyanurate are also derived from mineral or fossil fuel-based production methods [17]. Polystyrene relies on petrochemicals, while mineral-based insulations like glass and stone wool require high-energy manufacturing processes [19]. Furthermore, many of these materials have limited sustainability at the disposal stage of their material life due to limited recyclability and reuse [19].

In addition to the environmental impacts, some of these insulation materials also have the potential to be human health hazards. Hexabromocyclododecane (HBCD), a brominated flame retardant commonly used to treat expanded polystyrene and extruded polystyrene, has been implicated in causing potential reproductive, developmental, and neurological defects [20]. Better material alternatives are needed to address these issues.

1.3.2 Hemp and hempcrete

Hemp and its industrial byproducts offer a promising avenue for sustainable material development across multiple industries. Structurally, hemp consists of six distinct layers extending from its hollow core: pith, xylem, cambium, phloem, cortex, and epidermis [4]. The cambium is the differentiation point between bast fibers [4], which are used in textiles, and hurd, which can be processed into hempcrete. As an agricultural crop, hemp has a short growing cycle of 90–120 days [4], allowing for multiple grow cycles per season. Hemp can be produced

without pesticides and requires less water than other fibrous plants like cotton. This is significant because cotton accounts for approximately 2.4% of global cropland, but 11% of global pesticide use, and requires 2.4 times more water per unit of cultivated area than hemp [21], [22]. Hemp also potentially enhances soil nutrition through the natural weeding of smaller plants and the absorption of heavy metals from the soil [23]. Hemp also has high carbon dioxide reuptake, consuming 1.29 kg CO₂e to 1.851 kg CO₂e during plant growth [24]. Additionally, hemp has diverse applications, including hemp seeds as a consumable product with nutritional value similar to soy, fibers for textiles, and hurd for insulation [2], [21], [22].

Hempcrete is a carbon-neutral to carbon-negative insulation material commonly made from hemp hurd and lime, but can contain other binder materials. For additional information on binders, see section 1.3.3. Hempcrete offers a sustainable alternative to conventional insulation materials. The low operational and embodied carbon of hempcrete is achieved through carbon sequestration during hemp growth and the carbonation of the lime binder throughout its lifespan [2]. One life cycle analysis (LCA) for hempcrete, composed of hemp and lime, found that a 1 m², 300 mm thick wall with timber support can sequester 82.7 kg of CO₂ [25]. Carbon sequestration over hempcrete's material lifespan allows it to offset its emissions and achieve a net life cycle reduction in greenhouse gas emissions of 36.08 kg CO₂ over a 100-year timeline [25]. This aligns with other estimates of hempcrete's lifecycle CO₂e emissions being -16.0 kg CO₂e/FU [6]. It should be noted that the range of LCA values depends on the binder:hurd ratio, binder composition, LCA assumptions, expected life span, and other assumptions [6].

As a sustainable insulation material, hempcrete is advantageous for its acoustic properties, moisture buffering, and thermal conductivity [26]. The thermal conductivity value of

hemcrete varies from 0.06 to 0.18 W/mK for densities ranging from 200 to 800 kg/m³ [2]. This aligns with others' estimations for thermal conductivity of 0.117-0.183 W/mK for mixes using different compositions of hydrated lime, natural hydraulic lime, OPC, metakaolin, and lime-based commercial mixes using a 2:1 binder:hurd ratio with varying water: binder ratios [5]. Additionally, for low and medium-density wall mixes of 200 kg/m³ to 400 kg/m³, others have found R-1.25 of 0.22 Km²/W to R-2.3 of 0.40 Km²/W [2]. R values represent the thermal resistance, with higher thermal resistance indicating better performance as an insulation material. It should be noted that the thermal resistance of hemcrete decreases with the increase in binder content relative to hurd, regardless of the binder type, due to increased density [2].

While hemcrete is a promising sustainable insulation material, its relatively low thermal resistance compared to synthetic insulation and the poor compressive strength of traditional hemcrete, ranging from 0.2 to 1.15 MPa [26], limit its application to wall in-fill insulation. Enhancing the strength of hemcrete could expand its applications as a sustainable insulation alternative. High binder content and compaction have been used to address hemcrete's low compressive strength [3], [27]. However, both methods increase hemcrete's thermal conductivity due to increased material density, making it a less effective insulator [2], [6], [27]. The increased thermal conductivity of high-density mixes, which results in higher material requirements to achieve the same level of insulation, is particularly significant when cement is used as a hydraulic binder to improve early strength gain [6]. Elevated cement content raises hemcrete's embodied carbon [3] due to cement production's substantial contribution to human-related carbon emissions [7]. Increased cement content within the binder also reduces the availability of lime in the binder to participate in carbonation [6]. The elevated embodied carbon

and diminished carbonation potential in high cement content mixes undermine hempcrete's sustainability, potentially turning it into a net carbon emitter [6].

1.3.3 Hempcrete binders

In hempcrete, hydrated and hydraulic lime are commonly used as binder materials [2], [6]. Hydrated lime, which lacks clay impurities such as silica and alumina [2], is known for its low drying shrinkage and good vapor permeability [28]. Hydrated lime is 80-90% portlandite (calcium hydroxide) and can immediately participate in carbonation with atmospheric CO₂ to form calcium carbonate (**Figure 1**) [6], [28]. However, hydrated lime is limited by its slow hardening, low early strength, and dissociation with water [28]. In contrast, hydraulic limes containing dicalcium silicates that can undergo hydration have both hydraulic and air-hardening properties [2]. Hydraulic limes have better early strength gain, faster hardening speeds, and better water resistance than hydrated lime [28]. However, unlike hydrated lime, hydraulic lime cannot immediately participate in carbonation [6]. Hydraulic lime must first undergo hydration reactions with calcium silicate to form portlandite. After the formation of portlandite, hydraulic lime can then undergo carbonation with atmospheric CO₂ [6]. A common type of hydraulic lime is natural hydraulic lime (NHL), which is produced by firing and slaking clay-limestone [6], [28]. Hydraulic binders like Type I OPC and NHL are often used to improve the early mechanical properties of hempcrete in conjunction with hydrated lime [6]. Pozzolanic materials, which are siliceous and sometimes aluminous, have been combined with hydraulic binders due to their mutual need for water and ability to augment strength through forming C-S-H gels (**Figure 1**) [6].

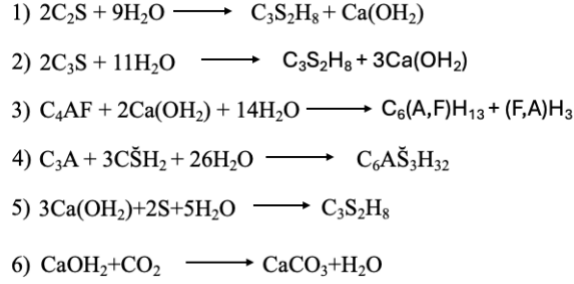


Figure 1: Cement binder chemistry for NHL, OPC, pozzolanas, and hydrated lime [6]. Equation 1 shows the primary hydration reaction for NHL. Equations 1 through 4 show the primary hydration reactions for type 1 OPC. Equation 5 shows the pozzolanic reaction with silica. Equation 6 shows the carbonation of portlandite present in hydrated lime and formed via the hydration reaction of hydraulic binders NHL and OPC.

Pozzolans are siliceous and sometimes aluminous materials. Pozzolanic materials such as metakaolin and blast furnace slag have been explored in previous studies to augment the strength of hempcrete and increase its sustainability, which can be diminished through the inclusion of OPC [3]. Pozzolanic binders can be combined with either hydraulic or non-hydraulic lime, but are more commonly used with hydraulic lime and OPC [3], [6]. Pozzolanas could potentially improve the compressive strength of hempcrete via the pozzolanic reaction. In this reaction, portlandite reacts with silica from the pozzolanas to form calcium silicate hydrate (C-S-H) gels (**Figure 1**) [6], [29], [30]. While conferring strength, the C-S-H formed from the pozzolanic reaction is distinct from that formed in cement hydration. C-S-H from the pozzolanic reaction is more polymerized and potentially less stable due to its lower Ca/Si ratio than C-S-H from OPC hydration [29], [31]. As seen in ultra-high-performance concrete, the pozzolanic reaction and improved particle packing behavior could potentially contribute to desirable strength benefits in hempcrete [30]. Reported strengths of hempcrete with the inclusion of pozzolanic binders range from 0.29 to 0.39 MPa at 1 year, with most strength development occurring between 5 and 28 days [3].

While the pozzolanic reaction may improve the strength of hempcrete, it decreases hempcrete's ability to sequester carbon, compared to mixes with higher hydrated lime binder content [6]. The reduction in hempcrete's carbon sequestration with pozzolanas is due to the consumption of portlandite during the pozzolanic reaction. Portlandite's carbonation potential is much higher than C-S-H. However, carbonation of C-S-H is still possible over time [6]. C-S-H's lower carbonation potential is significant for binders with high pozzolanic content due to the total carbonation potential of those mixes equaling the possible carbon uptake from C-S-H due to the total consumption of all portlandite available for carbonation [6]. The changes in carbon sequestration potential with different binder contents are relevant for optimizing the strength of hempcrete while maintaining its sustainability value as a carbon-neutral to carbon-negative insulation material.

1.3.4 Considerations for the inclusion of microbes

Microbially induced calcium carbonate precipitation (MICP) could potentially augment the strength of hempcrete via biomineralization. MICP is a biochemical process in which microorganisms change the chemistry of their surrounding environment to induce mineral precipitation [13]. MICP-capable ureolytic microbes have been used for oil and gas well sealing, subsurface stabilization, and cementation of porous media, such as sand, to form composite materials [11], [13], [32]. MICP is commonly facilitated by ureolytic microbes, which produce the urease enzyme. This enzyme catalyzes urea hydrolysis to ultimately produce carbonate and ammonium [13]. In the presence of calcium ions, carbonate can react to precipitate calcium carbonate (**Figure 2**) [13], [32].

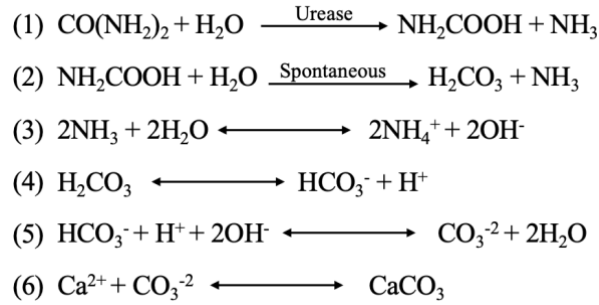


Figure 2: Shows the enzymatic hydrolysis of urea via urease [32]. Under elevated pH, the equilibrium shifts towards the formation of bicarbonate and carbonate (4) and (5). In the presence of excess calcium, calcium carbonate is formed.

While utilizing cellular functionalities within building materials may be desirable, environmental stressors such as pH, high heat of hydration, low porosity, and nutrient limitations challenge microbial viability within cementitious materials [13], [14]. In OPC, vegetative cells remain viable for several days to weeks, with variation among bacterial species. For example, vegetative *Sporosarcina pasteurii* cells in OPC have shown viability ranging from 1×10^5 CFU/g at 24 hours to as low as 1×10^4 viable MPN/g immediately after mixing, with only 0.01% of vegetative cells surviving [12], [33]. Additionally, efforts to preserve microbial viability within cementitious materials, such as using expanded clay, polyurethane membranes, and glass beads, have shown limited success and are often time- and cost-intensive [34]. The reported viability for *Bacillus pseudofirmus*, *Bacillus cohnii*, and *Bacillus halodurans* spores immobilized in expanded clay within white cement was approximately 120×10^3 CFU/g, 60×10^3 CFU/g, and 5×10^3 CFU/g, respectively, after seven days [35]. However, it should be noted that high pH can have a potentially inhibitory effect on the urease enzyme [36], [37]. Additionally, it has been suggested that even in materials where cells cannot maintain viability, microbial inclusion could confer strength benefits through acting as nucleation sites for hydration products formed during cement curing [33], [38].

Extending bacterial survival in cementitious materials could enable broader applications, including environmental sensing and responsiveness. Lowering the pH of these materials may enhance microbial activity, as OPC's high pH of 13.6 is detrimental to most bacteria [14]. Others have demonstrated that reducing pH to 11 for as little as four hours can significantly increase microbial survival and urease activity, which could enhance MICP efficiency [14]. Common industrial cement additives, such as metakaolin, silica fume, and fly ash, reduce the pH of cementitious materials by reacting with calcium hydroxide (noted as $\text{Ca}(\text{OH})_2$ or CH), also referred to as portlandite to form calcium silicate hydrate (C-S-H) and calcium aluminate silicate hydrate (C-A-S-H) gels [39], [40]. This process binds free portlandite, leading to a reduction in pH. Replacing 50% of the dry weight with silica fume can lower the pore fluid pH to below 11 [40]. Optimizing binder chemistry to support microbial viability in hempcrete could enhance long-term strength and sustainability through biomineralization processes.

CHAPTER TWO

SILICA FUME-RICH BINDERS IMPROVE THE
COMPRESSIVE STRENGTH OF HEMPCRETE AND
VIABILITY OF *S. PASTEURII*

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Abstract

Building operations such as heating and cooling have a large contribution to human-related carbon emissions. Hempcrete is a carbon-neutral to carbon-negative insulation material. It is a sustainable alternative to conventional insulation materials, which can have large carbon footprints. Hempcrete is commonly manufactured using lime as a binder. However, the low compressive strength of lime hempcrete limits its utilization. Improving the strength of hempcrete can be achieved using a partial replacement of lime with ordinary portland cement (OPC), but this reduces hempcrete's sustainability. This study investigated how hempcrete binders rich in silica fume, an industrial byproduct, and metakaolin affect the pH and strength of hempcrete. This study additionally evaluated the impact of these binders on the viability of biomineralizing microorganisms within hempcrete. This is relevant for potentially utilizing microbial functionalities in hempcrete, such as biomineralization, crack healing, or environmental sensing. High silica fume and moderate metakaolin binder compositions generated hempcrete with strengths comparable to hempcrete with higher OPC or lime content. These silica fume-rich mixes also had a lower pH (~ 10) than higher OPC or lime content hempcrete (~ 13), which may explain the higher viability of microorganisms ($\sim 10^6$) in paste samples immediately after mixing, compared to OPC ($\sim 10^3$). However, the viability at 24 hours was not maintained in paste samples compared to hempcrete, demonstrating that other factors beyond pH continue to challenge microorganisms in these materials. Together, these results signify that silica fume can be used to strengthen hempcrete without diminishing its sustainability. Additionally, pH reduction may improve microbial viability, which is important for future work in leveraging microbial functionalities.

2.1 Introduction

Buildings account for 39% of energy-related carbon emissions, with building-related operations, such as heating and cooling, accounting for 28% of those [1]. Sustainable building and insulation materials are needed to mitigate the current impact of buildings on anthropogenic carbon emissions and meet expected growth in infrastructure demand [1], [10], [13], [16]. Embodied energy and carbon represent the carbon and energy burdens generated from raw material acquisition, processing, transport, and construction [17]. For insulation materials, these impacts are weighed against the reduction in energy consumption (i.e., energy used to heat and cool buildings over the material's lifespan) [17]. Common insulation materials like expanded polystyrene, polyisocyanurate, perlite, and aerogels have high embodied carbon and energy [41] and are usually derived from extracted mineral or fossil-fuel sources [17]. By contrast, hempcrete is an emerging alternative to common insulation materials with the potential for greater sustainability. Hempcrete is conventionally made using hemp hurd, a byproduct of industrial hemp production, and a lime binder [2]. The low operational and embodied carbon of hempcrete is achieved through carbon sequestration during hemp growth and the carbonation of the lime binder throughout its lifespan [6]. While hempcrete is a promising insulation material, its applications are currently limited due to various reasons, including low strength [2].

Improving hempcrete's low compressive strength could expand its utility and durability [2]. Conventional hempcrete insulation is generally considered a non-load-bearing material, with mixes with primarily lime binders having a low compressive strength of around 0.12-0.20 MPa for a 2:1 binder to hurd ratio [3]. Because of its low compressive strength and the general need for setting with cementitious materials, hempcrete often requires external structural support,

including form work and a timber frame [3]. Increasing hempcrete's compressive strength could also allow for better durability and increase the building structural capacity, allowing for increased timber frame spacing [3]. Strategies to improve hempcrete's strength include compaction [27], changing the liquid: binder ratio [42], decreasing the percentage of hemp hurd [42], and including a percentage of ordinary portland cement (OPC) in the binder [3]. While increasing the binder content or mechanical compaction can improve hempcrete's strength, these strategies also increase density and thermal conductivity [5]. Similarly, while high OPC binder content has been shown to increase compressive strength and Young's modulus [43], it diminishes net carbon savings [6] and reduces the benefit of using hempcrete compared to other insulation strategies.

Alternative binder compositions that include pozzolanic materials, such as metakaolin and blast furnace slag, have been used in hempcrete binders (~20-40%) in previous studies to increase hempcrete's sustainability [2], [3]. These mixes can achieve strengths of ~0.3-0.4 MPa, displaying an improvement over binders that only include lime (**Table 1**). Silica fume is another candidate as a binder component for hempcrete. Silica fume, a pozzolanic material, is an industrial byproduct from the ferrosilicon industry [39], [44]. Pozzolanic materials can be used within cementitious materials as a sustainable filler to reduce cement content [39], [45]. Pozzolanic materials can potentially improve hempcrete's strength through the pozzolanic reaction. In the pozzolanic reaction, SiO_2 and Al_2O_3 within the pozzolanas react with Ca(OH)_2 (portlandite) to form calcium silicate hydrates (C-S-H) and calcium aluminate silicate hydrates (C-A-S-H) gels [39], [46]. Whether hempcrete binder compositions with high silica fume and

metakaolin relative to lime content achieve desirable strength properties has not been reported previously.

It may also be desirable to augment the strength of hempcrete or repair cracks within it through microbial-induced calcium carbonate precipitation (MICP). MICP is accomplished by hydrolyzing urea into carbonate species and ammonia via the urease enzyme produced by ureolytic microbes, such as *Sporosarcina pasteurii* (*S. pasteurii*) [13], [32]. MICP has been used for bio-cementing porous media such as sand to form composite materials, oil and gas well sealing, and subsurface stabilization [11], [32], [47], [48]. However, the pH of conventional hempcrete is very high, ~12.4-13.8, due to the high quantity of alkaline materials, e.g., lime and OPC [14], [49]. In similarly high pH cementitious environments, microbial viability of ureolytic organisms is usually very low [12], [14], [33], [35]. Also, high pH can inhibit the urease enzyme [36], [37]. Previous studies indicate that reducing pH in media from 13.6, which is representative of the pH of cement, to ~11 for four hours can increase microbial viability and urease production [14]. Since replacing alkaline materials in hempcrete binders with silica fume and metakaolin has the potential to reduce pH, these materials could result in higher viability of common biomineralizing microorganisms.

This study aimed to determine the effects of silica fume and metakaolin on the pH, strength, and viability of *S. pasteurii* in pastes and hempcrete. The hypothesis was that pozzolanic fillers, including silica fume and metakaolin, would improve hempcrete strength compared to hempcrete with primarily lime binders and improve microbial viability compared with higher cement binder mixes. Our results demonstrate the ability to increase the strength of hempcrete without the need for high cement content, which diminishes hempcrete's

sustainability. Lowering pH through the use of silica fume improves the initial, but not prolonged, microbial viability in hempcrete. These results also give new insights into how cement additives can improve microbial viability, which is significant for future applications that utilize cellular properties such as environmental sensing and self-healing.

Table 1: Compressive strength of hempcrete reported in the literature with variations in binder content, hurd content, and compaction.

Paper	Binder	Binder: hurd: liquid ratio (by weight)	Hurd content	Liquid: binder ratio	Strength properties	Thermal properties
(Sinka et al., 2014) [27]	60:40 DL60 dolomitic lime, metakaolin by mass, pure dolomitic lime	N/A	0.375/0.625 hemp/binder ratio by mass	0.66	0%, 25% and 50% displacement ratios with a maximum of 60mm displacement used. They found compressive strengths of 0.133, 0.154, 0.266 MPa respectively.	0%, 25% and 50% displacement ratios with a maximum of 60mm displacement used. They found thermal conductivities of 0.070, 0.076, 0.086 W/(m*K)
Bruijn et al. 2009 [43]	A: 25% hydrated lime and 75% hydraulic lime	N/A	0.28 hemp/binder ratio by mass	1.06	Compressive strength of 0.20 MPa	N/A
	B: 40% hydrated lime and 60% hydraulic lime		0.33 hemp/binder ratio by mass	1.33	Compressive strength of 0.15 MPa	
	E: 100% cement		0.22 hemp/binder ratio by mass	0.89	Compressive strength of 0.55 MPa	
Parcesepe et al. 2021 [50]	Natural hydraulic lime, silica sand, and dolomitic limestone aggregates	N/A	0.2-0.8	N/A	Compressive strength: 0.377, 0.620 MPa for a low water and high water-binder ratios respectively, specific ratios not reported	Thermal conductivity: 0.46 W/(m*K)

Table 1: Continued.

Paper	Binder	Binder: hurd: liquid ratio (by weight)	Hurd content	Liquid: binder ratio	Strength properties	Thermal properties
Walker et al. 2014 [3]	Builder's mix (BM): 70% lime, 20% NHL3, 10% OPC	2:1: 3.1	N/A	N/A	Compressive strength for all mixes (BM, CM, G, G+WR, M, M+WR) varied between 0.02 and 0.04 MPa at 5 days and 0.29 and 0.39 MPa at 1 year	N/A
	Commercial mix (CM): 100% commercial binder	2:1: 2.9				
	GGBS (G): 70% calcium lime, 30% GGBS	2:1: 3.1				
	GGBS+WR (G+WR): 70% calcium lime, 30% GGBS, 0.5% methyl cellulose	2:1: 3.1				
	Metakaolin (M): 80% calcium lime, 20% metakaolin	2:1: 3.3				
	Metakaolin +WR (M+WR): 80% calcium lime, 20% metakaolin, 0.5% methyl cellulose	2:1: 3.1				

2.2 Materials and Methods

2.2.1 Culturing of *S. pasteurii* starter cultures

Vegetative cells were cultured from cryovials containing American Type Culture Collection 11859 (ATCC) *S. pasteurii* at 30 °C and 150 RPM in Urea Yeast Extract (UYE) media (**Table 2**). The media was inoculated using a 1:100 ratio of inoculum to fresh media. Cells were cultured overnight and then transferred into fresh media at approximately 16 hours, with the transfer culture maintaining a 1:100 ratio of inoculum to fresh media.

Table 2: Material sourcing information for urea yeast extract media.

Material	Amount	Sourcing
Yeast extract	15g/L	Gibco Bacto Yeast Extract
Urea	20g/L	ACS certified pubchem ID: 1176
Ammonium sulfate	10g/L	ACS certified pubchem CID: 6097028
TRIS	15.75 g/L	Tris-(hydroxymethyl) aminomethane) pubchem CID 6503

2.2.2 Preparation of pastes

Preparation of pastes for the pH design of experiment. All samples were mixed by hand following the pH design of experiment (DOE) mix compositions (**A1, Table A1**). Mixes were prepared in a randomized order using 20 grams of total binder (**Table 3**), water, and a liquid: binder ratio of 0.7. Pastes were then cured for 24 hours at approximately 20°C and 30% humidity before testing.

Table 3: Material sourcing information for binder materials and hurd.

Material	Sourcing
Silica Fume	MasterLife SF 100
Lime	Western Miracle Type S Hydrated Masons Lime
Cement	Portland Type I/II sourced from GCC Trident Plant, Three Forks, MT
Metakaolin	MasterLife MK 828
Hemp Hurd	0.15–20 mm / 0.07–0.13 g/cm ³ fiber sourced from IND Hemp, Fort Benton, MT

Preparation of paste samples for the strength design of experiment. All mixes were combined in an Industrial Hobart A2000 mechanical mixer following strength DOE mix compositions (**A1**, **Table A2**). For paste strength testing, total cement and lime contents were included at a fixed 60:40 cement-to-lime ratio. Dry components (**Table 3**) were mixed until homogenous, and then water was added using a 0.7 liquid: binder ratio. Mixes were then cast in 2 in. x 4 in. disposable plastic cylinders and tamped using a metal rod. Mixes were then demolded at 48 hours. Samples were then cured in a standard concrete cure room, temperature-controlled with 100% humidity.

Preparation of SF39, SF60, and SF81 pastes for pH testing. Three mix designs were chosen to investigate further from the strength DOE. Mixes were designed with three different silica fume (SF) binder content compositions: SF39, SF60, and SF81 (**Table 4**). Five samples for each mix were prepared using 30 g of binder (**Table 3**) and a 0.7 liquid: binder ratio using tap water. Mixes were mixed by hand and were air-cured for 24 hours at approximately 20 °C and 30% humidity before testing.

Table 4: Mix compositions for testing the effects of binder composition on pH, strength, and microbial viability. The mix names reflect the quantity of silica fume by percent of the total binder weight.

Mix binder content	SF 39	SF 60	SF 81
Silica Fume	39%	60%	81%
Metakaolin	8%	8%	8%
Cement	32%	19%	7%
Lime	21%	13%	4%

Preparation of microbial pastes. Transfer cultures were cultured at 30 °C and 150 RPM in UYE for viability experiments using pastes. Before mixing, the bacterial culture was diluted with sterile UYE to adjust the optical density at 600 nm to 0.8. Optical density was measured using a Synergy HT plate reader (BioTek Instruments, Inc., Winooski, VT) with 200 μ L aliquots transferred to a 96-well plate. The average OD₆₀₀ of sterile UYE was subtracted from the OD₆₀₀ of the culture to account for background absorbance from the media and the plate. Pastes were mixed by hand in a biosafety cabinet using 30 g of binder (**Table 3**) following SF39, SF61, and SF81 mix compositions (**Table 4**) and a 0.66 liquid: binder ratio. The liquid consisted of either UYE-only or UYE inoculated with *S. pasteurii*. Bacterial viability was tested immediately after mixing and at 24 hours using the most probable number technique (MPN). Samples used to determine viability at 24 hours were cured at 100% humidity at 23.8 °C for 24 hours and then prepared for MPN. From each sample, 30 g of paste was used to determine microbial viability using MPN.

2.2.3 Preparation of hempcrete

Preparation of conventional hempcrete. Hempcrete mixes were prepared using SF39, SF60, and SF81 binder compositions (**Table 4**), and as 80:20 and 20:80 cement-to-lime mixes (i.e., no added silica fume or metakaolin). Hempcrete samples were mixed using a 0.802 liquid:

binder ratio and a 15:1 paste to hurd ratio. Hemp hurd was pre-soaked for 24 hours in water in 5-gallon buckets. For hempcrete, hurd is typically added dry; the effect of pre-soaking on strength was investigated and found to be negligible (A2). All hurd was sourced from IND Hemp, LLC, and sieve analyses were conducted between hemp hurd shipments to quantify any differences between batches (A3). Conventional hempcrete mixes used the “old” hurd sourced from IND Hemp, LLC (A3).

At 24 hours, mixes were transferred to a 32 in. by 60 in. by 11 in. Brentwood Mortar Box, where they were combined with dry binder ingredients (Table 3) according to the mix composition and homogenized using garden tools. For strength and pH testing, the mixes were cast into 4 in. by 8 in. cylinders and tamped using a pre-wetted 2 in. x 4 in. x 12 in. wood to achieve uniform compaction. Lids were placed on the molds to prevent moisture loss, and the specimens were demolded after 72 hours. The cylinders were then air-cured at approximately 20°C and 30% humidity until 28-day strength and pH testing. Other curing conditions were investigated, but this methodology achieved the highest 28-day strengths for otherwise identical mixes (A2).

Preparation of microbial hempcrete. Microbial hempcrete mixes were prepared using binder compositions SF39, SF60, and SF81 (Table 4) with a 0.802 liquid: binder ratio and a 15:1 paste to hurd ratio. The liquid consisted of either UYE-only or UYE inoculated with *S. pasteurii*. For the UYE *S. pasteurii*, starter cultures were added to two 5-gallon buckets sterilized with 70% ethanol, each containing 20 L of UYE using a 1:100 ratio of inoculum to media. UYE only controls were prepared similarly, but without the addition of the *S. pasteurii* starter culture. Hemp hurd was added to the media and soaked for 24 hours. Buckets containing media and pre-

soaked hurd were aerated using a Tetra Whisper Air Pump 60-gallon aquarium pump with 6-inch top fin aquarium air stones to increase oxygen availability while culturing. All mixes were soaked in a non-sterile environment with the ability to sequester microbes from both the non-sterile hurd and the surrounding environment. Microbial hempcrete used the “new” hurd (**A3**), sourced from the same manufacturer, IND Hemp, LLC.

The media for UYE-only controls and UYE with *S. pasteurii* mixes were refreshed with UYE supplemented with 48 g/L of calcium chloride dihydrate before mixing. Pre-soaked hurd and refreshed media were transferred to a 32 in. by 60 in. by 11 in. Brentwood Mortar Box, where they were combined with dry binder ingredients (**Table 3**) according to the mix composition and homogenized using garden tools. Similarly to the hempcrete samples prepared using water, for strength and pH testing, the mixes were cast into 4 in. by 8 in. cylinders and tamped using a pre-wetted 2 in. x 4 in. wood to achieve uniform compaction. Lids were placed on the molds to prevent moisture loss, and the specimens were demolded after 72 hours. The cylinders were then air-cured at approximately 20°C and 30% humidity until 28-day strength and pH testing. Other curing conditions were investigated, but this methodology achieved the highest 28-day strengths for otherwise identical mixes (**A2**). Samples for hempcrete viability testing were cast into 2 in. x 4 in. molds, tamped by hand to achieve uniform compaction, and demolded either immediately after mixing or after 24 hours. Lids were placed on the molds to prevent moisture loss. The material in these samples was then prepared following the MPN procedures to assess microbial viability.

2.2.4 Methods for assessing pH, strength, and viability

pH testing of pastes and hempcrete. For pH testing, either a Wagner Meters 880-R0007-001 Rapid RH Digital pH meter or an MSC Extech PH100 pH meter was calibrated using pH 4.0, 7.0, and 10.1 buffers. The Wagner Meters 880-R0007-001 Rapid RH Digital pH meter was used to assess samples from the pH DOE, and an MSC Extech PH100 pH meter was used to test the pH of all other paste and hempcrete samples. To test pH, 7 mL of DI water was added to the sample surface and allowed to sit for 2 minutes or until the pH readings stabilized. The pH readings were then recorded.

Strength testing of pastes and hempcrete. For pastes, strength testing was performed at 28 days using an MTS Criterion 60 Test System in accordance with the ASTM C39M-20 Standard Test Method. All tests were conducted using neoprene caps to distribute the load evenly across the specimen's cross-sectional area.

For compressive strength testing of hempcrete, either an MTS Criterion 60 Test System or a Test Mark Industries CM-400P-D was used. Both the MTS Criterion 60 Test System and Test Mark load frames were used in substantial accordance with ASTM C39M-20 Standard Test Method guidelines. All tests were conducted with neoprene caps to ensure even load distribution across the specimen's cross-sectional area.

Hempcrete mixes whose strengths were below the minimum detection limit are represented as the detection of the test frame used to test for compressive strength. The Test Mark load frame's detection limit is 5.3 kN (1200 lbs) over the cylinder area, which is approximately 0.658 MPa. The MTS machine's detection limit is 0.04 kN (10 lbs) over the cylinder area, which is approximately 0.0055 MPa. Lines in subsequent figures represent the minimum detection limits.

Assessment of microbial viability in pastes and hempcrete. Samples for viability testing were prepared using procedures adapted from Basaran et al. [12]. The paste and hempcrete samples for UYE-only controls and UYE inoculated with *S. pasteurii* were ground using a mortar and pestle until homogeneous. A 30 g portion of the ground sample was then measured and suspended in 40 mL of uninoculated UYE. Viability was additionally tested for UYE-only controls for pastes, and UYE inoculated with *S. pasteurii* from the transfer culture, which was used as the liquid content to mix microbial pastes. Viability was also determined for UYE-only controls and UYE inoculated with *S. pasteurii* with pre-soaked hurd before the addition of binder. All samples were tested following the same procedures listed below. All samples were then sonicated for 2 minutes, and the suspension was allowed to settle for 10 minutes. The supernatant was then removed and vortexed for 1 minute. The supernatant was used as the starting inoculum for viability testing.

Viability was estimated for samples inoculated with *S. pasteurii* or the UYE-only controls using the most probable number method (MPN). For each sample, a 10-fold serial dilution was performed in UYE. For each dilution level, 1 ml was transferred into glass culture tubes containing 9 ml of fresh UYE, with three replicate tubes per dilution. Tubes were incubated at 30 °C for 72 hours. Viability was then verified using optical density, with higher optical density readings indicating growth. Optical density was measured using a Synergy HT plate reader (BioTek Instruments, Inc., Winooski, VT) with 200 µL aliquots transferred to a 96-well plate. The average OD₆₀₀ of sterile UYE was subtracted from the OD₆₀₀ of the sample to account for background absorbance from the media and the plate. All culturing tubes were plated onto BHI agar with 2% urea as 10 µL lawns to confirm that the turbidity observed from optical density

readings was due to the presence of microbes rather than residual particulate matter from paste and hurd samples. Cell concentrations were estimated using the MPN index for the upper and lower limits of the 95% confidence interval. Samples where viability was not able to be detected are reported as the minimum detectable limit of 9.5 MPN/g. For an overview of samples tested, see (A4).

2.2.5 Data analysis

The pH and strength designs of experiments (DOE) were generated using a central composite design and evaluated using a multiple linear regression with backward elimination. For the pH DOE, the relationship between the percentage of binder content, including OPC, lime, silica fume, and metakaolin, on the paste pH was examined, with a predetermined alpha level of 0.1 for factor removal from the full quadratic model. The strength DOE assessed the effect of the binder composition percentages of metakaolin and silica fume on the 28-day strength and used an alpha of 0.1 for factor removal from the full quadratic model.

One-way analysis of variance (ANOVA) was used to test the impact of mix composition on the strength of hempcrete with water and paste pH. Two-factor ANOVA was used to evaluate the effects of mix composition and *S. pasteurii* on the pH and strength of hempcrete with UYE-only controls and UYE inoculated with *S. pasteurii*. Two-factor ANOVA was additionally used to assess the impact of mix composition and time on paste viability. Three-factor ANOVA was used to evaluate the effect of mix composition, time, and *S. pasteurii* on microbial viability within microbial hempcrete samples. All models were checked for residual normality and equal variance, with dependent variables transformed as necessary to meet the statistical assumptions. Statistical significance was defined a priori as a p-value of less than 0.05. Tukey's test was used

for post hoc testing of significant main effects with more than two levels. The Bonferroni procedure was used to test for post hoc differences in the case of significant interactions, where the critical alpha was 0.05 divided by the number of comparisons. All analyses were performed using Minitab version 19.

2.3 Results

2.3.1 Impact of binder composition on paste pH

The results of the pH design of experiment demonstrated that silica fume and metakaolin were able to reduce the pH of hardened paste (**Figure 3**). The pH was reduced from ~13 for OPC to as low as ~10 in 75% silica fume mixes measured 24 hours after mixing (**Figure 3A**). Multiple linear regression analyses showed that silica fume content had a pronounced influence on pH, likely driven by the high OPC and lime replacement levels. Metakaolin alone did not reduce paste pH at the replacement levels studied (**Figure 3B**). However, metakaolin enhanced the pH reduction when combined with high amounts of silica fume. The best-fit model was $\text{pH} = 11.755 + 0.0275 \text{ SF} + 0.0340 \text{ MK} - 0.000488 \text{ SF} \cdot \text{SF} - 0.001071 \text{ SF} \cdot \text{MK}$, with $R\text{-sq}(\text{adj})$ of 0.544. In the model, silica fume is denoted as SF, metakaolin as MK, and their interactions are denoted as multiplication signs.

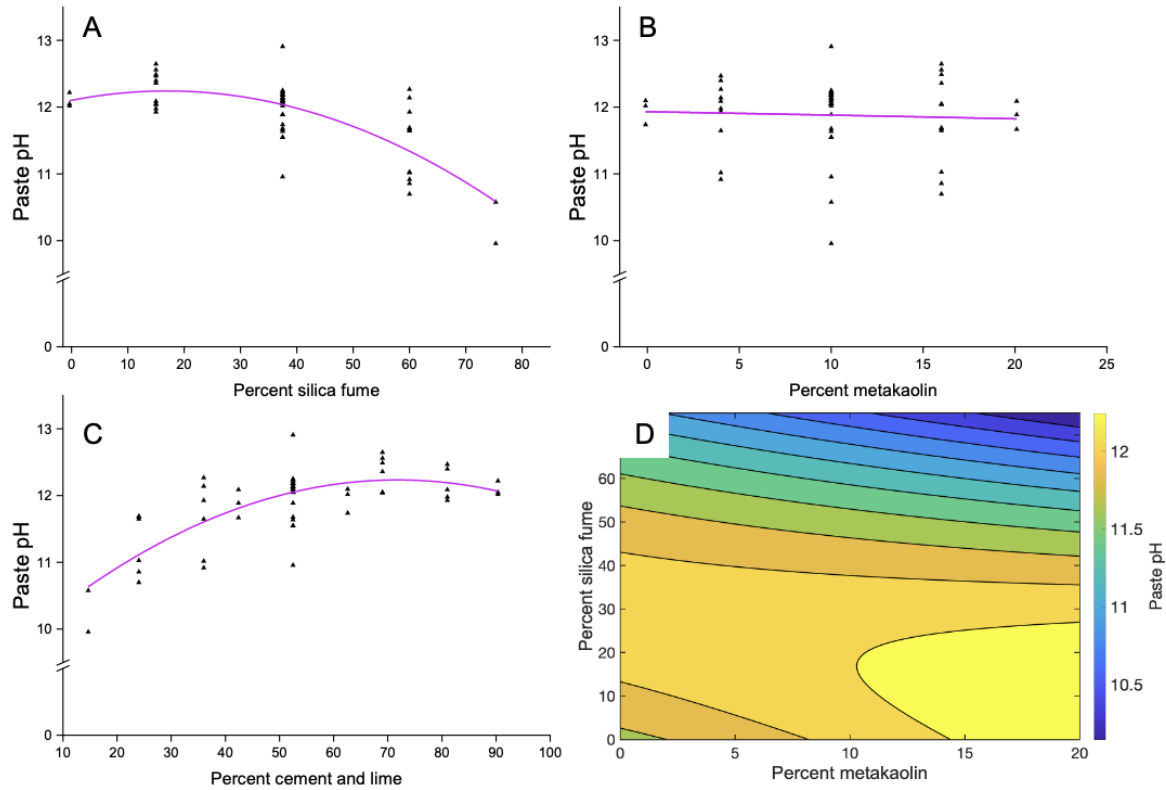


Figure 3: Paste pH measured 24 hours after mixing can be reduced with increased silica fume content. A) Impact of percent binder content silica fume on paste pH. B) Impact of percent binder content metakaolin on paste pH. C) Impact of percent binder content of the total cement and lime on pH with varying binder percentages (**Table A1**). D) The contour plot demonstrates the interaction between silica fume and metakaolin on paste pH, with color change indicating pH change.

2.3.2 Impact of binder composition on pH of SF39, SF60, and SF81 paste and hempcrete samples

At 24 hours post-mixing, paste pH was similar for OPC, SF39, and SF60 mixes, which had mean pHs of 13.27, 13.07, and 13.02, respectively (**Figure 4A**). SF81 had a mean pH of 10.61, demonstrating a significant main effect of binder composition on paste pH ($p < 0.05$). Post-hoc testing confirmed that SF81 had a significantly lower pH than all other groups.

For hempcrete cylinders, pH values were lower than those observed in cement pastes (**Figure 4B**). With increased silica fume content, pH decreased, as shown by SF81 exhibiting the

lowest pH with a mean pH of 9.31 and 9.37 for UYE inoculated with *S. pasteurii* and UYE-only control hempcrete samples, respectively. A significant interaction between binder composition and *S. pasteurii* addition was observed ($p = 0.001$), indicating that the effect of microbial inoculation on pH depended on binder composition. In SF60, *S. pasteurii* increased pH relative to UYE controls ($p < 0.05$), while no difference was observed for SF39 or SF81. For SF60, the mean pH was 10.65 and 9.81 for UYE inoculated with *S. pasteurii* and UYE-only controls. The mean pH for SF39 and SF81 was 12.18 and 9.34, respectively.

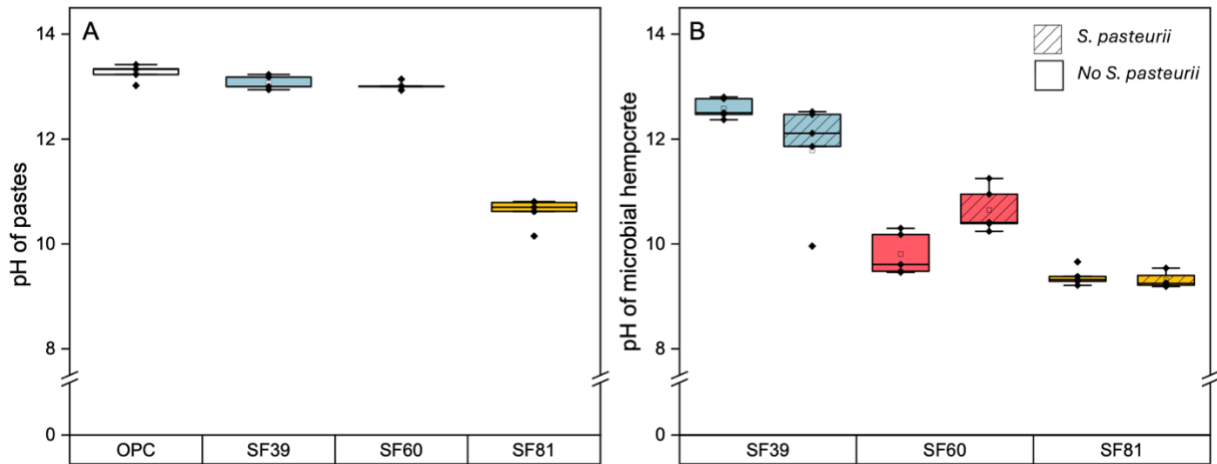


Figure 4: pH is decreased with increased silica fume content in SF39, SF60, and SF81 pastes and hempcrete. A) pH of pastes at 24 hours post-mixing in water. B) pH of hempcrete at 28 days post-mixing with hurd pre-soaked in UYE-only controls or UYE inoculated with *S. pasteurii*. The top and bottom of the box plots represent the 75th and 25th percentiles, respectively, and the whiskers represent 1.5 standard deviations from the interquartile range.

2.3.3 Impact of binder composition on compressive strength of hempcrete and pastes

First, the impact of metakaolin and silica fume on compressive strength was measured for hardened paste samples after 28 days of curing. As expected, large replacements of OPC with these fillers decreased strength (**Figure 5A**). However, silica fume combined with metakaolin

improved strength more than silica fume replacement alone (**Figures 5B and 5D**). The best-fit model was: Compressive strength = $18.77 + 0.1129 \text{ MK} - 0.04516 \text{ SF} - 0.00757 \text{ MK} * \text{MK} + 0.000396 \text{ SF} * \text{SF}$, with R-sq(adj) of 0.831. In the model, silica fume is denoted as SF, metakaolin as MK, and their interactions are denoted as multiplication signs.

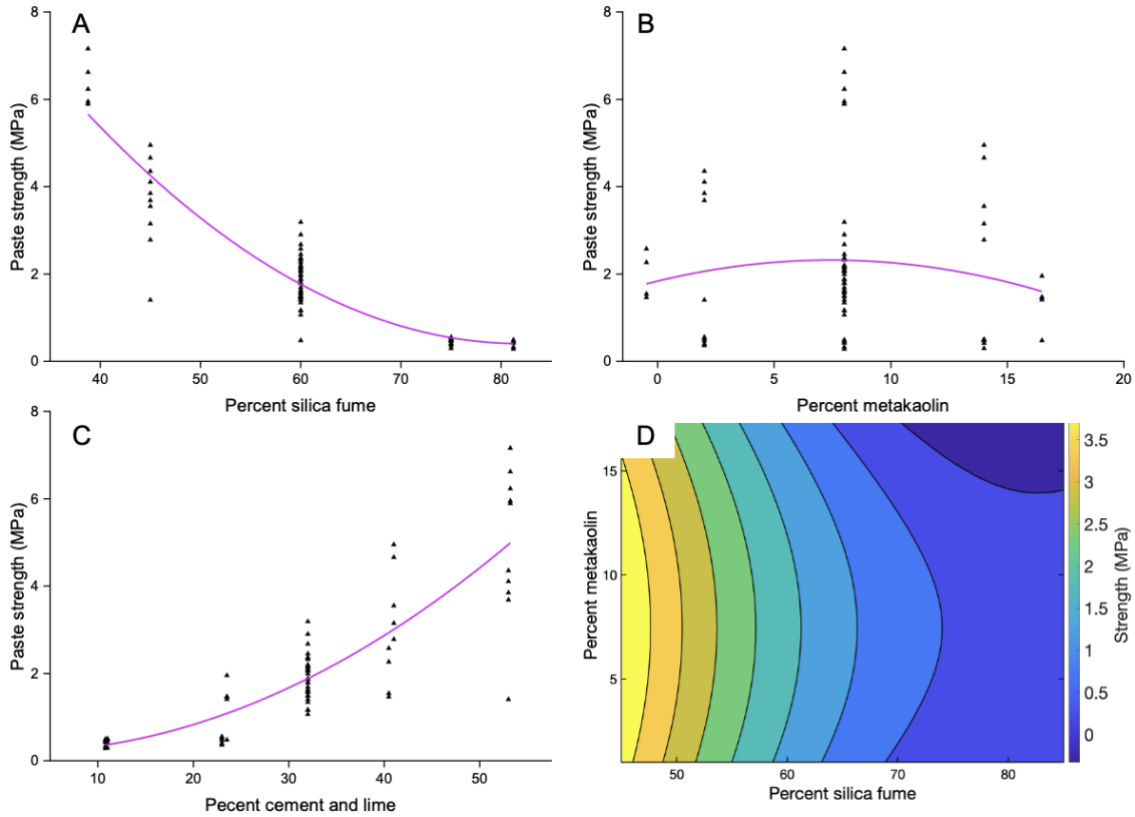


Figure 5: Increased silica fume content reduces paste strength at 28 days. A) Impact of percent binder content silica fume on paste strength. B) Impact of percent binder content metakaolin on paste strength. C) Impact of percent binder content of cement and lime on paste strength. D) contour plot demonstrating the interaction between silica fume and metakaolin on paste strength with varying binder compositions (**Table A2**).

Next, the impacts of binder composition on strength were assessed for hempcrete (i.e., with the inclusion of hemp hurd at a paste: hurd ratio of 15:1). For these experiments, several mix designs were selected from the strength DOE (e.g., SF39, SF60, SF81) to further investigate

the impacts of variation in silica fume and metakaolin on hempcrete strength. SF39 and SF30 mixes were chosen due to their strength, while SF81 was chosen due to its reduced pH. In these samples, the hemp hurd was soaked in water, which is conventional in hempcrete preparation. There was a significant effect of binder composition on strength for hempcrete ($p < 0.001$). Post-hoc testing revealed that strength was highest at 1.78 MPa for SF39, which has the least silica fume content. Strength significantly decreased with higher silica fume content, with the SF60 and SF81 mixes demonstrating mean strengths of 1.04 ± 0.14 MPa and 0.49 ± 0.08 MPa, respectively (**Figure 6A**).

Strength testing was also conducted on microbial hempcrete, where *S. pasteurii* was introduced by soaking the hemp hurd in nutrient media that supports microbial growth. Both microbial and media-only control samples were refreshed with media supplemented with 48 g/L calcium chloride dihydrate prior to mixing. A significant main effect of binder composition on the strength of hempcrete ($p < 0.001$) was observed. However, there was no significant effect of microorganism addition ($p > 0.05$) and no interaction between binder composition and microorganism addition ($p > 0.05$). Hempcrete with UYE-only controls and UYE inoculated with *S. pasteurii* showed increased strength compared to mixes with water (**Figures 6A and 6B**). The mean strengths for SF60 and SF39 hempcrete mixes with UYE inoculated with *S. pasteurii* were 1.29 MPa and 2.11 MPa, respectively. The mean strength for hempcrete with UYE-only controls was 1.36 MPa and 2.05 MPa for SF60 and SF39, respectively. Post-hoc testing confirmed that strength was highest for the mixes with the least silica fume content, and all binder composition groups had significantly different strengths. Notably, the compressive

strength of SF81 microbial hempcrete mixes was below the Test Mark load frame's detection limit, so the data were reported as the load detection limit of 0.658 MPa.

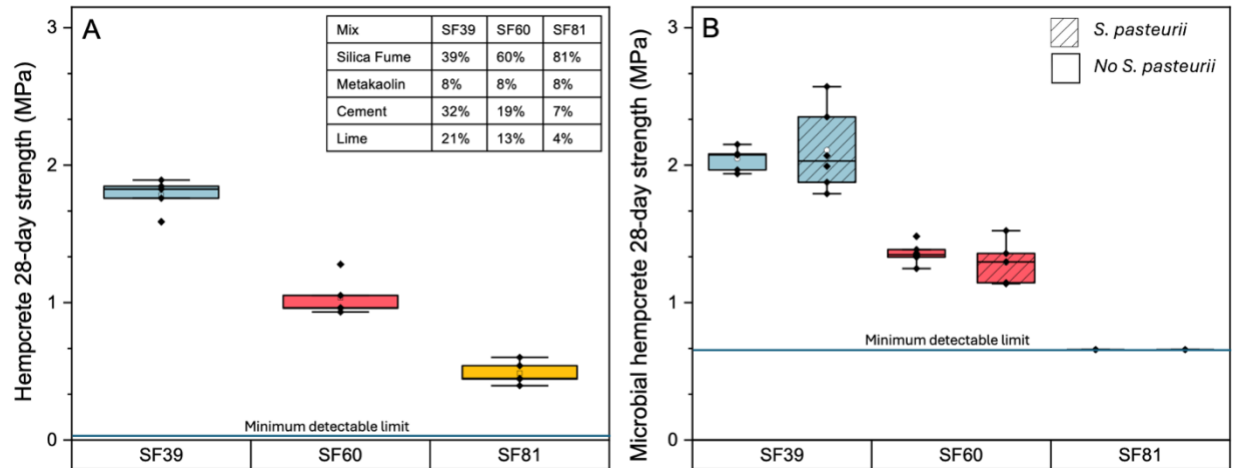


Figure 6: Increased cement replacement decreases hempcrete strength. A) Hempcrete mix strengths with hurd pre-soaked in water. B) Microbial hempcrete mix strengths with hurd pre-soaked in UYE-only controls or UYE inoculated with *S. pasteurii*. The top and bottom of the box plots represent the 75th and 25th percentiles, respectively, and the whiskers represent 1.5 standard deviations from the interquartile range. Blue lines represent the minimum detectable limits of the MTS and Test Mark, respectively.

Finally, compressive strengths were measured for hempcrete manufactured with either 80:20 or 20:80 lime: cement ratios after 28 days of curing. These mixes represent common adjustments to the binder in hempcrete that do not employ additional components such as silica fume or lime. These experiments were performed for a variety of paste: hurd and water contents (A5). Using the same paste: hurd and water: binder ratios as the hempcrete samples in this study, the 80:20 cement-to-lime mix achieved a mean strength of 2.95 MPa (Figure 7), while the compressive strength of the 20:80 mix was below the Test Mark load frame's display detection limit (i.e., below 0.658 MPa). There was a significant difference ($p < 0.001$) between the strength of the 80:20 cement-to-lime mixes and the minimum detectable strength of the Test Mark

machine, representing the upper limit for the potential strength for the 20:80 cement-to-lime mixes.

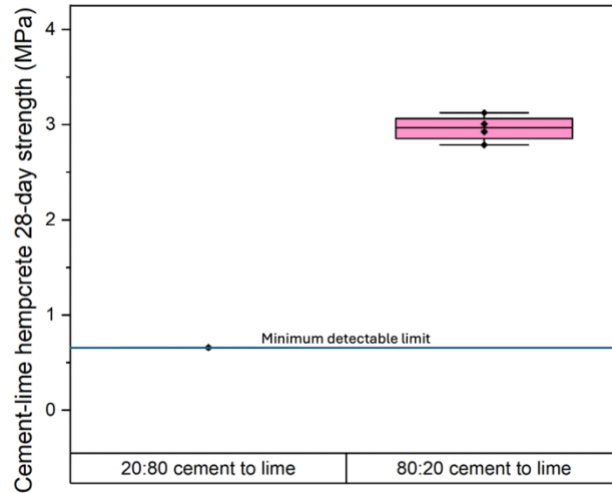


Figure 7: The strength of hempcrete is improved with the addition of cement, as shown by the 28-day strength of 20:80 and 80:20 cement to lime binder composition hempcrete. The top and bottom of the box plots represent the 75th and 25th percentiles, respectively, and the whiskers represent 1.5 standard deviations from the interquartile range. The 20:80 mix's max compressive strength was below the minimum detectable limit of the Test Mark load frame, which is represented by the blue line.

2.3.4 Impact of binder composition on microbial viability of SF39, SF60, and SF81 paste and hempcrete samples

Viability was examined for both pastes and hempcrete samples prepared using SF39, SF60, and SF81 mix compositions. In paste samples, bacterial viability immediately after mixing was similar for OPC, SF39, and SF60, each around $\sim 10^4$ MPN/g, but was notably higher in SF81 mixes at $\sim 10^6$ MPN/g (**Figure 8A**). As expected, no viability was observed in paste UYE-only controls at 0 or 24 hours post-mixing, indicating that if microbes were present, they were below the minimum detectable limit of 9.5 MPN/g. Statistical analysis showed a significant main effect of time on *S. pasteurii* viability ($p < 0.001$), with viability decreasing across all groups by 24

hours. However, mix composition did not significantly affect viability ($p > 0.05$), and the interaction between binder composition and time approached significance ($p = 0.055$).

A similar trend was observed for microbial hempcrete, with SF81 showing the highest viability for both mixes with UYE-only controls and mixes with UYE inoculated with *S. pasteurii* (**Figure 8B**). Additionally, it should be noted that UYE-only controls, while not inoculated with *S. pasteurii*, could sequester microorganisms from both the hurd and the surrounding environment. Initial viability for SF39, SF60, and SF81 hempcrete mixes was lower than in their corresponding hardened pastes; however, the addition of hurd increased viability at 24 hours compared to the paste samples, which were below detectable limits for some paste mix compositions. A significant three-way interaction between mix composition, time, and liquid content used to pre-soak hurd (i.e., UYE-only and UYE inoculated with *S. pasteurii*) was observed for microbial hempcrete ($p < 0.05$).

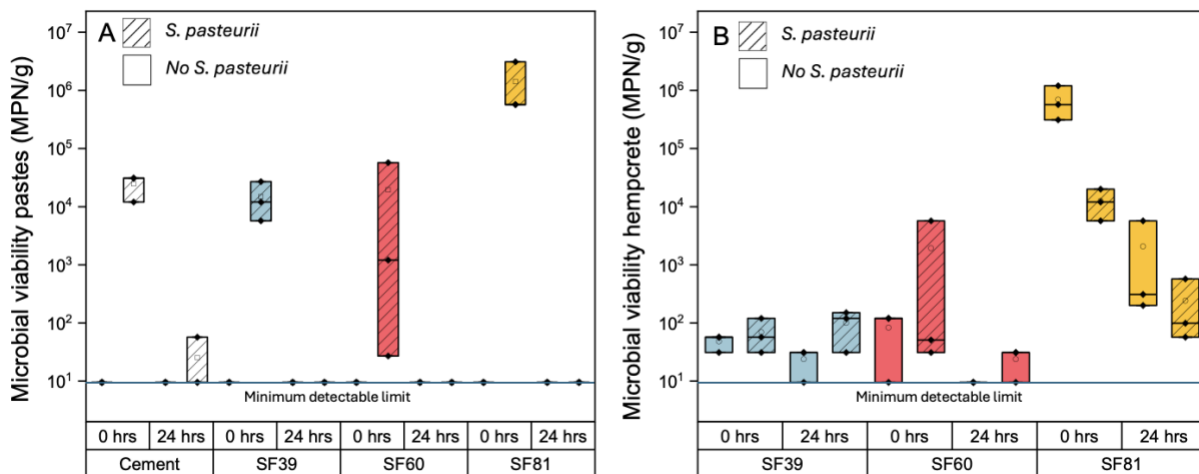


Figure 8: Microbial viability evaluated at 0 and 24 hours post-mixing in microbial pastes and hempcrete. A) Microbial viability within pastes using UYE-only controls or UYE with *S. pasteurii*. B) Microbial viability within hempcrete mixes with hurd pre-soaked in UYE-only controls or with UYE inoculated with *S. pasteurii*. The top and bottom of the box plots represent the 75th and 25th percentiles, respectively, and the whiskers represent 1.5 standard deviations from the interquartile range.

2.4 Discussion

This study aimed to expand the potential applications of hempcrete by modifying its binder composition to enhance strength while minimizing cement use, as well as creating a more supportive environment for microbial life. Silica fume, a byproduct of the ferrosilicon industry, and metakaolin, both pozzolanic materials, were selected as novel binder components for hempcrete. Both silica fume and metakaolin can react with portlandite in cement and hydraulic lime to create calcium silicate hydrates, potentially contributing to increased strength [6]. This study examined the effect of varying binder compositions of silica fume, metakaolin, cement, and lime on material strength and the viability of microorganisms within pastes and hempcrete. The key results are that incorporating high levels of silica fume improves compressive strength and lowers the pH of hempcrete compared to traditional hempcrete mixes. Additionally, lower-pH mixes can improve the immediate but not prolonged viability of ureolytic microorganisms that are commonly employed for biomineralization.

A central result of this study was that hempcrete binders that minimize cement and instead have high quantities of silica fume achieve strengths comparable to or exceeding traditional lime-based hempcrete used for insulation (e.g., 0.2–1.15 MPa [26]). The strongest mix, which included 39% and 32% cement silica fume (i.e., SF39), achieved ~60% of the strength of a binder with 80% cement, 20% lime, using the same paste: hurd, and liquid: binder ratios (**Figure 6 and Figure 7**). This is notable since the SF39 composition has 40% of the cement content as the 80:20 mix but still achieves ~60% of the compressive strength. Further, mixes that contain the nutrient broth (i.e., UYE) are even stronger; SF39 with UYE or UYE and microbes achieve 69-72% of the 80:20 mix. This could be due to various contributors, including

hurd size (**A3**), media salts, or the inclusion of microbes within these mixes. Moderate levels of metakaolin were seen from the paste experiments to interact with silica fume to contribute to the strength improvement; however, this study did not determine the specific mechanisms by which this is accomplished. These results demonstrate that utilizing large replacement percentages of common concrete fillers such as silica fume and metakaolin may provide feasible alternatives for improving the strength of hempcrete, potentially reducing the need for substantial additions of cement, which significantly contributes to anthropogenic carbon emissions [1].

The inclusion of viable biomineralizing microorganisms within hardened paste and concrete is desirable for crack healing, densification, or applications like environmental sensing, but has been challenging to achieve, in part because of the excessively high pH of these materials [13], [14], [32]. Achieving microbial viability within hempcrete would be of interest for similar reasons. Cement and lime have a high pH (**A6**). Silica fume substitution reduces the pH of hempcrete to improve immediate microbial viability. The pH reduction was highest for the SF81 mixes, but this binder composition was not sufficiently strong for realistic applications. The SF39 and SF60 mixes had favorable strengths and considerably reduced pH for hempcrete (**Figure 4**). The pH reduction achieved with silica fume content was greater for hempcrete than for hardened paste, perhaps because of the interaction between the hurd and the matrix, differences in measurement times (24 hours for pastes, 28 days for hempcrete), or the further progression of hydration reactions, with the pozzolanic reaction, consuming portlandite. Prior work demonstrated that even small pH reductions can be useful for improving microbial viability for *S. pasteurii* [14]. Outside microbial hempcrete, decreasing the pH of cementitious materials

could be useful for other applications where pH leaching is detrimental, such as waste repositories [40].

Our study confirmed the findings of others that vegetative *S. pasteurii* has low viability in OPC, whether measured immediately after mixing or at 24 hours (**Figure 8A**) [33]. In hardened paste, binder compositions that achieve the lowest pH (i.e., SF81) also increase the viability of microorganisms tested immediately after mixing (**Figure 8**). However, in these hardened paste samples, viability was not improved compared with OPC for the other binder compositions tested in this study 24 hours after mixing. These results suggest that other stressors (e.g., changes in material density and heat of hydration) also impact microbial viability in these materials. Encouragingly, the inclusion of hemp hurd (i.e., hempcrete) achieves larger decreases in pH and larger improvements in microbial viability at 24 hours. All binder compositions achieved microbial viability immediately after mixing for hempcrete samples. The differences between hardened paste and hempcrete samples may be attributable to the decreased pH for hempcrete or potentially other reasons, such as changes in porosity or differences in nutrient availability. Importantly, in hempcrete samples, other microbial species besides *S. pasteurii* would be expected to be present, given that samples were prepared at the scale of gallons and maintaining sterile conditions was not possible. The presence of other microorganisms is also supported by the different colony morphologies observed for samples plated from MPN tubes to confirm growth (**A7**). Positive viability within the media-only (i.e., control) hempcrete samples without added *S. pasteurii* confirms the hospitability of these materials to other microorganisms. These results are encouraging since it may be advantageous to use microbes other than *S. pasteurii*

depending on the desired functionalities of the microorganisms, such as bioremediation, biomineralization, or other activities [9], [11], [32], [51].

There are important limitations to this study. The MPN method relies on confidence intervals rather than direct cell counts, introducing some inherent uncertainty in the results, including the fact that there may be viable cells within the sample that may be below detectable limits [52]. Inherently destructive sample preparation processes, such as sonication and the use of a mortar and pestle, could artificially lower the observed viability of microorganisms. Additional work is needed to determine the metabolic activity and biomineralization potential of microorganisms within these mixes or to explore the viability of other species capable of biomineralization. Additionally, this study did not assess how these mix compositions affect hempcrete's density or thermal conductivity, which are positively correlated and relevant to its performance as an insulation material.

In conclusion, this work tested the hypothesis that silica fume and metakaolin can improve the strength of hempcrete and reduce the pH to improve microbial viability. These findings demonstrate the potential of utilizing silica fume-rich binders to enhance the strength of hempcrete and the immediate viability of microorganisms within the material. We found that the inclusion of silica fume is able to notably increase hempcrete strength at 39% weight binder content compared to hempcrete with primarily lime binders. We also found that pH reduction is important for immediate viability, but reduced viability at 24 hours in pastes compared to hempcrete suggests that other stressors, such as porosity, still impact microbial viability within these materials. These results are significant for potentially expanding the utility of hempcrete through strength augmentation and progressing towards engineering more hospitable

environments for microbes capable of improving the functionality and sustainability of these materials.

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CHAPTER THREE

CONCLUSIONS AND FUTURE DIRECTIONS

Concluding Remarks

This work aimed to expand applications of hempcrete by improving its compressive strength while maintaining its desirability as a carbon-neutral to carbon-negative insulation material. This was accomplished by using silica fume and metakaolin to enhance strength as well as reduce pH to create a more supportive environment for microbial life. Key results showed that incorporating high levels of silica fume improves compressive strength and lowers the pH of hempcrete compared to traditional mixes. These lower-pH mixes supported greater immediate viability of ureolytic microorganisms capable of biomineralization, though prolonged viability remained limited.

The first key result of this work was that mixes with high silica fume content (39-60% of the binder weight) achieved strengths comparable to or exceeding traditional lime-based hempcrete [26]. The strongest binder composition investigated herein, SF39, reached approximately 60-72% of the strength of an 80:20 cement-to-lime binder mix made with the same liquid: binder and paste: hurd ratios. Strength variation was observed for different pre-soak treatments, with increased strength of approximately .29 MPa for microbial hempcrete compared to mixes with water for SF39 and SF60. The observed strength variations could be due to various contributors, including hurd size (**A3**), media salts, or the inclusion of microbes within these mixes. This finding is significant because SF39 mixes contained less than half the cement content of the 80:20 cement to lime hempcrete mix. These findings highlight silica fume's

potential for creating low load bearing hempcrete without the use of high cement content, which is a significant carbon emitter [1], [7], or mechanical compaction, which can increase hempcrete's thermal conductivity and require specialized equipment [2], [5]. This work represents a first, but important, step toward expanding hempcrete's applications by enhancing strength while maintaining its role as a carbon-neutral to carbon-negative building material.

The second key result of this work was that these alternative binder compositions successfully reduced the pH of pastes and hempcrete. The pH reduction of high silica fume mixes was greater for hempcrete than for hardened pastes. This is potentially due to differences in curing time before pH measurements, with pH measured at 24 hours for pastes as opposed to 28 days for hempcrete mixes. For high silica fume mixes, pH was significantly reduced in pastes and hempcrete. Notably, while SF81 achieved the lowest pH, its low compressive strength indicates that it is not a viable binder for hempcrete applications. The pH reduction achieved within 39%-60 % SF contents was still considerable and may be advantageous for improving microbial viability within hempcrete.

The final key result of this work was that the inclusion of silica fume improved the viability of microorganisms immediately after mixing for both pastes and hempcrete. With pastes, we saw diminished viability at 24 hours. However, all hempcrete mixes demonstrated immediate and 24-hour viability, as confirmed by plating. This finding suggests that additional factors other than pH still affect viability within these mixes, such as diminished porosity during curing. Notably, within hempcrete, we saw the viability of microbes sequestered from the hard and/or surrounding environment in UYE-only controls, i.e., mixes not inoculated with *S. pasteurii* (A7). This is encouraging because it suggests the ability to incorporate other microbes

into these mixes apart from *S. pasteurii*, a microbe commonly used in cementitious materials due to its tolerance to high pH environments.

Future Directions

While this thesis describes how silica fume and metakaolin can augment the strength of hempcrete and improve microbial viability through pH reduction, it does not address how changes to the binder material change the insulative properties of hempcrete. Changes to the binder material, such as adding silica fume or metakaolin, can alter the porosity of hempcrete. From the literature, it is known that pozzolanas change the hydration products and curing of OPC [29], [39]. Additionally, pozzolanic materials have been shown to decrease the porosity of concrete and silica fume has been thought to improve the strength of high-performance concrete by filling in void space, thereby increasing density, in addition to other strengthening mechanisms [30], [39]. Reducing the porosity of hempcrete is significant for its ability to act as an insulation material. Reduced porosity decreases thermal resistance and increases the material requirement needed to achieve the same insulation value. Future work should quantify the insulative properties of these mixes.

Additionally, it would be beneficial to perform a life cycle analysis (LCA) on these mixes. While silica fume and metakaolin enhance strength, they require both water and portlandite and thus are often combined with cement or hydraulic lime [6]. Increased hydraulic lime and cement content limits carbonation and reduces overall carbon sequestration, compared to binders with higher compositions of hydrated lime, which can directly participate in carbonation and does not require water [6]. Additionally, the consumption of portlandite, via the pozzolanic reaction, may further limit carbonation, thereby reducing hempcrete's overall

sustainability [6]. An LCA would be beneficial for optimizing the strength of these materials while considering how they affect hempcrete's insulative properties. Additionally, an LCA would allow for a holistic look at optimizing both the cost and sustainability of hempcrete over its material lifespan from a material and energy efficiency perspective.

This work represents the first step toward using biomineralizing bacteria to improve the strength of hempcrete. Future research is needed to investigate the spatial distribution of hydration products due to their ability to affect both mechanical and thermal performances. Mineralization differences between the edge and center of hempcrete samples should be explored, as spatial variation is common in other biomineralized materials, like sand, depending on the method of microbial incorporation. Determining these differences could involve using scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS). EDS could be used to show elemental composition across a cross-section to help qualitatively determine calcium localization. SEM could qualitatively assess differences in void space and microstructure [33]. However, it should be noted that these methods do not differentiate between biotic and abiotic calcium carbonate. Prior work with *B. megaterium*, *S. pasteurii*, and *B. cohnii* has shown their ability to act as nucleation sites, resulting in increased hydration products and microstructural differences in pastes [38], despite not all strains being capable of biomineralization. It may be beneficial to look at the biomineralization spatial distribution and quality between live, autoclaved, and control samples. Additionally, while *S. pasteurii* has often been used for biomineralization experiments, it may be advantageous to look at other biomineralizing microorganisms with higher pH or heat tolerance [13].

Future works should determine whether the improved strength gain observed for microbial hempcrete results from bacterial biomineralization or if cells act as nucleation sites for hydration products. Previous studies have noted that bacteria may not always remain metabolically active or capable of participating in MICP [14], [33]. A challenge in evaluating ureolytic activity within cementitious materials is that assays like the Jung assay may be disrupted by the high pH of cement and lime, which affects NH_3/NH_4 ratios and colorimetric indicators. However, comparing biomineralization differences among live, autoclaved, and control samples, including those with abiotic urease or non-biomineralizing bacterial strains, could provide insight into whether observed strength differences stem from active biomineralization or cells acting as nucleation sites during the hydration of cement.

While pH detrimentally affects microbes, other factors, such as decreased porosity, nutrient availability, and high heat of hydration for cementitious materials, also reduce microbial viability [13], [14]. This is reflected in the conclusion that, although reduced pH improves immediate viability in paste, other factors still impact microbial viability within these mixes. The reduced pH observed in 28-day cured hempcrete compared to 24-hour paste samples suggests that exploring different methods for incorporating microbes within hempcrete after curing might be beneficial. Surface treatments or submerging cured samples into biomineralizing media may allow microbes to mineralize the surface without requiring long-term viability within the material. This approach could also enable multiple rounds of biomineralization. If this line of inquiry was pursued, it would be beneficial to investigate the depth of mineralization achieved through these treatments due to hempcretes' increased porosity relative to other cementitious

materials and whether repeated applications enhance performance, as seen in other biomineralized materials such as sand.

More broadly, exploring how cellular mechanisms such as biofilm formation affect microbial viability within these systems would be valuable. Biofilms can enhance microbial resilience by facilitating increased cellular communication and metabolite exchange [53]. They serve as natural defense mechanisms from stressors such as UV, temperature, pressure, salinity, poor nutrient availability, etc. [53]. However, it is not fully understood how these protective mechanisms can be leveraged to increase the viability of microbes in the context of building materials. This could be studied by examining well-studied biofilm components such as curli in *E. coli*. In the *E. coli* biofilm, amyloid protein fibers (i.e., curli) are the primary proteinaceous components facilitating biofilm aggregation and persistence [53]. Additionally, it would be valuable to consider how other technologies, such as bioprinting, could allow for greater spatial control and surface functionalization, which could be advantageous for building materials.

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APPENDIX:

SUPPLEMENTAL INFORMATION FOR CHAPTER TWO

A1: Design of experiments (DOE)
for paste pH and strength testing

The pH DOE design was generated in Minitab using a central composite design with three replicates and three continuous variables. The dependent variable was the pH of hardened cement paste at 24 hours. The continuous independent variables were the proportions of silica fume, metakaolin, and the proportion of cement in the remaining dry binder. The percentage of lime in the dry binder was determined based on the cement content, ensuring that the total binder content equaled 100%. Each continuous factor was assigned high and low values, which were then used to generate the DOE. The DOE consisted of 60 total mixes with 24 cube points, 18 center points within the cube, 18 axial points, and an alpha of 1.682. Mixes were prepared according to the pH DOE mix compositions (**Table A1**).

The strength DOE design was generated in Minitab using a central composite design, with the 28-day compressive strength for pastes as the response variable. The continuous independent variables were the percentages for silica fume and metakaolin. The remaining dry binder, not specified as an independent variable in the DOE, consisted of cement and lime at a fixed 60:40 ratio. This resulted in 70 total mixes with 20 cube points, 15 center points in the cube, 20 axial points, and 15 axial midpoints, with $\alpha = 1.41$. Mixes were prepared according to the strength DOE mix compositions (**Table A2**). Mix 2, the center point of the DOE, was tested repeatedly to examine spatial relationships between variables. To match the mix sizes of the other tests, mix 2 was split into six batches to maintain consistent mix sizes.

Table A1: Mixes for pH design of experiment.

Mix	Silica fume (%)	Metakaolin (%)	Lime%	Cement %
1	75.3	10	7.3	7.4
2	60	4	7.2	28.8
3	37.5	10	26.3	26.2
4	60	16	19.2	4.8
5	15	16	55.2	13.8
6	37.5	10	0	52.5
7	37.5	0	31.3	31.2
8	15	4	16.2	64.8
9	37.5	10	52.5	0
10	0	10	45	45
11	37.5	20	21.3	21.2
12	15	16	13.8	55.2
13	15	4	64.8	16.2
14	60	16	4.8	19.2
15	37.5	10	26.3	26.2
16	60	4	28.8	7.2

Table A2: Mixes for strength design of experiment.

Mix	Silica fume (%)	Metakaolin (%)	60:40 Cement Lime Mix (%)
1	45	14	41
2	60	8	32
3	75	14	11
5	75	2	23
7	45	2	53
9	38.8	8	53.2
11	60	0	40
13	60	16.5	23.5
14	81.2	8	10.8

A2: Effect of pre-soaked hurd and cure conditions on hempcrete strength.

To evaluate how pre-soaking the hurd affects strength, SF60 cylinders were mixed using a liquid-to-binder ratio of 0.802 and a 15:1 paste-to-hurd ratio. The cylinders were removed from their molds after 72 hours and air-cured for 28 days. For strength testing, an MTS Criterion 60

Test System was used to detect the max compressive strength in accordance with ASTM C39M-20 Standard Test Method guidelines. All tests were conducted with neoprene caps to ensure even load distribution across the specimen's cross-sectional area. Overall, it was found that adding dry hurd to the dry binder before introducing water has no significant difference in strength ($p=0.456$) compared to using pre-soaked hurd as the sole water source in air-cured hempcrete cylinders at 28-day testing (**Figure A1.A**).

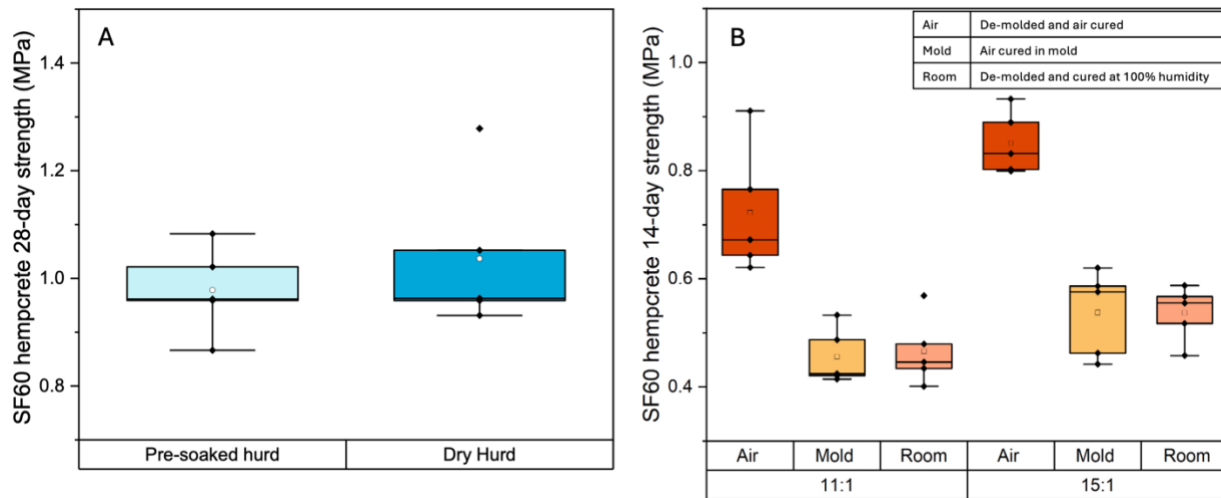


Figure A1: A) Differences in observed strength for pre-soaked or dry hurd using a 15:1 paste to hurd ratio. B) Differences in observed strength for air, mold, and standard cure room conditions with 11:1 and 15:1 paste-to-hurd ratios.

To evaluate how curing conditions and the paste-to-hurd ratio affect strength, SF60 cylinders were mixed using a liquid-to-binder ratio of 0.802 and hurd pre-soaked in water. Both 15:1 and 11:1 paste-to-hurd ratios were evaluated. The cylinders were removed from their molds after 72 hours and subjected to three different curing methods: air curing, mold curing, and curing in a standard concrete cure room, temperature-controlled with 100% humidity. For strength testing, an MTS Criterion 60 Test System was used to detect the max compressive

strength in accordance with ASTM C39M-20 Standard Test Method guidelines. All tests were conducted with neoprene caps to ensure even load distribution across the specimen's cross-sectional area.

The compressive strength of the SF60 hempcrete cylinders at 14 days was significantly influenced by the varying curing conditions. Air curing resulted in the highest compressive strength compared to both mold curing and temperature-controlled curing at 100% humidity using a cure room, regardless of whether a paste-to-hurd ratio of 11:1 or 15:1 was used (**Figure A1.B**). The air-cured cylinders with a 15:1 paste-to-hurd ratio exhibited the highest strength overall. Statistical analysis confirmed significant main effects of both curing conditions ($p < 0.001$) and paste-to-hurd ratio ($p = 0.002$) on the 14-day compressive strength of the hempcrete. However, no significant interaction was found between curing conditions and paste-to-hurd ratio ($p = 0.661$). Post hoc comparisons indicated that air curing led to significantly greater strength, with the 15:1 paste-to-hurd ratio yielding the highest strength among all samples. For the 15:1 paste to hurd ratio, the mean strength was 0.85 MPa, 0.54 MPa, and 0.54 MPa for air cured, mold cured, and room cured samples, respectively. For the 11:1 paste to hurd ratio, the mean strength was 0.72 MPa, 0.45 MPa, and 0.46 MPa for air cured, mold cured, and room cured samples, respectively.

A4: Size comparison of hurd sourced from IND Hemp.

Two batches of hemp hurd used in this study and sourced from IND Hemp, Fort Benton, MT, were compared to ensure similarities in hurd and to maintain hempcrete's performance within its workability, density, and mechanical properties. Samples were weighed on a digital balance, and particle size distribution was measured using an RO-TAP mechanical sieve shaker.

Two sets of samples (100 g: 1a and 2a, 150 g: 1b and 2b) were prepared to assess the influence of sample weight on sieve analysis. Samples 1a and 1b were collected from the “new” hurd, and samples 2a and 2b were collected from the “old” hurd. Sieves with mesh sizes ranging from 0.85 mm to 12.5 mm were used, with shaking durations of 10 minutes for 100-gram samples and 20 minutes for 150-gram samples. Retained materials were weighed to construct the particle size distribution profile (**Figure A2**). The sieve analysis data were used to calculate the percentage and cumulative percentage of material retained on each sieve, and the particle size distribution curve was plotted.

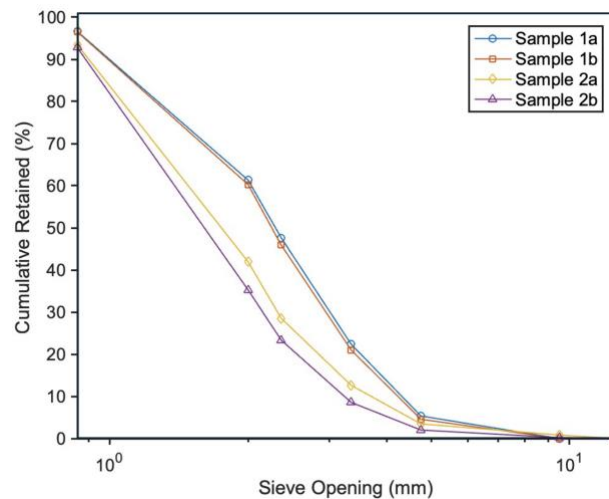


Figure A2: Graph of the cumulative retained hurd. Sample 1a and 1b are 100 and 150 grams of the “new” hurd. Sample 2a and 2b are 100 and 150 grams of the “old” hurd.

The sieve analysis results indicated that most hemp hurd particles were retained on sieves with smaller openings, exhibiting a consistent distribution trend across both sample sizes. More than 50% of the particles were within the 0.85 mm to 2 mm size range, demonstrating a significant proportion of fine fractions in the overall material composition. It should be noted that the presence of hemp fibers may have affected the sieving results, with varying degrees of

fiber accumulation on each sieve that may have prevented particle passage. However, the fiber weights were negligible compared to the total weights of the samples. The particle size distribution trend is consistent for both samples, with the new hurd particles trending slightly smaller.

A4: Summary of samples tested.

Table A3: Overview of samples tested. DOE = design of experiment; UYE = urea yeast extract.

Study	Groups	Mix details	Cure conditions	Testing details
<u>pH of pastes</u>	pH DOE mixes with water	Prepared using a 0.7 liquid:binder ratio and 20 g of binder. Central composite design with 24 cube points, 18 center points, and 18 axial points. Binder compositions included metakaolin (0–20%), silica fume (0–75.3%), lime (0–64.8%), and cement (0–64.8%). Pastes mixed by hand.	Air cured in open plastic weigh boats for 24 hours at ~20°C and 30% humidity.	Wagner Meters 880-R0007-001 Rapid RH Digital pH Meter.
	OPC with water	Prepared using a 0.7 liquid:binder ratio and 30 g of binder.	Air cured in open plastic weigh boats for 24 hours at ~20°C and 30% humidity.	MSC Extech PH100 pH Meter.
	SF39 with water	Pastes mixed by hand.		
	SF60 with water			
	SF81 with water			

Table A3: continued.

<u>pH of hempcrete</u>	SF39 with UYE	Prepared using a 0.802 liquid:binder ratio and 15:1 paste:hurd ratio.	Cylinders demolded at 72 hours and air cured at ~20°C and 30% humidity.	MSC Extech PH100 pH meter.
	SF39 with <i>S. pasteurii</i> and UYE	Hemp hurd pre-soaked for 24 hours in UYE only controls or UYE with <i>S. pasteurii</i> .		
	SF60 with UYE	Media refreshed with new media supplemented with 48 g/L calcium chloride dihydrate before mixing.		
	SF60 with <i>S. pasteurii</i> and UYE	Hempcrete homogenized by hand and cast into 4×8 in. cylinders.		
	SF81 with UYE			
	SF81 with <i>S. pasteurii</i> and UYE			
	20:80 cement to lime with water	Prepared using a 0.802 liquid:binder ratio using water and 15:1 paste:hurd ratio.	Cylinders demolded at 72 hours and air cured at ~20°C and 30% humidity.	MSC Extech PH100 pH meter.
	80:20 cement to lime with water	Hurd pre-soaked 24 hours in water. Hempcrete homogenized by hand and cast into 4×8 in. cylinders.		
<u>Strength of pastes</u>	Strength DOE mixes with water	Prepared with a 0.7 liquid:binder ratio Central composite design with 20 cube points, 15 center points, 20 axial points, and 15 axial midpoints. Binder compositions included metakaolin (0–16.5%), silica fume (45–81.2%), and 60:40 cement:lime (10.8–53.2%). Mixed using Industrial Hobart A2000 mixer, and cast into 2×4 in. cylinders.	Cylinders were demolded at 48 hours and cured in a standard cure room at ~23.8°C and 100% humidity.	Strength testing performed at 28 days using an MTS Criterion 60 Test System.

Table A3: continued.

<u>Strength of conventional hempcrete</u>	SF39 with water	Prepared using a 0.802 liquid:binder ratio and 15:1 paste:hurd ratio.	Cylinders demolded at 72 hours and air cured at ~20°C and 30% humidity.	Strength testing performed at 28 days using an MTS Criterion 60 Test System.
	SF60 with water	Hemp hurd pre-soaked for 24 hours in water.		
	SF81 with water	Hempcrete homogenized by hand and cast into 4×8 in. cylinders.		
	20:80 cement to lime with water	Prepared using a 0.802 liquid:binder ratio and 15:1 paste:hurd ratio.		
<u>Strength of microbial hempcrete</u>	80:20 cement to lime with water	Hemp hurd pre-soaked for 24 hours in water.	Cylinders demolded at 72 hours and air cured at ~20°C and 30% humidity.	Strength testing performed at 28 days using a Test Mark Industries CM-400P-D machine.
		Hempcrete homogenized by hand and cast into 4×8 in. cylinders.		
	SF39 with UYE	Prepared using a 0.802 liquid:binder ratio and 15:1 paste:hurd ratio.		
	SF39 with <i>S. pasteurii</i> and UYE	Hemp hurd pre-soaked for 24 hours in UYE only controls or UYE with <i>S. pasteurii</i> .		
<u>Strength of microbial hempcrete</u>	SF60 with UYE	Media refreshed with new media supplemented with 48 g/L calcium chloride dihydrate before mixing.	Cylinders demolded at 72 hours and air cured at ~20°C and 30% humidity.	Strength testing performed at 28 days using a Test Mark Industries CM-400P-D machine.
	SF60 with <i>S. pasteurii</i> and UYE	Hempcrete homogenized by hand and cast into 4×8 in. cylinders.		
	SF81 with UYE			
	SF81 with <i>S. pasteurii</i> and UYE			

Table A3: continued.

<u>Viability in pastes</u>	SF39 with UYE SF39 with <i>S. pasteurii</i> and UYE SF60 with UYE SF60 with <i>S. pasteurii</i> and UYE SF81 with UYE SF81 with <i>S. pasteurii</i> and UYE	Prepared using a 0.66 liquid:binder ratio and 30 g binder. Liquid content for pastes was either UYE-only or UYE inoculated with <i>S. pasteurii</i> Pastes mixed by hand in a biosafety cabinet.	Cured in open plastic weigh boats under standard cure room conditions ~23.8°C and 100% humidity.	Viability assessed by MPN immediately after mixing and after 24 hours of curing.
<u>Viability in hempcrete</u>	SF39 with UYE SF39 with <i>S. pasteurii</i> and UYE SF60 with UYE SF60 with <i>S. pasteurii</i> and UYE SF81 with UYE SF81 with <i>S. pasteurii</i> and UYE	Prepared using a 0.802 liquid:binder ratio and 15:1 paste:hurd ratio. Hemp hurd pre-soaked for 24 hours in UYE only controls or UYE with <i>S. pasteurii</i>. Media refreshed with new media supplemented with 48 g/L calcium chloride dihydrate before mixing. Hempcrete homogenized by hand and cast into 4×8 in. cylinders.	Cylinders demolded at 24 hours.	Viability assessed by MPN immediately after mixing and after 24 hours of curing.

A5: Strengths of 80:20 and 20:80 cement to lime
binder composition, hempcrete mixes

Table A4: 80:20 and 20:80 cement to lime hempcrete strengths.

Mix	Hurd: Binder: liquid	Binder Composition (Cement: Lime)	liquid: Binder	Paste: Hurd	Cure Conditions	Dry Density (kg/m ³)	Compressive Strength (MPa)
1	1:3:2.5	80:20	.83	5.5:1	Air cured	664.77	1.41
2	1:6:5	80:20	.83	11:1	Air cured	885.82	2.57
3	1:7:4	80:20	.57	11:1	Air cured	1065.22	2.31
4	1:3:2.5	20:80	.83	5.5	Air cured	631.13	1.34
5	1:7:4.8	20:80	.68	11.8:1	Air cured	865.00	1.12

A6: Effect of 80:20 and 20:80 cement to lime
binder compositions on hempcrete pH

80:20 and 20:80 cement-to-lime mixes were prepared using a 0.802 liquid: binder ratio, with hurd pre-soaked in water for 24 hours before mixing. For mixing, soaked hurd and liquid content were transferred to a 32 in. by 60 in. by 11 in. Brentwood Mortar Box, where they were combined with dry binder ingredients and homogenized by hand. The mixes were cast into 4 in. by 8 in. cylinders, tamped using a pre-wetted 2 in. x 4 in. piece of wood to achieve uniform compaction. Lids were placed on the molds to prevent moisture loss, and the specimens were demolded after 72 hours. The cylinders were then air-cured at approximately 20°C and 30% humidity until 28-day pH testing. An MSC Extech PH100 pH meter was calibrated for pH testing in pH 4.0, 7.0, and 10.1. A 7 mL sample of water was added to the hempcrete surface and allowed to sit for approximately 2 minutes or until the pH readings stabilized.

The mean pH for the 20:80 and 80:20 hempcrete mixes were 12.96 and 13.42, respectively (**Figure A3**). However, there was no significant difference ($p < 0.05$) between the pH of the 20:80 and 80:20 cement to lime hempcrete mixes at 28 days.

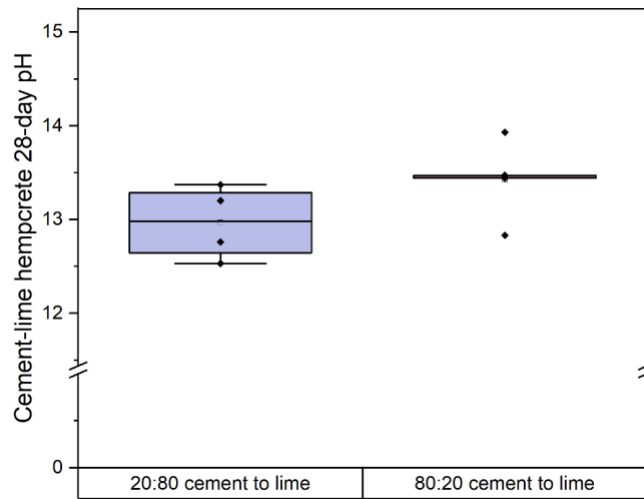


Figure A3: 28-day pH of 20:80 and 80:20 cement to lime hempcrete cylinders.

A7: A representative plate from MPN is used to confirm growth.

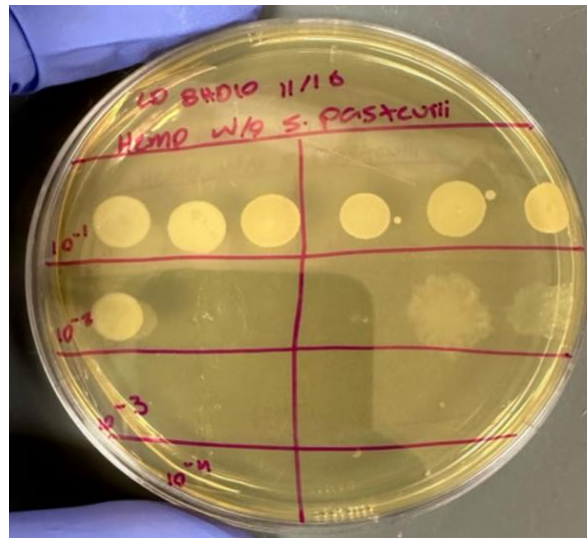


Figure A4: Representative plate used to confirm growth from MPN. After 72 hours of incubation, 10 μ L aliquots from MPN tubes were plated on BHI agar supplemented with 2% urea.