

BIO-BASED RHEOLOGY MODIFYING AGENTS FOR CEMENT-BASED MATERIALS

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BIO-BASED RHEOLOGY MODIFYING AGENTS FOR CEMENT-BASED MATERIALS

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To my family and friends

ABSTRACT

Recent developments in the construction sector lead to the possible implementation of advanced manufacturing techniques of cement-based materials. Such concrete production led to the development of high-performance concrete mixes (HPC) with superior fresh state qualities over conventional concrete. HPC mix design requires fresh-state properties like homogeneity, flowability, and high workability. These properties cannot be achieved by relying on natural rheological properties of cement-paste itself. These parameters can be obtained through the use of various chemical and mineral additives to increase flowability without causing segregation and bleeding in concrete. These additives could be generally named “Rheology Modifying Agents” including superplasticizers, high range water reducers (HRWR), and viscosity of modifying agents (VMA).

While the list of synthetic rheology modifying agents are extensive, a few novel bio-based natural polymers like welan gum and diutan gum are now being used in cement-based materials as VMAs. Not only they enhance the rheological performance by improving yield stress, they also provide the stability and segregation resistance in cementitious systems. These biopolymers naturally contain long polysaccharide molecular structure and high molecular weight which increases water retention. Recent studies also show that extracellular polysaccharides and cell walls extracted from bacterial cells could also be used as an alternative VMA. Previously, we also showed that species of *Sporosarcina pasteurii*, *Bacillus megaterium*, *Bacillus subtilis*, and *Peanibacillus polymxa* can increase both yield stress and viscosity at low w/c mixes when

they were used at optimum dosages. However, the efficiency of these cells was almost eliminated at w/c higher than 0.45.

This thesis aims to develop a bio-based viscosity modifying agent (bio-VMAs) for cement-based materials particularly for highly flowable mixes. We follow 2 different approaches to achieve this goal. First, bacterial cells and biofilms formed by *Peaenibacillus polymxa*, and *Bacillus amyloliquefaciens* species were used as bio-VMAs. Then, a 2-part bio-VMA was developed by immobilizing *S.pasteurii* cells on locally available minerals such as diatomaceous earth, metakaolin, sepiolite, and pumice. The influence of PCE based superplasticizer and fly ash were evaluated based on the impact of bio-VMA dosage and water to cement ratio. The performance of designed bio-VMAs was mainly evaluated through rheological analysis and mini-slump test. A further compressive strength testing was done for mortar samples containing 2-part bio-VMA. Our results showed that the yield stress of cement paste could be increased by the addition of bio-VMAs. The use of 2-part bio-VMAs was more promising in terms of improving rheology without sacrificing performance.

ÖZETÇE

İnşaat sektöründeki son gelişmeler, çimento bazlı malzemelerin ileri üretim tekniklerinin olası uygulamasına olanak sağlamıştır. Bu tür beton üretimi, geleneksel betona göre üstün taze beton özelliklerine sahip yüksek performanslı beton karışımlarının (HPC) geliştirilmesine yol açmıştır. Yüksek performanslı beton tasarımı homojenlik, akışkanlık ve yüksek işlenebilirlik gibi taze beton özellikleri gerektirir. Bu özellikler çimento hamurunun doğal reolojik özelliklerine dayanarak elde edilemez. Betonda ayrışmaya ve terlemeye neden olmadan akışkanlığı arttırmak çeşitli kimyasal ve mineral katkı maddeleri kullanılarak elde edilebilir. Bu katkı malzemeleri genel olarak dağıtma gücü yüksek akışkanlaştırıcılar (HRWR), süperakışkanlaştırıcılar ve viskozite düzenleyici katkı malzemelerinin (VDK) gibi reoloji düzenleyici katkı malzemeleri olarak adlandırılabilir.

Sentetik reoloji düzenleyici katkı malzemelerinin listesi geniş olmakla birlikte, welan sakızı ve diutan sakızı gibi biyolojik bazlı doğal polimerler VDK olarak çimento bazlı malzemelerde kullanılmaktadır. Bu katkı malzemeleri sadece akma gerilimini arttırarak reolojik performansı arttırmakla kalmaz, aynı zamanda çimento bazlı sistemlerde kararlılığın (stabilizasyonun) ve ayrışma direncinin de artmasını sağlarlar. Bahsedilen biyolojik bazlı polimerler uzun polisakkarit molekül yapısına ve yüksek molekül ağırlığına sahiptir ve bu sayede su tutma oranını arttıırırlar. Son dönemlerde yapılan çalışmalar bakteri hücrelerinden elde edilen hücre dışı polisakkaritlerin ve bakteri hücre duvarlarının da alternatif VDK olarak kullanılabileceğini göstermektedir. Daha önceki çalışmalarda *Sporosarcina pasteurii*, *Bacillus megaterium*, *Bacillus subtilis* ve *Peanibacillus polymxa* türlerinin optimum dozajlarda kullanıldığında düşük su/çimento

karışımlarında hem akma gerilimini hem de viskoziteyi attırabileceği gösterilmiştir. Ancak su-çimento oranı 0.45'ten daha yüksek olması durumunda hücrelerin VDK olarak verimi neredeyse ortadan kalkmıştır.

Bu tez çimento esaslı malzemelerde özellikle yüksek derecede akışkan karışımlar için biyolojik bazlı viskozite düzenleyici katkı malzemesi (VDK) geliştirmeyi amaçlamaktadır. Bu hedefte iki farklı yaklaşım izlenmiştir. İlk olarak *Peanibacillus polymxa* ve *Bacillus amyloliquefaciens* türleri tarafından oluşturulan bakteri hücreleri ve biyofilmler, biyolojik bazlı VDK olarak kullanılmıştır. Daha sonra iki bileşenli biyolojik VDK üretilmesi amacıyla *S.pasteurii* hücreleri, diatomlu toprak, metakaolin, lületaşı ve ponza üzerine sabitlenmiştir. Süperakışkanlaştırıcı ve uçucu külün eklendiği karışımlar hazırlanmıştır ve farklı hücre ve biyofilm dozajları ile farklı su oranlarının etkisi incelenmiştir. Tasarlanan biyolojik VDK'lar reolojik analizler ve mini-yayıma testleri ile değerlendirilmiştir. 2 bileşenli katkı malzemesi içeren harç numuneleri için basınç dayanımı testi uygulanmıştır. Elde edilen sonuçlar çimento hamurunun akma stresinin biyolojik bazlı VDK'ların eklenmesiyle arttırılabileceğini göstermiştir. 2 bileşenli biyolojik katkı malzemelerinin kullanımı, performanstan ödün vermeden çimento hamurunun reolojik performansının geliştirilmesine olanak sağlamıştır.

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CHAPTER I

1.1 Introduction

For the last decade, the demand for high-performance concrete required comprehensive studies and a better understanding of the fresh and hardened states of concrete. The simplest composite structure of conventional concrete consists of cement paste, fine and coarse aggregates. However, high workability, strength, and durability needs lead the chemical admixtures to become one of the foremost materials of modern concrete mixes. Modern construction technologies require superior properties in mix design compared to conventional concrete mixes. Such that, the possibility 3-D printing of concrete mixes revolve new manufacturing paradigms of flow behavior and rheology of cement-based materials [1].

Extrudable cement-based materials have higher cementing content due to its limited aggregate size and aggregate packing factor. In addition, the fresh-state properties of printable cement-based materials are generally adjusted by using admixtures to facilitate the manufacturing proceeds without any formwork [2]. Various admixtures such as superplasticizers, rheology controlling admixtures (RCAs), viscosity modifying admixtures (VMAs), accelerators, and retarders can be employed to obtain the desired properties including extrudability, pumpability, and buildability. The superior qualities of the concrete develop with stable, cohesive structure that can resist bleeding and segregation. To provide all these prospect properties, a comprehensive mixture proportioning process should be carried starting from the material selection and use.

According to Khayat, flowability depends on the ability of the liquid phase's viscosity to provide a minimum separation of constituents and enhance the homogeneity and hardened properties of the material [3]. Workability and flowability could be better explained by fundamental rheological parameters such as yield stress and plastic viscosity. Yield stress is the stress above which the material flow initiated [4]. Yield stress is an indicator of pumpability, extrudability as well as stability [5]. Plastic viscosity is the resistance to flow which is an important parameter for robustness against segregation. Cement-based materials are known to be shear-thinning materials where their viscosity decreases with increasing shear rate. Besides, cement-based materials also time-dependent structural recovery termed thixotropy, which is defined by the decrease in apparent viscosity with time under shear and recovery when shear is removed [6]. This property can be indicative of stability and generally correlated with an increase in yield stress over time [2].

The scientific approach of rheology focuses on these pointed parameters, enables researchers to understand and solve the complex relationship of stress, strain, and time relationship [7]. To satisfy the current demand for cement-based materials, viscoelastic materials such as cement-paste does not only include cement and water. Chemical and mineral admixtures are commonly used to tailor the abovementioned rheological properties of cement-based materials. Throughout the literature, the parameters affecting the rheological behavior of cement-based materials are defined as shape and size distribution of particles, surface properties of solids, solid concentration and solid interaction capacity within matrix [7]. The variety of mineral and chemical admixtures dispersed inside the mix water creates a distinctive rheological profile with each type and dosage of admixture added since the interparticle forces depend on molecular interaction [8]. Complex systems like these hydrodynamic interactions [9] and thixotropic behavior

of cement-based matrixes make the process prone to experimental evaluation rather than computational modeling [7]. Even so, these particle properties could also be used as an indicator of the rheological behavior while designing the experiments. Rheology of cement-based materials generally influenced by its composition, volume fraction of the binding system, and aggregate content (particle size and shape). Presence of admixtures, supplementary cementitious materials (SCMs), and mineral additives can be used to tailor the rheological properties. As such, superplasticizers will decrease the yield stress by increasing the interparticle spacing while VMAs will increase the plastic viscosity of concrete.

VMAs are commonly used in concrete mixes to control the workability. The most commonly used VMAs used in cement-based systems are cellulose ether derivative, like welan gum and Diutan gum, as well as anionic polyacrylamides [2]. Clays are also being used VMAs to increase the viscosity and thixotropy of the materials [10]. Additive manufacturing requires a cement-based system exhibiting high thixotropy and stability, which could be even more possible when chemical admixtures and mineral additives used together.

The emerging concrete technology not only requires a robust mix design formulation but also a sustainable system that meets the rheological and mechanical performance needs of additive manufacturing. The greenhouse gas emission of concrete production is a worldwide accepted fact causing direct or indirect consequences such as climate change, damaged agricultural fields, reduced natural resources, and increased energy needs [11]. Many studies involve designing mortar mixes with sustainable binders, however, another point that has to be considered is the significant use of chemical admixtures [11]. Even though, production of commonly used admixtures results with a significantly high Global Warming Potential (GWP). Such that, GWP of production 1 kg

of superplasticizers is almost equivalent to the production of 1 kg of Ordinary Portland Cement (OPC) clinker [12]. Thus, it is crucial to adopt a sustainable strategy against admixtures since the necessity to use admixtures is still unchanged due to the need to enhance the above-mentioned efficiency of cement-based materials when CO₂ emission rates and GWP of chemical admixtures are taken into consideration [13]. These non-renewable, toxic chemicals are polluting the air and aquatic life during concrete production and demolition processes [14]. Synthetic polymers are proved to release toxic species into the environment as well as being a costly material, while relatively low cost, sustainable alternatives of bio-based admixtures are presented as an alternative throughout the literature [15]. Therefore, the performance-based approach to use bio-based admixtures within cement-based materials can be a sustainable, more environmentally friendly solution compared to chemical-based production [13] and enhance the rheological performance of concrete through cement-paste.

1.2 Goal and Objectives

The goal of this thesis is to develop a bio-based viscosity modifying agent (bio-VMAs) for cement-based materials particularly for highly flowable mixes. Two different approaches were followed to achieve this goal. First, bacterial cells and biofilms formed by *Peanibacillus polymxa*, and *Bacillus amyloliquefaciens* species were used as bio-VMAs. Then, a 2-part bio-VMA was developed by immobilizing *S.pasteurii* cells on locally available minerals such as diatomaceous earth, metakaolin, sepiolite, and pumice. The specific objectives can be listed as below:

- 1- Identify the possible use of bacterial cells and biofilms as VMAs by evaluating their influence on yield stress, plastic viscosity, and slump

- 2- Design a 2-part bio-VMA by immobilizing the selected bacterial strain on natural minerals
- 3- Evaluate the performance of the bio-VMA through rheological analysis by evaluating their influence on yield stress, plastic viscosity, and slump
- 4- Investigate the influence of 2- part bio-VMA on the compressive strength of cement-based materials.



CHAPTER II

LITERATURE REVIEW

Concrete is the most used construction material consisting of cement paste, fine and coarse aggregates, mostly modified with pozzolans and admixtures. Consequently, the performance of concrete varies based on the used ingredients [16]. The performance of concrete is mostly evaluated as in two stages as a fresh and hardened state. While the fresh state performance is the property criteria before the final set, the hardened state performance governs the strength and durability aspects of hardened concrete [16]. Both stages require different qualifications even though the hardened state qualities depend on the plasticity of fresh performance [7]. The foremost term used for quality assessment of fresh state concrete is workability. Workability is simply the ease of working with concrete in terms of mixing, transporting, pumping, placing, and finishing. Besides workability, the other properties influencing fresh state performance are segregation and bleeding resistance, cohesiveness and adhesion quality of the material, which might require further understanding on particle to particle interactions and internal friction forces developed during casting [16]. Therefore, scientist employ the rheological analysis of the material to model and understand these complex parameters which will satisfy the “workability” requirements of concrete.

2.1 Rheology of Cement Paste

Rheology is defined as the science investigating the deformation and flow characteristics of materials [7]. The flow of concrete has been a quantitative study since Abrams introduced a slump test in 1920 to measure workability [16].

A slump is a conventional tool that can estimate the workability by measuring the flow behavior of fresh concrete. The slump test is a crude test that could be applied in any construction site however slump may cause large errors due to non-homogenous mixes and human error. This might lead to insufficient results to assess the pumpability and placing of concrete. In addition, due to the methodology slump test might not guarantee good workability. Such that, in slump test, the material is sheared under its own weight, which might be less than the force applied during casting and pumping of concrete. Therefore, a test methodology applied with higher shear forces might be more adequate to evaluate the fresh state properties [17].

The science of rheology can define workability as the combined effect of parameters yield stress and viscosity [18]. Another frequently used rheological material property is thixotropy. The attempt to measure rheological performance with rotating vane and the coaxial cylinder is been in practice since the 1970s [16]. The rheology of cement-based materials relies on measuring the time-dependent response of the material to applied stresses [19]. Then, flow curves are generated based on various rheology models which show the relationship between shear stress induced in the material to the applied shear rate. Thus, due to the presence of various phases rheology of cement paste will not be the same with the rheology of mortar or concrete. The presence of aggregate will increase the applied shear on the material. Recently, emerging 3D printing of concrete increased interest in rheological analysis. There is an extensive amount of studies on mostly rheological properties of cement paste [20]–[23].

Cement paste is a complex Non-Newtonian fluid. The most suitable definition of the state of cement paste is stated to be a “suspension” [7], [24], [25]. Researchers emphasize the “suspension” description is to provide a distinct evaluation of Non-Newtonian and Hookean fluid behavior of solid suspensions for cement- paste. The flow

of the cement paste is defined with shear, stress, and strain rate generated from the torque and flow rate of equipment [19]. While Newtonian fluids demonstrate a directly proportional relationship between shear stress and shear rate and the plastic viscosity is defined with the constant slope of the graph [26], the behavior of complex Non-Newtonian suspensions could be different. Cement paste as being a non-Newtonian fluid exhibit a point of yield stress [19], and pseudoplastic behavior [17], also referred to as shear-thinning behavior which defines the increasing viscosity measurements with increasing shear rate [19]. The linear measurements of cement paste are partly attained at high shear rates generally when agglomerated particles are deflocculated [17]. Cement pastes exhibit a viscoelastic behavior similar to elastic solids at shear stresses lower than yield stress ($\tau < \tau_o$) [7]. An accurate yield point can be obtained by direct stress measurements from rheometers. Stress ramps and implementing controlled stress can determine precise rheological properties [17]. The rheological behavior of cement paste can be modelled through the Bingham model.

$$\tau = \tau_o + \mu\dot{\gamma} \quad (1)$$

Where τ_o represents the yield stress (Pa), μ for the plastic viscosity (Pa.s), and $\dot{\gamma}$ is the shear rate (s^{-1}) [7].

The structural breakdown of cement paste suspensions is quantified with a single point called “yield stress” [17], which is the stress required to initiate flow [7]. Rheological measurements of neat cement paste are controlled by solid particle concentration, which mostly depends on the water to cement ratio (w/c) [9]. Decreasing w/c will result in increasing solid particle interactions leading to stronger flocculation, which will increase the yield stress [7]. The opposite trend is also valid for increasing

w/c. Thus, the yield stress of cement pastes is given particular importance to determine the flow properties of the material [17], [18].

For stress levels below the yield stress, cement pastes exhibit plastic deformation with strain recovery[18]. Once the required torque or shear force is achieved the material starts to flow (yield). After the flow initiation, i.e. further stress application above the yield stress, the flow behavior is controlled by the viscosity of the material. Based on the Bingham model (equation 1), the y-intercept of the linear line indicates the yield stress of the material while the slope of the line represents the viscosity. The viscosity of concrete directly affects the correlation between stability, cohesion, pumpability, and finishing characteristic of the material [27]. Cement-based systems, like cement paste, mostly exhibits shear thinning behavior, where the viscosity of the material decrease with increasing shear rate. For some mixes, this phenomenon invalids the accuracy of the Bingham model, and in such a case a linear term is introduced to the Bingham model as a modification (Modified Bingham Model) [28].

Another phenomenon influencing material flow is thixotropy [7]. Thixotropy is a gradual decrease in viscosity due to applied shear stress and substantial recovery of the structures when the stress is removed [29]. A true thixotropic material will maintain the same initial viscosity values upon recovery. For cement paste, the thixotropic behavior mostly depends on coagulation, dispersion, and agglomeration of cement particles. Tattersall and Banfill define this process as structural break-down and build-up, rather than fully reversible thixotropy[7]. When particles in cement particles interact with water, a thin hydrated membrane immediately covers the particle which increases the flocculation. When cement paste is sheared, these flocs will be broken and recovery will be maintained by the formation of new flocs [7]. Thus, the thixotropic behavior of cement

paste depends on the shear history of pastes, including the mixing process, before experimental measurements. High thixotropy will indicate good stability upon casting, good segregation resistance, and reduced entrapped air content.

2.1.1 Measuring Rheology of Cement-Paste

Accuracy of rheological analysis depends on model assumptions and material quality such that the cement paste should be homogeneously mixed without any segregation. In addition, it also depends on the rheometer specifications and apparatus[30] as well as an experimental technique [7] used during the measurement. Generally, the practical evaluation of the rheology of cement-based materials could be done with simple field tests like slump, v-funnel test, etc. However, it is possible to conduct more complex analyses with viscosimeters and rotational rheometers in the laboratory. In its current state technology, these types of equipment induce shear rate with various methods like rotational or oscillatory and measure the time-dependent response of the materials. These equipments provide sensitive analysis regarding material rheology. However, the methodology is very prone to errors such as material segregation and slip phenomena[30]. Both static behavior (hydrodynamic interactions)[17] and dynamic behavior (response to the rotational stresses) of the material might induce a water-rich layer at smooth walls of the measuring system due to the segregation of cement particles. This results with inaccurate measurements which do not represent the actual behavior of the cement paste suspension [30]. Among all the measuring geometries vane geometry is the most efficient in terms of eliminating the slip since it provides a localized shearing within the paste and procuring less disruption of matrix[30]. However, it might be possible to observe experimental errors similar to slip in very stiff mixes, where there

is an air gap between the paste and vane surface leading to lower viscosity measurements [31].

2.2 Rheology Modifying Admixtures for Cement-based Materials

The rheological properties of cement-based materials can be controlled by the mix design such as volume fraction of the binders, type, and composition of the binder, presence of supplementary cementitious materials (SCMs); in case of mortar and concrete aggregate size and shape can be effective. Admixtures such as superplasticizers and viscosity modifying agents (VMAs) are now regularly used in concrete to control the workability. Admixtures have been used in the cementitious materials even in earlier centuries which enabled the development of currently used modern, complex polymers. Sumerians used bitumen as an organic binder and using clays as a water repellent at 3000 B.C. [32]. Romans, on the other hand, were able to identify and use chemicals to modify cement-based materials. Yet, the best-known example is Vitruvius (84-10 B.C.) used animal proteins for set retardation of gypsum and dried blood for air-entrainment [32]. But today, at the current state of science and technology, scientists can present much more fundamental categorization and parameters over the synthetic and bio-based admixtures. The list of admixtures is extensive depending on the required concrete properties. Herein, we will only focus on admixture on those that are used to modify the rheological properties.

2.2.1 Synthetic Admixtures

The deformation and flow behavior of cement pastes becomes more complex with the presence of admixtures. For providing adequate workability without sacrificing from performance, it will be critical to alter rheological properties through using suitable

admixture, or combination of more than one admixture. Most of these synthetic admixtures are counted to be chemically inert without interfering hydration reaction. However, they induce physicochemical consequences, such as superplasticizers can affect hydration by inducing particle repulsion. The most commonly used synthetic rheology modifiers are superplasticizers, a.k.a dispersing agent. Superplasticizers can be lignosulfonate (LS), polynaphthalene sulfonate (PNS), and polycarboxylate ether (PCE) based additives reducing the yield stress and viscosity of materials at constant binder to water ratio [33]. Most of these dispersing agents are surfactants and work through either electrostatic repulsion, steric effects, or both [33]. The most commonly used superplasticizers are PCE based additives and these polymers have an anionic backbone with hydrophilic side chains. The backbone is absorbed on the particle whereas the side chains prevent the particle interaction through steric hindrance.

Another rheology modifying agent used in cement-based materials is VMAs. VMAs are used to induce higher particle flocculation resulting in an improvement in water retention, and reduction in risk of segregation particularly for highly flowable concretes. VMAs can increase the yield stress of the material and reduce the deformation due to the weight of the material. The most commonly used VMAs in cementitious mixes are cellulose-ether derivatives (welan gum and diutan gum) and anionic polyacrylamides. While polyacrylamides are synthetic, welan gum and diutan gum are bio-based additives. VMAs increase the yield stress by entrapping mixing water as well as simultaneously being adsorbed on several cement particles and bridge them. The efficiency of VMAs is governed by their molecular weight and affinity with the cement particles [34], [35].

2.2.2 Mineral Admixtures

In general, mineral admixtures are used to improve both the fresh and hardened state performance of cement-based materials through improving workability, strength, flow capacity and durability. Besides the increasing demand for mineral admixtures due to sustainability and economic considerations, recent studies showed that the incorporation of mineral additives can be used as a rheology modifying agent [36], [37].

Mineral additives are ground insoluble materials from natural sources and by-products [38]. Though each mineral possesses unique morphology, particle size, shape, and texture which causes diversity in solid volume concentration, particle size distribution, interparticle spacing and solid particle network [9]. It should be noted that high range water reducers like superplasticizers are commonly used in cement-based materials to provide adequate flow capabilities to the material instead of increasing the water to cement ratio of mix. Thus, it is highly important to evaluate the compatibility of viscosity modifying agents or mineral admixtures compatibility with plasticizers.

Fine inorganic particles that hold high surface area are recognized as VMA since they increase the fine particle content and thixotropy[3]. Ferraris et al.[27] studied the influence of mineral additives on the rheological performance of cement pastes. The target performance was to improve workability by incorporating ultra-fine fly ash addition; and samples exhibited a decreasing yield stress results without using superplasticizers inside mixture [27]. Materials like silica fume have a high surface area and increase water demand, thus enhances the water retention capacity of material [3]. Park et al. [39]studied the rheological effect of blast furnace slag, silica fume resulted with decreasing yield stress and plastic viscosity. Libre et al.[40] studied the incorporation of limestone and fly ash on improving workability. On the contrary, addition of silica

fume enhanced yield strength and plastic viscosity through densifying the microstructure and increase the particle to particle interaction [39]. Increasing pozzolan content with metakaolin addition having a high surface area in cement grouts enhanced yield stress, viscosity, and flow time of material [41]. Similarly, Mansour et al.[42] showed that increasing metakaolin content in cement pastes results with an increase in the flow of pastes; which was attributed to the filler effect of metakaolin.

Metakaolin is a calcined clay and a commonly use VMA in cement-based systems. Clays are hydrophilic, which can absorb water and induce a more flocculated structure. Among the different families of clays, attapulgite, bentonite, kaolinite, and sepiolite are known as rheology modifiers for cement-based materials [43]–[45]. These clay minerals induce shear thinning behavior due to the presence of strong opposing surface charges which could be broken down at high shear rates. Melo et al.[46] studied the combined effects of sepiolite and metakaolin on rheology of cement paste. The results showed that the incorporation of sepiolite and metakaolin increased the water retention capacity resulting with an increase in yield stress. This action was attributed to the change in pore radius and increasing matrix density [46]. Also, Jarabo et al.[47] presented improved rheological properties of the mixture by incorporating acicular, fibrous sepiolite. It was stated the addition of sepiolite provided additional floc formation inside the matrix. Additionally, incorporation of natural zeolite onto grouts presented pseudo-plastic behavior while increasing yield stress and plastic viscosity [48]. Hassanzadeh and Sun [49] studied the rheological impact of diatomaceous earth (DE) in cement-based materials. It was found that when incorporation of DE increased the viscosity of the material.

2.2.3 Bio-Based Agents

Viscosity modifying agents are capable of increasing the cohesiveness of cement-based materials. When VMAs are used with HRWRs, the mixture can present high flowability yet decreases the material separation, segregation, and bleeding tendency due to VMAs[50]. It should be noted that apart from the aimed performances, the compatibility of the admixture with various materials inside the matrix, controls the demand on the market. Bio-based admixtures are referred to as the materials which are biopolymers or products of biotechnological processes [32]. The majority of bio-based admixtures are preferred within cement-based matrixes but other than that, painting and coating materials, gypsum wallboards, joint fillers, and grouts also benefit from the advantages these admixtures provide [32].

The most common type of VMAs is synthetic polymers and biologically derived polysaccharides[51]. Polysaccharides are water-soluble polymers that are used as VMAs[3]. The fermentation of the biological admixtures is commonly from bacteria or fungi, but it is necessary to point out on some occasions, these natural products can be chemically modified to gain adequate properties like adjustment of molecular weight[32], though it adds additional cost to the process.

Water-soluble polysaccharides bind the mix water and increase the viscosity of water[3]. The concentration of the polymers depends on the type of admixture; therefore, each bio-based additive inhibits unique behavior [48]. There is no specific limitation on the size of bio-admixtures, but the relative activity of materials proved to differ related to its chemical structure, chain length, and molecular weight. The investigations on bio-based viscosity modifying agents reveal 3 vital action mechanisms, that are accepted throughout the literature: adsorption, association, and intertwining[3], [51]. Adsorption

mechanism is referred to as the hydrophilic part of the polysaccharide, constituting hydrogen bonding with the free water molecules inside the material; thus, increasing the viscosity of water and matrix. The association mechanism is dedicated to the attractive forces between adjacent polysaccharide chains while disabling the motion of water when creating a gel-like structure to increase the viscosity. And lastly, intertwining arises with the initiation of shear forces, causing long chains of polysaccharides to entangle, leading to higher viscosity [51], [52]. Apart from these three mechanisms, some bio-VMAs have hydroxyl and carboxyl side groups that are capable of binding with Ca^{2+} ions of cement thus further enhancing the rheological properties of cement-based materials. But when superplasticizers are absorbed onto cement particles, it decreases the surface tension of water, competes over the Ca^{+2} bonding with polysaccharides and peptidoglycans [52]. However, it should be noted that these action mechanisms which enhance the viscosity of cement-based systems, can be broken inducing shear-thinning behavior of cement-based materials. Commonly used bio-based admixtures are explained briefly as below.

- Sodium gluconate, commonly used as set retarders for cement, has a small molecular size but negatively affects the early strength of concrete [32], It has the capability to enhance the dispersion effect when used with superplasticizers in cement-based systems[53].
- Lignosulfonate is a salt produced from processing wood waste, that acts as a retarder [54]. It has a macromolecular form, can enhance the flowability of cement-based systems [32].
- Cellulose ether is a polysaccharide commonly used as a water-retention agent. CE improves workability and can be used as VMA for cement-based systems [36]. Rheological studies conducted to observe the influence of cellulose ethers suggests improved particle network due to CE, thus improved apparent viscosity results of pastes

[55]. It increases the adhesiveness, air-entrainment, and yield stress of materials[32]. Cellulose ethers are derived from cell walls of plants [51].

- Chitosan is a bio-admixture derived from the exoskeleton of crabs and shrimps known as crustaceans [51]. Incorporation of chitosan inside the cement pastes and mortars, improved cohesion capacity of material, and enhanced viscosity and consistency to pastes.[56], [57]. But, Commonly used polysaccharide cellulose ethers are reported to have high product cost, as chitosan is specified to have 75% high-priced over cellulose ether [51].
- Xantham gum is a natural polymer[3], that has a high molecular weight, derived from *Xanthomonas campestris* with an anionic structure [58]. Apart from mortar and concrete, Xantham gum is also used inside injection grouts[32]. It can enhance the yield stress and viscosity of paste when incorporated inside the matrix[58]. Xantham gum produces shear-thinning property of cement-based matrixes while reducing the bleeding and segregation of material[32].
- Diutan gum is microbial polysaccharide [52], fermented from *Sphingomonas* sp. [58], possesses an anionic character [59]. It has a higher molecular length than welan gum, both compatible with cement-based systems so diutan gum is also studied to evaluate the ability to enhance the bleeding and segregation capacity of mixture[59]. They are stated to be efficient when used as VMA, act as a thickening and stabilizing agent [52], [58]. Since it tends to increase water retention capacity as well as yield stress and the viscosity [58], [59].
- Welan gum is a bacterial polysaccharide; a fermentation product of bacterium *Sphingomonas* species with an anionic charge [51]. The molecular weight of welan gum is stated to be higher than diutan gum [59]. Welan and Diutan gum are studied to prove their efficiency as VMA while presenting stabilizing and cohesive effect on cement pastes

[51], as well as both having stable behavior at a high pH environment of cement-based systems [52]. High demand on both welan and diutan gum is the proof of effective use for the rheological performance of cement-based materials while being among the most expensive admixtures [51].

- Starch is a polysaccharide that can enhance the viscosity of cement-based materials [32]. Starch is a non-ionic compound and possesses a similar chemical structure to cellulose[52].
- Nopal mucilage and marine brown algae were proposed as an efficient viscosity-enhancing admixture. The addition of bio-polymers provided stable, cohesive, and homogeneous SCC [60].
- Direct addition of *Sporocarcina pasteurii* cells onto mixture water, increased the viscosity and yield stress of cement pastes. The addition of cells in cement paste indicated compatible results with superplasticizer as well as fly ash [31].
- *Bacillus megaterium* and *Bacillus subtilis* cells presented efficient results on improving the rheological performance of cement pastes, when directly added to mixture water. Both cells presented compatibility with superplasticizer, thus the efficiency of *B.megaterim* was also stated with fly ash incorporated pastes [61].
- *P.polymyxa* cells and biofilms of *Bacillus amyloliquefaciens* were directly added to mix water of cement paste and enhanced the rheological performance of cement pastes while being compatible with superplasticizer and fly ash[62].
- Extracellular polysaccharide(EPS) derived from *Paenibacillus* species was used in cement-based mortars to evaluate its efficiency as VMA. Results have shown the capability of EPS on preventing segregation and air entrapment while providing homogeneous structure [63].

- The bacteria cell wall of *Bacillus subtilis* was used to study the rheological effect on cement pastes due to the peptidoglycan component. Cell walls were found efficient in terms of increasing the viscosity. Cell walls of *B.subtilis* possess anionic charge and water-retention capacity[51].

It should be noted that the advanced technologies of the construction industry base on 4 major components: cost, reduced labor activity, workability, and environmental effects. With the unique properties' bio-admixtures offer, they have solid potential to surpass the market demand of synthetic polymers.



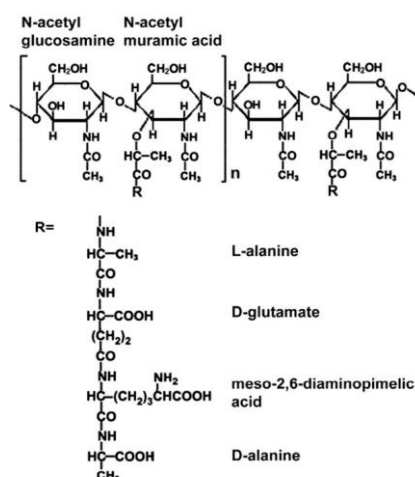
CHAPTER III

3.1 Introduction

As mentioned in Chapter 2, the interaction of VMAs targets the water and cement particle interaction inside the matrix by triggering mechanisms of adsorption, association, and intertwining. These 3 types of action mechanisms can increase the viscosity at low shear rates and mostly exhibit shear-thinning behavior in cement-based materials with increasing shear rate.

The working mechanism of well-known bio-based VMAs such as welan gum and xantham gum were known to be based on their high molecular weight[64]. Diutan gum has been used as a viscosity modifier for cement-based grouts by increasing the pseudoplastic behavior of the material [59]. The assessment of chitosan presented a satisfying behavior as a viscosity modifier also due to its high molecular weight [57]. The successful experiments based on using these bio-based VMAs proves their ability to resist the pH environment of cement-based materials. The molecular structure of peptidoglycan, welan gum, and diutan gum demonstrate a chemical structure with long-chain polysaccharide backbones and their side groups (*see* Figure 3.1). Due to this molecular structure, these bio-based molecules can increase the viscosity by bonding with cement particles with their hydroxyl and carboxyl groups as well as adsorption, association, and intertwining of chains in the water phase [51].

a) Peptidoglycan



b) Welan gum and diutan gum

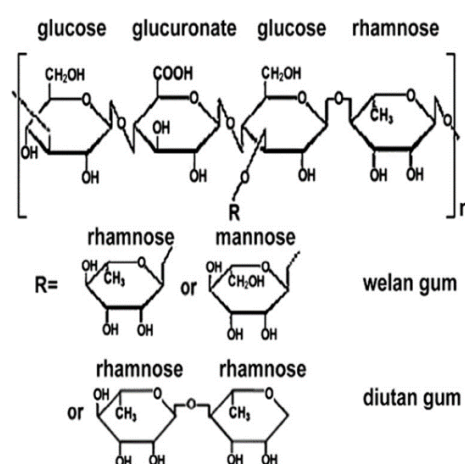


Figure 3.1: Representation of the chemical structure of (a) peptidoglycan and (b) welan and diutan gum [51].

A novel alternative to this bio-based VMAs could be bacterial cells and biofilms formed through these bacterial cells. Similarly, these cells and biofilms present long polysaccharide chains and peptidoglycan backbones, which can resemble the behavior of VMAs.

Biofilms are defined as surface-attached multicellular communities, which happen to resemble polymer gel. It is composed of exopolysaccharides, water, ions, proteins released from bacterial cells [65]. These biological structures have high molecular weight and large surface areas, which made them particularly interesting as VMAs. However, previous studies also suggested that separating EPS from bacterial cells is a labor-intensive process since EPS and biofilms were insoluble, [66]. Even so, polysaccharides are proven effective regarding the enhancement of rheological behavior when used as VMA inside cement-based materials. This chapter summarizes an experimental study where *Peanibacillus polymxa*, *Bacillus amyloliquefaciens* cells, and biofilms of these species were used as bio-VMAs in cement paste.

3.2 Materials and Methods

3.2.1 Bacterial Growth of EPI 60 (*Bacillus amyloliquefaciens*)

B. amyloliquefaciens cells (EPI 60) were first cultured on sterilized solid modified nutrient medium. The cells were inoculated on nutrient agar plates containing yeast extract (5 g/L), sodium chloride (NaCl, 5 g/L), peptone (10 g/L), and agar powder (12.5 g/L) at 37°C for overnight. The pH of the solid medium was adjusted to 7. Then, the cells were collected from the plates and suspended in 10 mL of liquid modified nutrient medium with 15% glycerol solution. The liquid modified nutrient medium contained peptone from caseine (10 g/L), NaCl (5 g/L), and yeast extract (5 g/L). The pH of the liquid medium was also adjusted to 7. Then, the cells were stored at -20°C until testing. In case where bacterial cells were needed, the frozen culture was incubated liquid modified nutrient medium.

Biofilm was cultured in a medium containing wheat (160 g), bran (103 g), 10 mL corn steel liquor, potassium phosphate (10 g), calcium chloride (0.5 g), sodium chloride (0.5 g) and glucose monohydrate (9.5 g) to 1-liter DI water. First, the nutrient medium was sterilized at 121° C for 45 minutes. Then, the medium was poured in sterilized SS 304 stainless steel plates (Ø: 11.8 cm). A 100µL of inoculum was spread on the plated and cultured at 37°C for 6 days. Then, the biofilm was collected from the plated by sterilized spatulas.

3.2.2 Bacterial Growth of EPI 61 (*Peanibacillus polymyxa*)

P. polymyxa (EPI 61) is gram-positive bacteria, which has thick cell walls containing peptidoglycans and polysaccharides [67]. In addition, *P. polymyxa* cells can also produce extracellular polysaccharides (EPS) during growth, which might also

contribute to the VMA mechanism. The cells were grown on modified nutrient medium agar plates containing peptone from caseine (10 g/L), NaCl (5 g/L), and yeast extract (5 g/L). The pH of the liquid medium was also adjusted to 7. Then, the cells were collected from the plates, suspended in 10 mL of liquid modified nutrient medium with 15% glycerol solution, and stored at -20°C until needed.

When EPI 61 culture was needed, the cells were inoculated from the frozen stock on a specified growth medium. *P. polymyxa* cells were cultured in a sterilized nutrient medium including corn-steep liquor (CSL, 12.5 g/L), K₂HPO₄ (5 g/L), sucrose (10 g/L) and agar (12,5 g/L). The pH of the medium was adjusted to 7. The cells were cultured on solid media for 72 hours at 30°C. Then, the cells were collected from the plates and used in the experiments within the following 3 days.

3.2.3 Biofilm Formation on Solid Medium

To grow the biofilm, EPI 60 strain was first cultured on solid agar plates containing yeast extract (5 g), NaCl (5 g), peptone (10 g), and agar powder (12.5 g) to 1-liter DI water and pH was adjusted to 7. EPI 60 cells were grown on sterilized solid medium. Then, they were collected from the plates and suspended in 10 mL of 15% glycerol solution. Then the cells were stored at -20°C until testing. Biofilm was cultured in a medium containing wheat (160 g), bran (103 g), 10 mL corn steel liquor, potassium phosphate (10 g), calcium chloride (0.5 g), sodium chloride (0.5 g) and glucose monohydrate (9.5 g) to 1-liter DI water. First, the nutrient medium was sterilized at 121 °C for 45 minutes. Then, the medium was poured in sterilized SS 304 stainless steel plates (Ø: 11.8 cm). A 100µL of inoculum was spread on the plated and cultured at 37°C for 6 days. Then, the biofilm was collected from the plated by sterilized spatulas and used

in the experiments within the following 3 days. Abovementioned EPI 60, EPI 61 cells and biofilms were produced by Episome Biotechnologies.

3.2.4 Preparation of Cement Paste Samples

The cement paste samples were prepared with Portland cement CEM I 32.5 N with a Blaine fineness of 500-5500 cm²/g according to the norm EN 197-1. Three different water to cement ratios (w/c) as 0.36, 0.43, and 0.50 were used. To assess the compatibility of bio- VMAs with superplasticizer and fly ash, a polycarboxylate superplasticizer (by 0.33 kg/kg cement) and F-type fly ash (by 15% of the cement weight) was added to cement pastes. The 45 µm sieve residue of the fly ash was reported as 29%. The number of cells was adjusted in terms of percent weight of cement, such as 0.10%, and 0.30% by cement weight. The cement paste samples were prepared according to ASTM C305-14 Standard Practice for Mechanical Mixing of Hydraulic Cement Pastes and Mortars (ASTM C305 2011). The cells were added to mixing water prior to mixing, homogenized with hand mixing for the 30s, and then mixed with cement. In case of superplasticizer addition, the cells were added to the initial 2/3rd of the mix water and the superplasticizer was added to the 1/3rd of the mix water. The last 1/3rd portion of the mix water including superplasticizer was added during the last 60s of the mixing. In total, the 4-minute mixing procedure of ASTM C305-14 standard was followed to 81 cement paste samples in total.

Table 3.1 Mix design of samples. EPI 60: *B.amyloliquefaciens*; EPI 61: *P.polymyxa*; FA: Fly Ash; SP: Superplasticizer. Control samples: No additive; 0.3% of superplasticizer incorporation; 15% replacement of cement with fly as by cement weight. w/c: 0.36, 0.43 and 0.50.

Sample Name	Type of bio-VMA (% by weight of cement)				Additives(% by weight of cement)	
	<i>B. amyloliquefaciens</i>	<i>P. polymyxa</i>	<i>B. amyloliquefaciens</i> biofilm	<i>P. polymyxa</i> biofilm	Superplasticizer	Fly ash
Control_ (w/c)	-	-	-	-	-	-
Control_SP_ (w/c)	-	-	-	-	0.33	-
Control_FA_ (w/c)	-	-	-	-	-	15
EPI 60_0.1%_ (w/c)	0.1	-	-	-	-	-
EPI 60_0.1%_SP_ (w/c)	0.1	-	-	-	0.33	-
EPI 60_0.1%_FA_ (w/c)	0.1	-	-	-	-	15
EPI 60_BF_0.1%_ (w/c)	-	-	0.1	-	-	-
EPI 60_BF_0.1%_SP_ (w/c)	-	-	0.1	-	0.33	-
EPI 60_BF_0.1%_FA_ (w/c)	-	-	0.1	-	-	15
EPI 60_0.3%_ (w/c)	0.3	-	-	-	-	-
EPI 60_0.3%_SP_ (w/c)	0.3	-	-	-	0.33	-
EPI 60_0.3%_FA_ (w/c)	0.3	-	-	-	-	15
EPI 60_BF_0.3%_ (w/c)	-	-	0.3	-	-	-
EPI 60_BF_0.3%_SP_ (w/c)	-	-	0.3	-	0.33	-
EPI 60_BF_0.3%_FA_ (w/c)	-	-	0.3	-	-	15
EPI 61_0.1%_ (w/c)	-	0.1	-	-	-	-
EPI 61_0.1%_SP_ (w/c)	-	0.1	-	-	0.33	-
EPI 61_0.1%_FA_ (w/c)	-	0.1	-	-	-	15
EPI 61_BF_0.1%_ (w/c)	-	-	-	0.1	-	-
EPI 61_BF_0.1%_SP_ (w/c)	-	-	-	0.1	0.33	-
EPI 61_BF_0.1%_FA_ (w/c)	-	-	-	0.1	-	15
EPI 61_0.3%_ (w/c)	-	0.3	-	-	-	-
EPI 61_0.3%_SP_ (w/c)	-	0.3	-	-	0.33	-
EPI 61_0.3%_FA_ (w/c)	-	0.3	-	-	-	15
EPI 61_BF_0.3%_ (w/c)	-	-	-	0.3	-	-
EPI 61_BF_0.3%_SP_ (w/c)	-	-	-	0.3	0.33	-
EPI 61_BF_0.3%_FA_ (w/c)	-	-	-	0.3	-	15

3.2.5 Rheological Measurements

The rheological measurements were conducted by ANTON PAAR Rheoloab QC rotational rheometer (Anton Paar, Graz, Austria) at room temperature (23°C). The measurements were conducted using a coaxial vane geometry. The data output and analysis were based on AntonPaar Rheoplus software.

After samples were prepared at Hobart Mixer, a mixing process applied at 100 s^{-1} for 60 seconds to ensure homogeneity and minimize the possible errors. After reinstating the homogeneity, the measurement proceeded with a constant shear rate of 100 s^{-1} for 60 s described as a pre-shear stage. The rheological evaluation is based on a decreasing shear

rate analysis from 100 s^{-1} to 1 s^{-1} covering the viscosity and shear stress measurements of this downward curve. The yield stresses and viscosity results are recorded where the rheological behavior of cement pastes was evaluated with the Bingham model (1).

$$\tau = \tau_o + \mu \dot{\gamma} \quad (1)$$

Where τ_o is the yield stress (Pa), μ is the plastic viscosity (Pa.s), and $\dot{\gamma}$ is the shear rate (s^{-1}).

3.2.6 Mini-Slump Test

For the measurements of the initial spread of cement pastes, a cone-shaped mold filled with samples and placed on smooth plexiglass. The cone is specially manufactured stainless-steel cone with a height of 57.2 mm, an upper diameter of 19 mm, and a lower diameter of 38.1 mm [3]. Figure 3.2 shows the schematic of the mini-slump cone. After filling the samples into the cone placed on the center of plexiglass immediately after mixing, the cone was lifted to measure the flow. The diameters at four right-angle positions were measured and the average diameter was calculated.

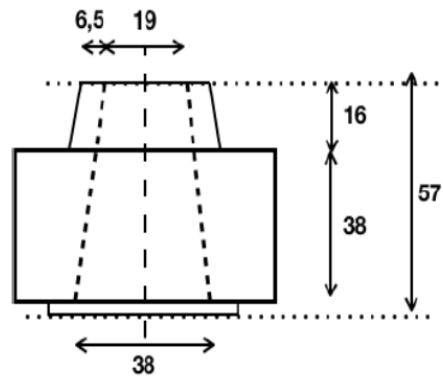


Figure 3.2: Schematic of the mini-slump cone test.

3.3 Results and Discussion

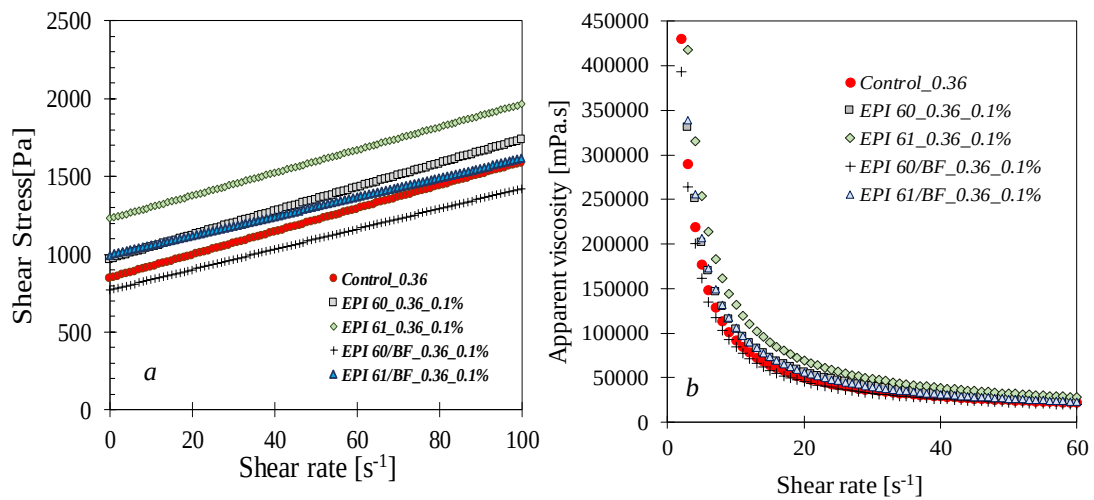
3.3.1 Influence of Bio-VMAs on the Rheology of Cement-paste

This study was undertaken to evaluate the influence of *B.amyloliquefaciens* (EPI 60), *P.polymyxa* (EPI 61), and their biofilms as bio-VMAs for cement paste samples. The rheological performance assessment included the viscosity and yield stress evaluation followed by mini-slump tests at 3 different w/c's. Our results showed that biofilms were more efficient as bio-VMAs in terms of increasing yield stress and viscosity.

Figure 3.3 summarizes the shear and viscosity curves for cement paste samples containing EPI 60, EPI 61, and their biofilms at 3 different w/c's. The cell and biofilm dosages were kept at 0.1% of cement weight. The remaining viscosity curves regarding chapter 3 were presented in Appendix A. All cement paste samples exhibited a shear-thin and the rheological behavior was evaluated based on the Bingham model as it was stated in Section 3.2. Yield stress values were calculated from the intersection of the regression line and the viscosity was defined by the slope of the line. Table 3.2 summarizes the yield stress and mini-slump values obtained from cement-paste samples at 3 different w/c's.

Even though the EPI 61 cells were found to be effective in terms of increasing the yield stress of the material at low w/c (0.36), their efficiency was reduced by increasing w/c. At high w/c (0.50), the only biofilm obtained from EPI 60 was effective in increasing yield stress. Compared to EPI 60 cells, EPI 61 was found to be a more effective bio-based agent when only the increase in yield stress was considered. EPI 60 was a gram-positive bacteria *B. amyloliquefaciens* having a close affinity with *Bacillus subtilis* as well as having long chains of molecules [68]. Whereas, EPI 61 was an anaerobic, gram-positive bacterium *P.polymyxa*, which is stated to have a high production rate of exopolysaccharide substances (EPS) with high molecular weight and complex structure

[69]. Bacterial polysaccharides are known to have high water solubility, inhibiting enhanced viscosity, thickening, and stabilizing effect when incorporated inside the matrix [70]. Biofilms are microorganism product, have the potential to become an important bio-VMA since they possess a three-dimensional microbial-derived extracellular matrix, enables adhesion to surfaces [71]. Biofilms are composed of both cells [72] and predominant contents of key macromolecules such as extracellular polymeric substances (EPS), proteins, peptidoglycan, etc. and water [66], [73]. These compounds forms communities and these communities are presented to range between micrometers to several centimeters[65]. In fact, while EPI 61 was effective when it is used as bacterial cells at low w/c, while it was more effective as a biofilm with increasing w/c. This shows that it is possible to use both intercellular and extracellular polysaccharides can be used as bio-VMA if they exist in cell structure. EPS were more effective in highly flowable mixes, which was attributed to their higher molecular weight.



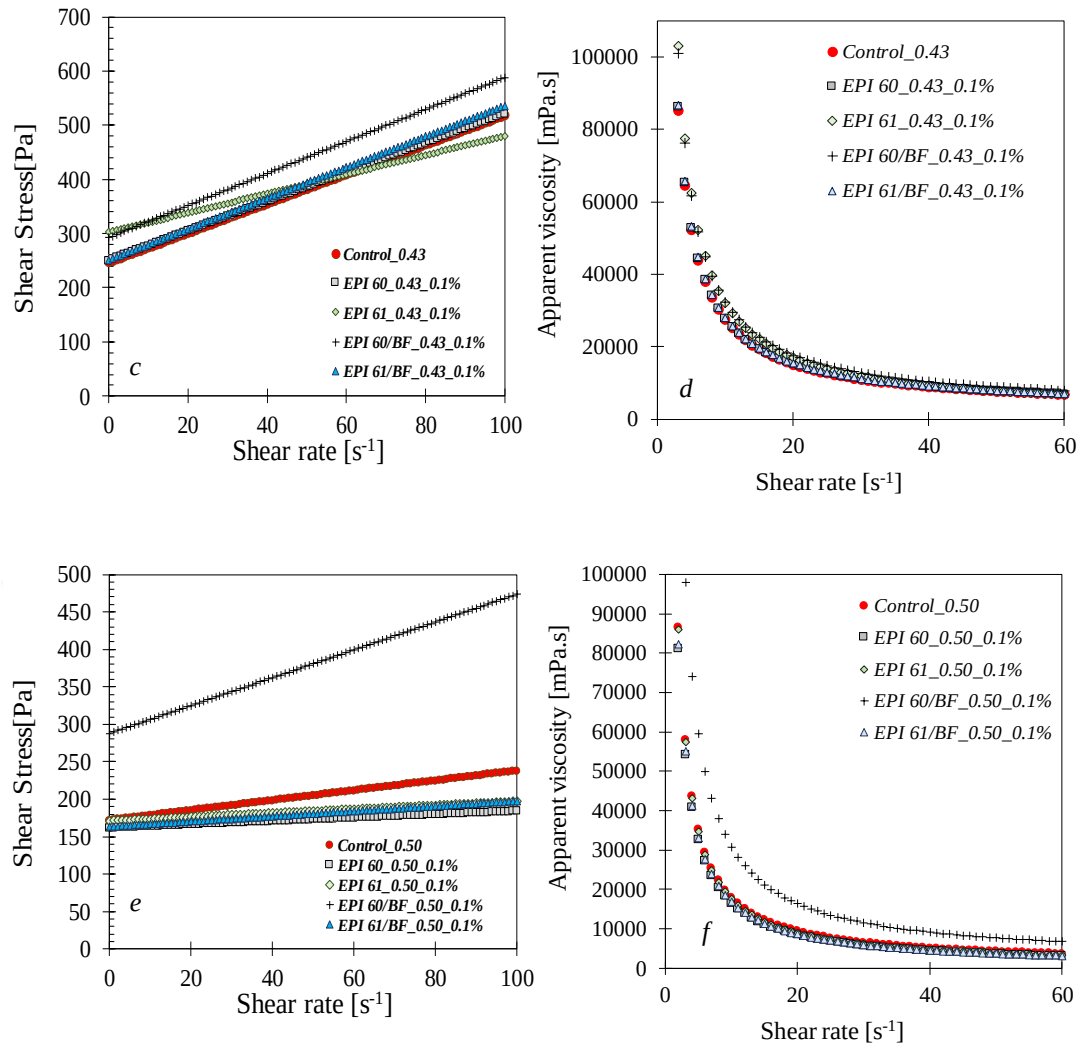


Figure 3.3 Rheological test results for cement paste samples. (a), (c), (e) Shear stress response with increasing shear rate; (b), (d), (f) Change in viscosity with increasing shear rate. Control sample: No additive; EPI 60: *B.amyloliquefaciens*; EPI 61: *P.polymyxa*; BF:biofilms ; VMA: 0.1% by cement weight; w/c: (a),(b)0.36, (c),(d) 0.43 and (e),(f) 0.50.

Besides the rheological tests, the workability of the mixes was evaluated by a mini-slump test [74]. At low w/c, the slump value of the samples was rather inconsistent such that there was an increase in the slump value of samples where there was an increase in yield stress. Even though incorporation EPI 61 cells and biofilm resulted with an increase in yield stress compared to the control sample, the presence of cells did not influence the mini-slump value compared to their counterpart control neat paste samples. Throughout the literature mini-slump test was correlated with the yield stress of the

material. With the incorporation of a VMA, it was expected to observe a decrease in mini-slump diameter along with an increase in yield stress. The difference in the mini slump test and rheological analysis could be attributed to the difference in applied shear rates. The mini-slump cone test was employed with a significantly lower shear rate than that was imposed during the rheological analysis [5]. Considering the pseudo-plastic behavior of cement paste, the material response to these two different shear modes and rates was not the same. An increase in w/c leads to a more flowable paste along with a more consistent slump reading. For samples having w/c's of 0.43 and 0.50, incorporation of bio-VMAs also decreased the slump diameter even there was not any change in the yield stress (i.e. EPI 60/BF_0.50_0.1%). The slump results also suggest that the proposed bio-VMAs resemble VMA characteristics.

Table 3.2 Yield strength and average slump values of cement paste with 0.1% of bio VMA addition by cement weight. Control sample: No additive; EPI 60: *B.amyloliquefaciens*; EPI 61: *P.polymyxa*; BF: biofilms; w/c: 0.36,0.43 and 0.50

Sample Name	Mini Slump (cm)	Yield Stress [Pa]
Control_0.36	4,1	845,4
EPI 60_0.36_0.1%	4,5	968,2
EPI 61_0.36_0.1%	6,0	1231,6
EPI 60/BF_0.36_0.1%	4,0	772,9
EPI 61/BF_0.36_0.1%	4,0	997,3
Control_0.43	5,5	247,2
EPI 60_0.43_0.1%	5,4	250,2
EPI 61_0.43_0.1%	5,5	302,9
EPI 60/BF_0.43_0.1%	5,3	293,0
EPI 61/BF_0.43_0.1%	5,2	251,7
Control_0.50	7,5	171,7
EPI 60_0.50_0.1%	7,2	161,8
EPI 61_0.50_0.1%	7,0	171,5
EPI 60/BF_0.50_0.1%	7,4	288,3
EPI 61/BF_0.50_0.1%	7,8	163,8

At w/c of 0.43 with the addition of EPI 61 cells and EPI 60/BF also increased the yield stress compared to control samples, along with the biofilms used as bio-VMAs.

Interestingly, the presence of EPI 60 biofilms revealed its thickening effect with high dosages of w/c's. Han et al. [70] have shown promising results on using EPS extracted from *B.amyloliquefaciens* (EPI 60) as VMAs with high intrinsic viscosity results and dense entangled mesh structure. Thus, the observed results might be due to having a higher molecular weight and longer chains of polysaccharides of biofilm, which possess stronger molecular attraction.

Regardless of the bio-VMA used in the mix, the cement paste exhibited a shear-thinning behavior as expected. This might be due to the action mechanism of bio-VMAs such that incorporation of polysaccharides and peptidoglycans in the cells structure leading to higher water retention particularly at low shear-rates due to intertwining of polysaccharides and peptidoglycans chains [75]. Increasing shear rate might break the chains disabling the adsorption of water molecules [75] also causing the previously entrapped water molecules to be released. A similar observation at low shear rates was stated by Sonebi [59] with the addition of diutan gum, attributed to water absorption inside cement paste together with entanglement and intertwining of adjacent polymer chains [59].

Through the literature, shear rates of 17, 20.4, 27.2, and 34 s⁻¹ were used to analyze the behavior of cement-based materials observed in casting and placing of concrete [27]. To evaluate the performance of the mix at low shear rates, an analysis of apparent viscosity made at shear rates of 17 and 34 s⁻¹, which are particularly lower and upper boundaries of these specified shear rates. Figure 3.4 shows the apparent viscosity values in samples and Table 3.5 summarizes the relative increase in apparent viscosity for samples containing bio-VMAs compared to their counterpart control neat paste at shear rates of 17 and 34 s⁻¹. The results were in line with yield stress findings, where incorporation of EPI 61 cells in low w/c cement paste samples showed a higher apparent

viscosity values at shear rates 17 and 34 s⁻¹ whereas the addition of EPI 60 biofilm was more effective in increasing apparent viscosity with increasing w/c.

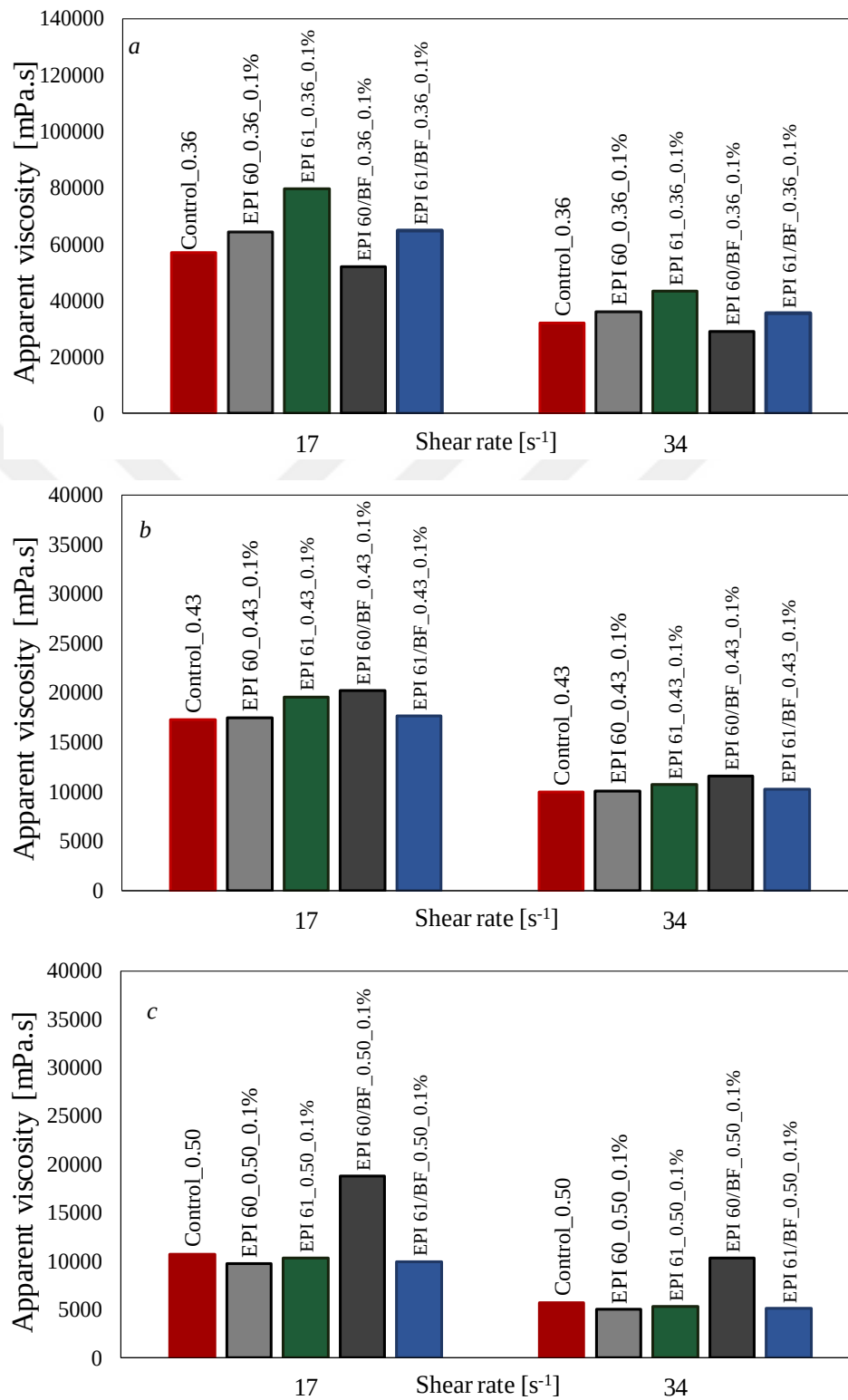


Figure 3.4: Apparent viscosity values for samples at shear rates of 17 and 34 s⁻¹. Control: No additive. EPI 60: *B.amyloliquefaciens*; EPI 61: *P.polymyxa*; BF:biofilms; VMA: 0.1% by cement weight; w/c: (a)0.36, (b)0.43 and (c)0.50

Increasing water content leading to a higher fluidity which may require a higher volume of polysaccharides for increasing the yield stress and viscosity. Biofilms contain syntrophic consortium of microorganisms where cells stick to each other and these adherent cells become embedded within a slimy extracellular matrix of EPS. Thus, it could be possible that for higher w/c both intercellular and extracellular polysaccharides might contribute to the mechanism. Biofilms can provide enhanced rheological performances for cement-based materials with high pH durability, ionic strength, and dense molecular structure [70]. Thus, increase molecular weight of polysaccharides and peptidoglycans and leading to a higher viscosity and yield stress values at lower shear rates. The structure of EPS in biofilms permits a variety of applications as biomaterials, rheology modifiers, and flocculants [76]. There is not any clear evidence why the effects of bacterial cells were more pronounced compared to biofilms at lower w/c. However, one possible explanation could be is that the working mechanism of EPS mainly governed by water retention particularly at low shear-rates due to the intertwining of polysaccharides and peptidoglycans chains. Thus, in a case where the water content is limited ($w/c < 0.40$), the effects of water retention on rheology could be limited. While the bacterial cells can also increase the water retention due to the presence of intercellular polysaccharide, they can also interact with calcium [Ca^{+2}] ions present in the environment due to their negative surface charges [77]. The cells could bind [Ca^{+2}] on the surface of cement particles. The results might suggest that at low w/c where water content is rather limited the working mechanism of these bio-VMAs mostly rely on cell-cement attraction whereas increasing water content leads to more predominant water retention by EPS matrix. The interaction between cells and cement-based matrix at low w/c should be further investigated.

To evaluate the influence of bio-VMA dosage on the rheological properties, the amount of bio-VMAs was increased to 0.3% by cement weight (see Figure 3.5). Herein, the response of the cement pastes presented different behavior compared to when bio-VMA was used 0.1% by cement weight. In contrast to what was observed at 0.1% bio-VMA addition at w/c of 0.36, the viscosity and yield stress were only increased when EPI 61 biofilm was added by 0.3% cement weight. Table 3.3 summarizes the yield stress and mini-slump values for samples containing 0.3% bio-VMA. The influence of the dosage was more pronounced in bacterial cells rather than biofilms. Such that, increasing the bio-VMA dosage to 0.3% from 0.1%, increased the efficiency of EPI 60 and 61 cells particularly at higher w/c's. This idea also supports that incorporation of bacterial cells governs 2 different mechanisms (i) surface charge attraction (ii) intertwining of chains. Thus, increasing dosage improves the efficiency of bio-VMAs. Whereas, increasing the dosage of biofilms did not affect their performance as it was in cells. This might suggest that biofilms work through increasing water retention which also depends on the availability of water phase.

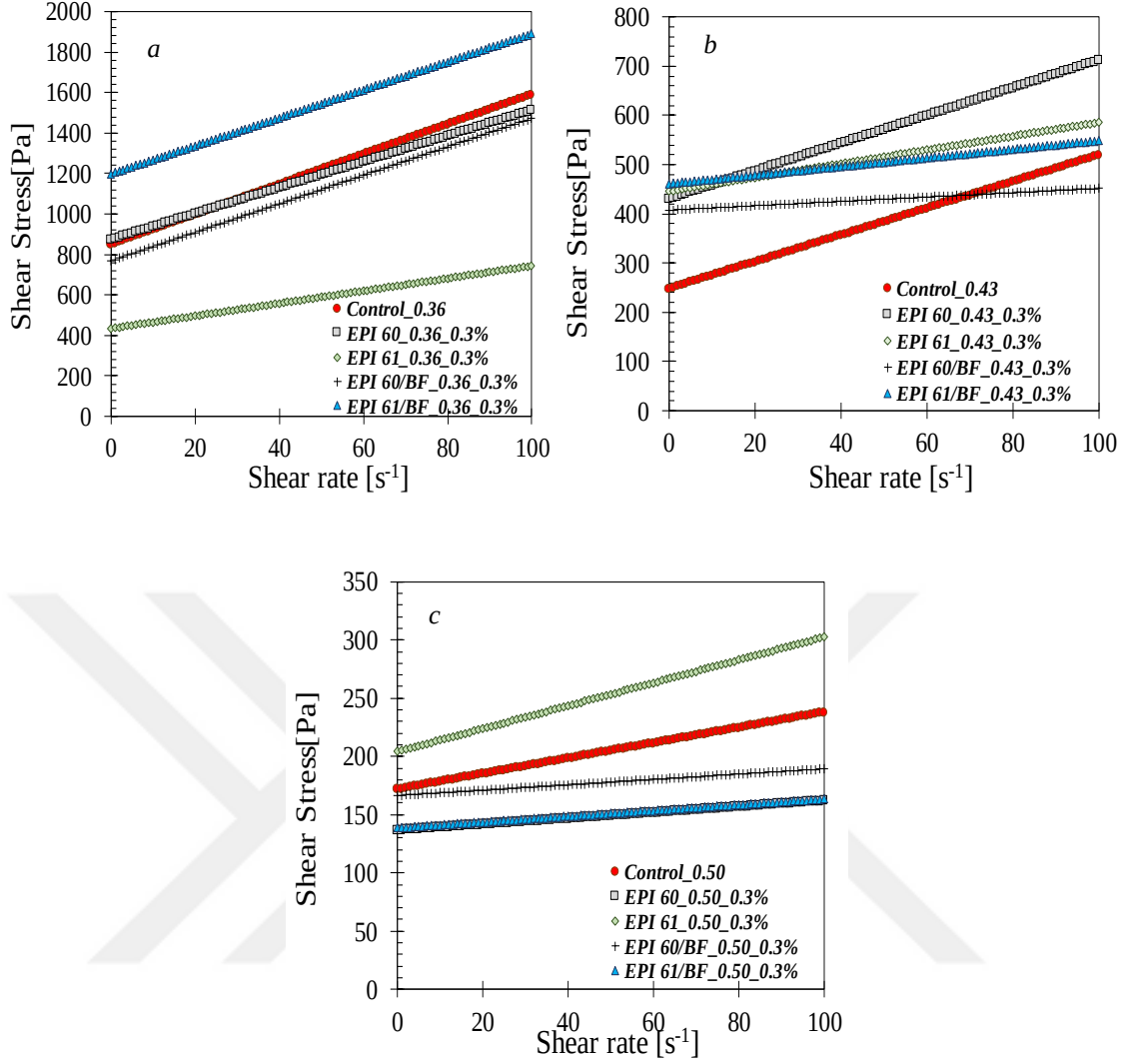


Figure 3.5: Rheological test results for cement paste samples. Control sample: No additive; EPI 60: *B.amyloliquefaciens*; EPI 61: *P.polymyxa*; BF:biofilms ; VMA: 0.3% by cement weight; w/c: (a) 0.36, (b) 0.43 and (c) 0.50.

While the incorporation of 0.1% EPI 61 cells significantly increased the yield stress at w/c of 0.36, increasing dosage to 0.3% completely resulted with an opposite behavior. In fact, the incorporation of cells reduced the yield stress and act as a dispersant. Further evaluations have to be done to understand if this is rather an experimental error for that specific bacterial culture inoculation. *P. polymyxa* cells, a.k.a EPI 61, was also used in our previous studies in which performance of 4 different cells was evaluated in terms of improving rheology. Similar behavior was observed when *P. polymyxa* cells were used in cement paste by 0.05, 0.1, and 0.5% by weight of cement [113]. Increasing the

dosage of *P. polymyxa* cells reduced the yield stress and viscosity even lower than the control neat paste at w/c of 0.36. Increasing w/c to 0.40 and 0.50 reduced this dispersive effect and the cells again resembled VMA behavior. This behavior was only observed in *P. polymyxa* cells while the rest of the strains were selected from *Bacillus sp.* A possible reason for this response could be an experimental error such that a low w/c increasing number of cells might increase the viscosity of the material. It might possible that the material might not be able to flow immediately around the rotating vane at low shear rates thus leaving an air gap between the vane and the matrix. The presence of this gap reduces the shear stress transfer to the surrounding cement paste thus resulting with both lower yield stress and viscosity, which interferes with the data measures. Further evaluations have to be done to understand the exact mechanism[78].

Table 3.3 Yield strength and average slump values of cement pastes with 0.3% of bio VMA addition by cement weight. Control sample: No additive; EPI 60: *B.amyloliquefaciens*; EPI 61: *P.polymyxa*; BF: Biofilms ; w/c: 0.36, 0.43 and 0.50.

Sample Name	Mini Slump (cm)	Yield Stress [Pa]
Control_0.36	4,1	845,4
EPI 60_0.36_0.3%	4,8	870,7
EPI 61_0.36_0.3%	4,9	434,0
EPI 60/BF_0.36_0.3%	4,1	767,6
EPI 61/BF_0.36_0.3%	4,0	1197,5
Control_0.43	5,5	247,2
EPI 60_0.43_0.3%	5,3	429,6
EPI 61_0.43_0.3%	4,9	446,2
EPI 60/BF_0.43_0.3%	5,1	407,9
EPI 61/BF_0.43_0.3%	5,20	461,5
Control_0.50	7,5	171,7
EPI 60_0.50_0.3%	7,1	136,5
EPI 61_0.50_0.3%	5,6	204,3
EPI 60/BF_0.50_0.3%	7,0	166,6
EPI 61/BF_0.50_0.3%	7,6	138,9

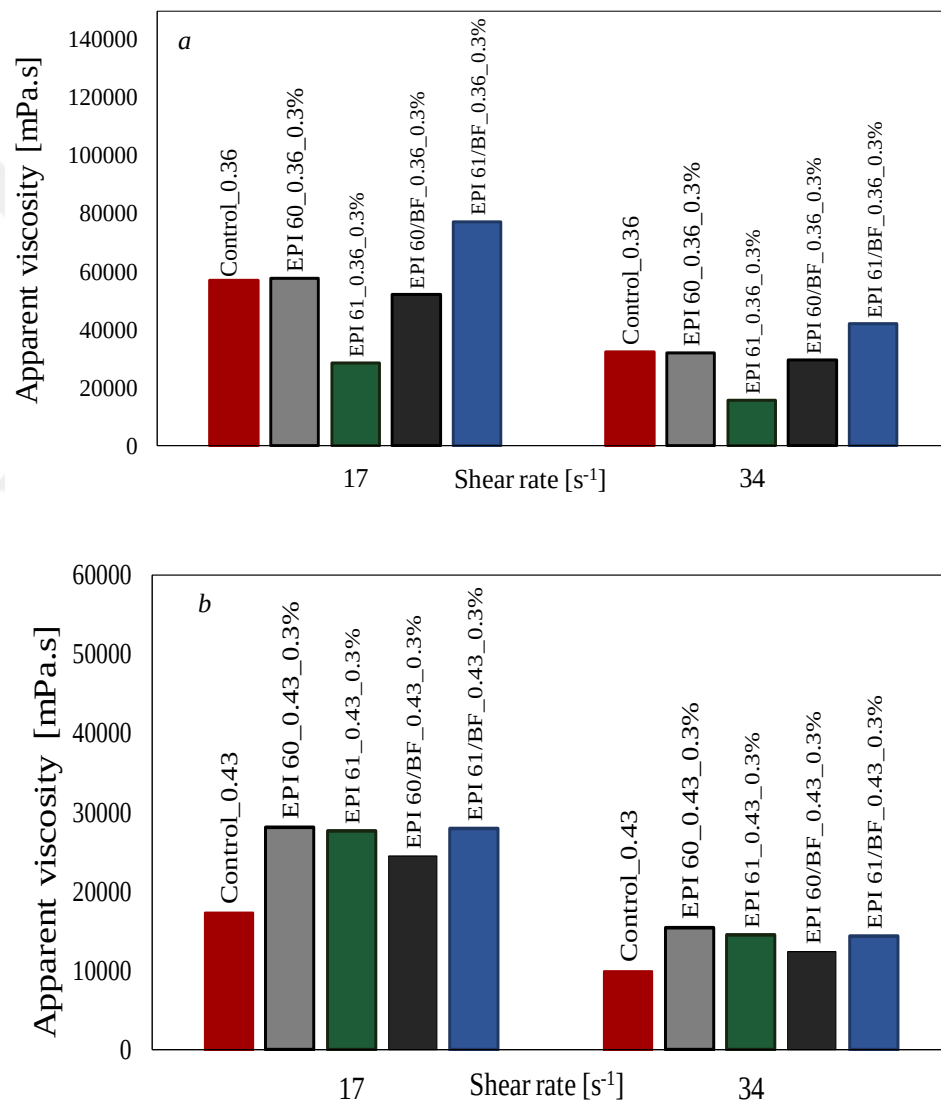
Figures 3.6 presents the influence of the EPI 60 (*B. amyloliquefaciens*) and EPI 61 (*P.polymyxa*) cells and biofilms on apparent viscosity at 17 and 34 s⁻¹. As expected,

the behavior of samples was in line with the increase in yield stress. At low w/c (0.36), incorporation of EPI 61 Biofilm by 0.3% of cement weight resulted with approximately 35% increase in viscosity compared to neat control paste. At low w/c, the action mechanism of peptidoglycan and polysaccharides structure of cells exhibits a high-water retention capacity based on chain intertwining at high concentration. The anionic structure of biofilms might be strengthening the internal bonding with water and cement inside the paste [67].

The increase in viscosity became less predominant with increasing shear rate which was again attributed to the working mechanism of biofilms. Increasing shear rate breaks the intertwined polysaccharide chains and released the entrapped water to the mix. This reduces the apparent viscosity of the mix.

Increasing water content resulted with a higher efficiency of both bacterial cells and biofilms in terms of increasing apparent viscosity. When bio-VMAs were used by 0.1% of cement weight at w/c of 0.43, there was not a drastic change in apparent viscosity neither 17s^{-1} nor 34s^{-1} . But all the bio-VMAs used by 0.3% of the cement weight resulted with approximately 60 ± 5 % increase in apparent viscosity at 17s^{-1} at a w/c of 0.43. A point that should be noted is the % change in apparent viscosity with increasing shear rate. But when the shear rate was increased to 34s^{-1} , the change in apparent viscosity was almost 20% in the control neat paste sample, while the change was 40 ± 3 % in samples containing bio-VMA. This might suggest that at this specified mixing proportions the biological agents increased the shear-thinning response of the material. In fact, this sudden decrease in apparent viscosity at 34s^{-1} was observed at any w/c where there was an increase in apparent viscosity at 17s^{-1} (see EPI 61_BF_0.36_0.3% and EPI 61_0.50_0.3% samples). Thus, bio-VMAs could be an alternative additive for additive manufacturing applications where a strong shear-thinning property is required during

shear. To be noted, the analysis was done by decreasing shear rate, which might be also an indicative of viscosity recovery with decreasing shear rate. This can help the material remain in place after extrusion. Further analysis has to be done to evaluate the thixotropic behavior of mixes prepared with bio-VMAs to evaluate the structural recovery upon shearing.



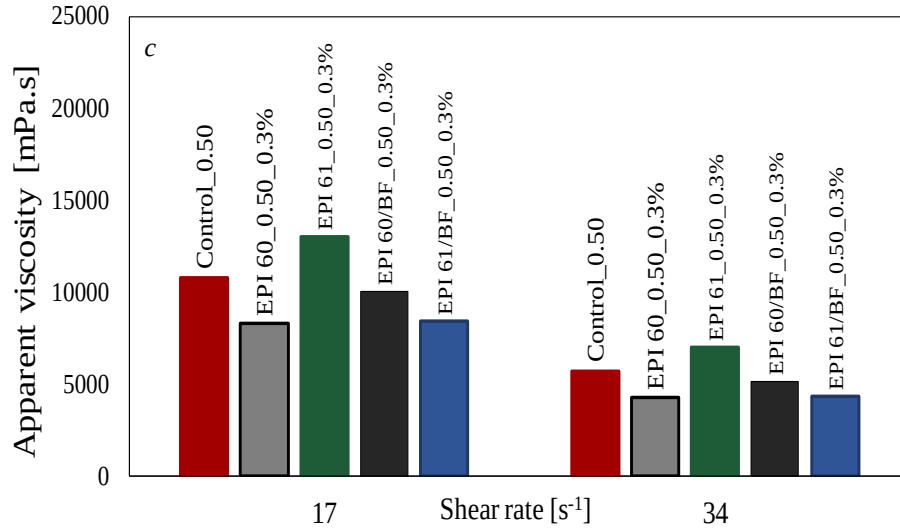


Figure 3.6: Apparent viscosity values for samples at shear rates of 17 and 34 s⁻¹. Control: No additive. EPI 60: *B.amyloliquefaciens*; EPI 61: *P.polymyxa*; BF:biofilms; VMA: 0.3% by cement weight; w/c: (a)0.36, (b)0.43 and (c)0.50.

3.3.2 Influence of Superplasticizer on the Efficiency of Bio-based VMAs

Superplasticizers, a.k.a. dispersing agents are used to improve workability and fresh state performance of concrete without increasing water content [16] and to reduce the bleeding of concrete [79]. Eventually, superplasticizers can reduce the yield stress and viscosity at constant w/c and solid content [16]. The polycarboxylate ether (PCE) superplasticizers have a polymer backbone structure consisting of carbon atoms with anionic carboxylate acids and nonionic long-chain polyether side groups. Anionic carboxyl acids absorb cement particles; when in close contact with one another, causes electrostatic repulsion because of a reduction in entropy [16].

Commercially available VMAs are commonly used along with superplasticizers to reduce segregation and provide stability after casting. Most of these commercially available admixtures used for concrete contains anionic polyelectrolytes that enable their adsorption onto positively charged surfaces, i.e. cement particles, and induce required functions [80]. Relatedly, the absorption of PCEs can be impeded through a “competitive adsorption” action mechanism. The ions in cement pore solution or other present

admixtures can occupy the positively charged sites on cement particles, preventing the absorption of PCEs resulting with reduced dispersion efficiency due to weak steric repulsion among the cement particles. Previous studies showed that depending on the type of chemical admixture the steric repulsion could be hindered due to competitive absorption [80]. Considering the fact that the cells might interact with cement particles through surface charges, it is important to identify the compatibility of this novel bio-VMAs with superplasticizers.

To evaluate their performance, a PCE based superplasticizer was added to the mixes by 0.33% of cement weight. Figure 3.7 exhibits the shear curves for cement paste samples containing superplasticizers and 0.1% bio-VMAs (by weight of cement). Superplasticizer weakens the van der Waals attraction among particles [41], therefore the dense structure of cement paste diffuse. A major impact of superplasticizer use is the decrease in yield stress along with a decrease in viscosity. Table 3.4 summarizes the yield stress and mini-slump measurements for cement-paste samples containing 0.1% bio-VMAs by weight of cement.

At w/c 0.36, almost all bio-VMAs, EPI 60, EPI 61 cells, and EPI 61 biofilm were able to improve yield stress and viscosity when these bio-additives were used at 0.1% of cement weight without any PCEs (see Figure 3.3). But when PCEs were added to the same mixes by 0.33% of cement weight, EPI 61 biofilm was able to improve the rheological parameters, yield stress, and viscosity. It should be noted that EPI 60 was used for *B. amyloliquefaciens* cells and EPI 61 was used as an abbreviation for *P. polymxa* cells. As was stated above, both EPI 60 and EPI 61 cells were more efficient at low w/c and their efficiency was reduced with increasing w/c. This behavior was more pronounced when these cells were used by 0.1% of cement weight rather than it was when cells were used by 0.3% of cement weight. The incorporation of PCE superplasticizers completely

interfered with the working mechanisms of cells. This might be due to the possible working mechanism of cells where it was governed by surface attraction. Cells are negatively surface charged, and it was possible that they increase the yield stress and viscosity by attracting positively charged cement particles. This mechanism resembles a surfactant mechanism that was also observed in PCE superplasticizers through steric hindrance. Thus, in such a case strong PCE polymers were absorbed on the particles and might reduce the surface attraction between cells and cement particles. At low w/c, only the addition of EPI 61 biofilms was able to withstand electrostatic repulsion and steric hindrance effect of superplasticizer. This suggests that water retention mechanisms can still be induced in the presence of superplasticizers. Especially at high concentrations of cement pastes, this action mechanism catalyzes the increase of apparent viscosity results [59].

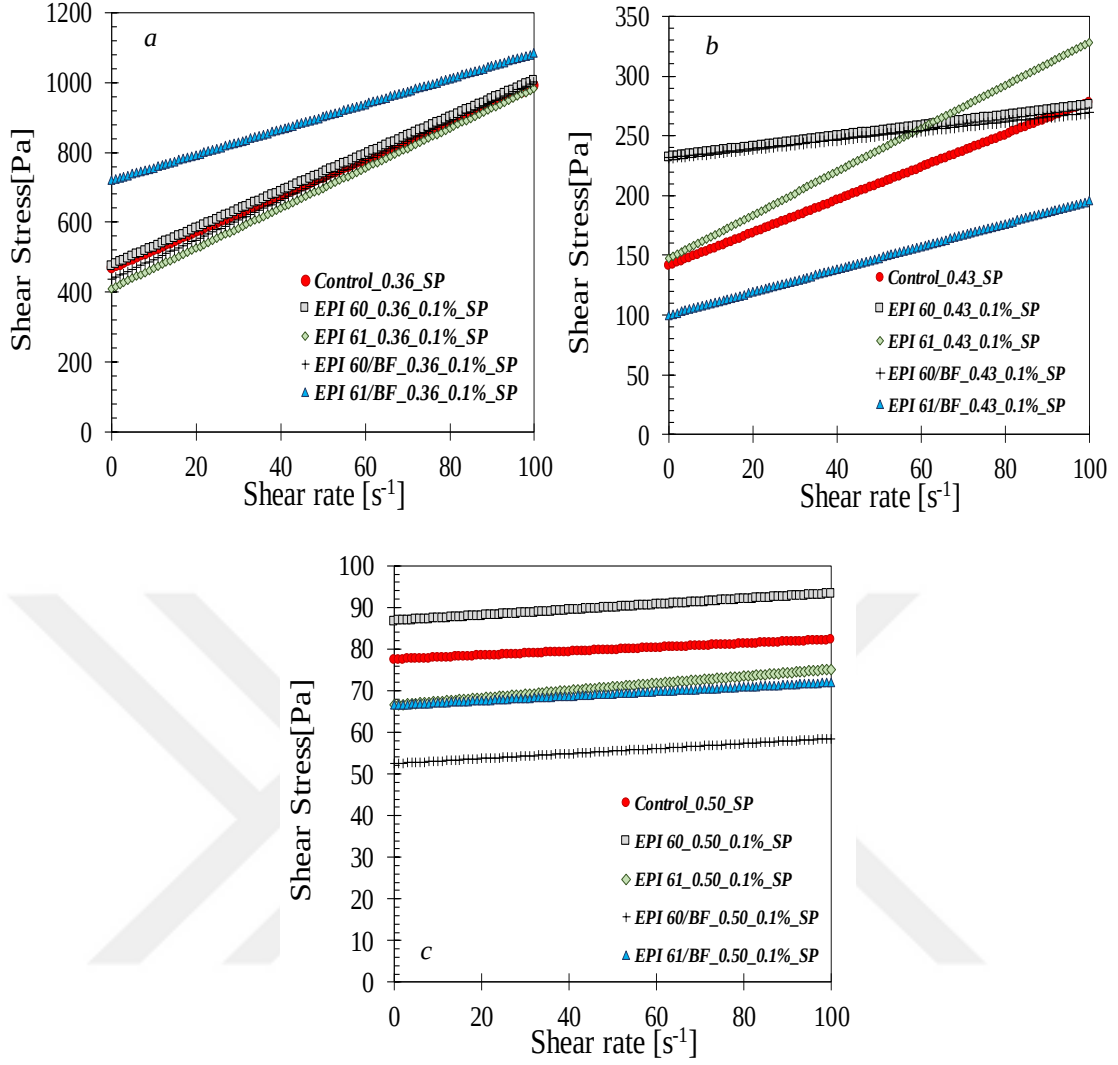


Figure 3.7: Rheological test results for cement paste samples. Control sample: Control SP: Cement paste containing 0.33% of superplasticizer; EPI 60: *B.amyloliquefaciens*; EPI 61: *P.polymyxa*; BF:biofilms ; VMA: 0.1% by cement weight; w/c: (a) 0.36, (b) 0.43 and (c) 0.50.

In contrast to what we have observed previously in samples did not contain any superplasticizers, the efficiency of cells was rather increased with increasing w/c. A similar trend was also observed in our previous studies [31]. Even though the interaction between cells and PCE superplasticizers are not exactly known, this might suggest that the cells can also affect the yield stress and viscosity due to their intercellular polysaccharides.

The mini slump measurements of cement paste samples were again not consistent with the yield stress values at low w/c which was attributed to very low workability of cement paste. Low w/c might decrease the flowability of the mix, thus the slump diameter was almost the same in all samples. Increasing w/c reveals more accurate results in slump test. Particularly at a w/c of 0.50, the slump measurements were in line with yield stress values.

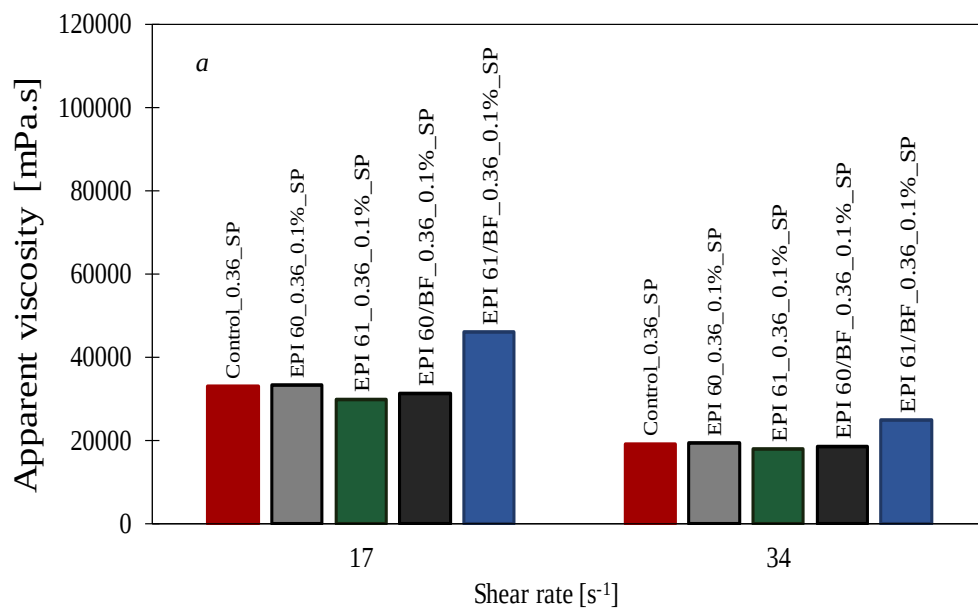
Table 3.4 Yield strength and average slump values of cement pastes with 0.1% of bio VMA addition by cement weight. Control sample: Contains 0.33% superplasticizer by cement weight; EPI 60: *B.amyloliquefaciens*; EPI 61: *P.polymyxa*; BF:biofilms ; w/c: 0.36, 0.43 and 0.50.

Sample Name	Mini Slump (cm)	Yield Stress [Pa]
Control_0.36_SP	4,5	471,3
EPI 60_0.36_0.1%_SP	4,4	475,7
EPI 61_0.36_0.1%_SP	4,6	411,5
EPI 60/BF_0.36_0.1%_SP	5,2	437,6
EPI 61/BF_0.36_0.1%_SP	4,7	720,6
Control_0.43_SP	8,5	140,9
EPI 60_0.43_0.1%_SP	7,4	232,4
EPI 61_0.43_0.1%_SP	6,1	148,0
EPI 60/BF_0.43_0.1%_SP	6,9	231,9
EPI 61/BF_0.43_0.1%_SP	8,8	100,3
Control_0.50_SP	9,9	77,4
EPI 60_0.50_0.1%_SP	9,7	86,7
EPI 61_0.50_0.1%_SP	10,0	66,6
EPI 60/BF_0.50_0.1%_SP	12,0	52,5
EPI 61/BF_0.50_0.1%_SP	10,7	66,6

Figure 3.8 represent the apparent viscosity results for cement pastes containing 0.1% of bio-VMAs by cement weight, at the shear rates 17 s^{-1} and 34 s^{-1} . Again, the change in apparent viscosity values were in line with the increase in yield stress. A slight viscosity increase was observed in samples in which an increase in yield stress was observed. Incorporation of superplasticizers increased the flowability of the mix and

reduce the efficiency of bio-VMAs compared to counterpart samples without any superplasticizers.

Studies showed that welan gum, a biological polymer with high molecular weight, exhibit a different stabilizing mechanism compared to synthetic VMAs such that welan gum induces stabilizing affect by only increasing the yield stress [81]. This was attributed to working mechanism of welan gum where water retention was maintained by stretching of rigid welan gum chains. However, bio-VMAs, i.e. EPI 60/61 cells and biofilms, affects rheology both increasing yield stress and viscosity. Such that incorporation EPI 61 BF in low w/c mix (EPI 61/BF_0.36_0.1%_SP sample) led to a 53% increase in yield stress and 40% increase viscosity compared to neat paste. This almost equivalent increase in viscosity was observed in all samples in which yield stress was increased. This shows that these bio-VMAs interfere with the rheology of cement-paste through more than one action mechanism.



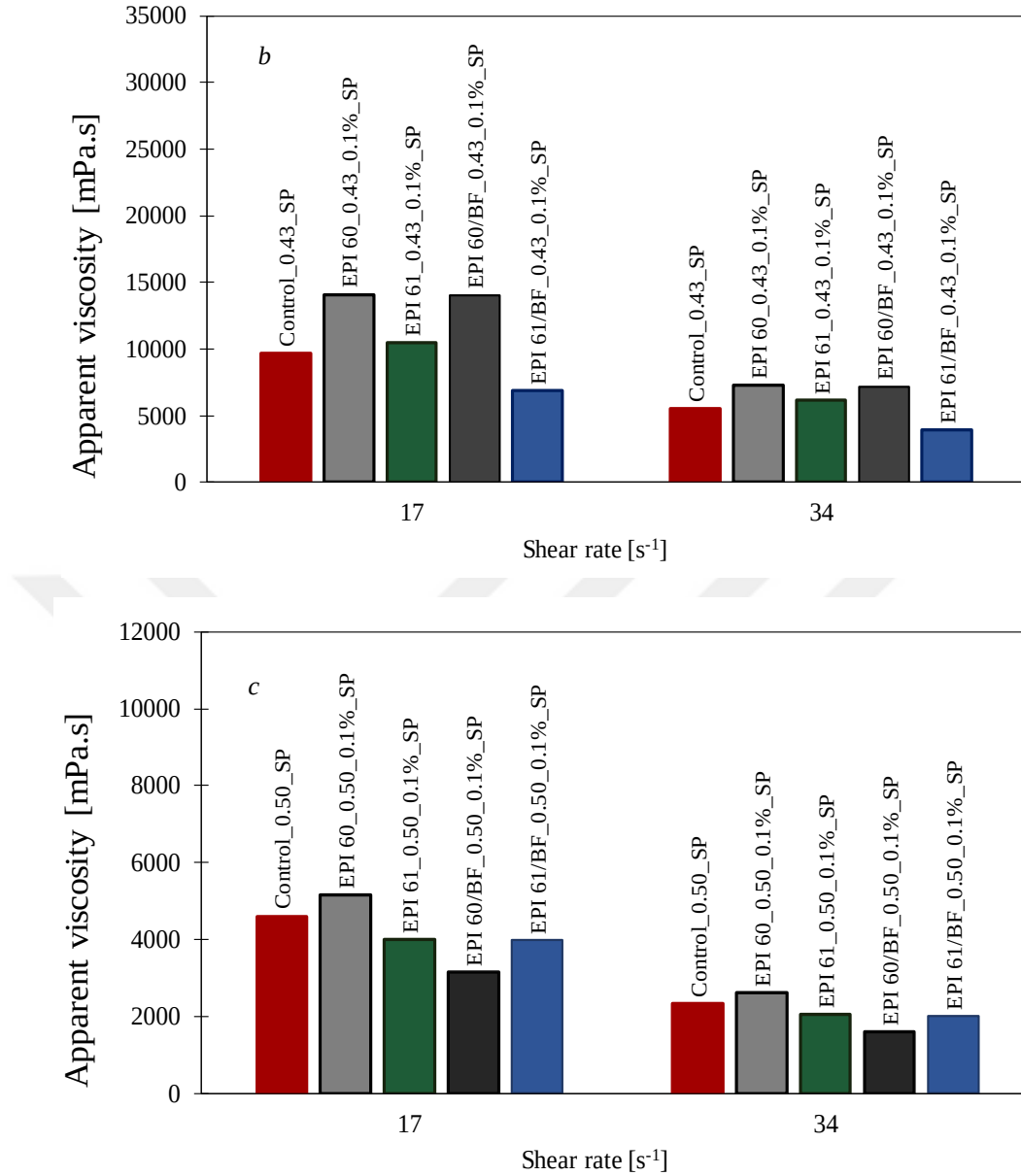


Figure 3.8: Apparent viscosity values for samples at shear rates of 17 and 34 s⁻¹. Control SP:0.33% superplasticizer by cement weight. EPI 60: *B.amyloliquefaciens*; EPI 61: *P.polymyxa*; BF:biofilms; VMA: 0.1% by cement weight; w/c: (a)0.36, (b)0.43 and (c)0.50.

At last but not the least, the viscosifying effect of those bio-VMAs which could improve both yield stress and viscosity was more pronounced in the presence of PCE superplasticizers. Table 3.5 summarizes comparison of some representative samples with and without superplasticizers. As an example, comparing *EPI 61/BF_0.36_0.1%* sample with *EPI 61/BF_0.36_0.1%_SP*, incorporating PCEs almost tripled the increase in

viscosity. Similar trends were observed in all representative samples. While there was not any change in apparent viscosity of EPI60_0.43_0.1% and EPI60_0.5_0.1% samples, incorporation of superplasticizers to these mixes resulted with a significant increase in viscosity. This might indicate that this PCE might have low anionic surface charge and stabilizing affect still mostly dominated by bio-VMAs [80]. However, there were few samples where an opposite trend was observed. Further experimental analysis has to be done to understand which mechanism was predominant in mixes containing superplasticizers.

Table 3.5 The percent increase of apparent viscosity measurement for samples at shear rates at 17 and 34 s⁻¹ referenced to control samples, Control sample: No additive; EPI 60: *B.amyloliquefaciens*; EPI 61: *P.polymyxa*; SP: Superplasticizer; BF: biofilms; VMA: 0.1% by cement weight; w/c: 0.36, 0.43 and 0.50

Sample Name	% increase relative to control neat paste	
	17s ⁻¹	34s ⁻¹
EPI 61/BF_0.36_0.1%	13%	10%
EPI 61/BF_0.36_0.1%_SP	40%	30%
EPI 60_0.43_0.1%	-	-
EPI 60_0.43_0.1%_SP	46%	32%
EPI 60/BF_0.43_0.1%	17%	16%
EPI 60/BF_0.43_0.1%_SP	45%	31%
EPI 60_0.50_0.1%	-	-
EPI 60_0.50_0.1%_SP	75%	81%

Throughout the literature, welan gum and similar biological polymers exhibited viscosifying properties at lower dosages and showed a dispersing affect with increasing dosage [80]. Figure 3.9 shows the shear profiles for cement paste samples containing 0.3% bio-VMAs and 0.33% PCE superplasticizers by weight of cement. Increasing bio-VMA dosage improved the stabilizing efficiency of these additives at w/c of 0.36 and 0.43 (see Table 3.7). However, any of the bio-VMAs used in the study could improve rheological properties at w/c of 0.50 when they were used at 0.3% of cement weight with PCEs. In this case, bio-VMAs exhibited a dispersing affect in cement paste samples. A similar effect was also observed in several VMAs, such that when cellulose ether based

VMAs were used more than 0.2% of water weight (0.1% by cement weight If w/c was assumed to be 0.5), their efficiency as a stabilizer would be significantly reduced. This was mainly attributed to their molecular weight and affinity with cement particles [2].

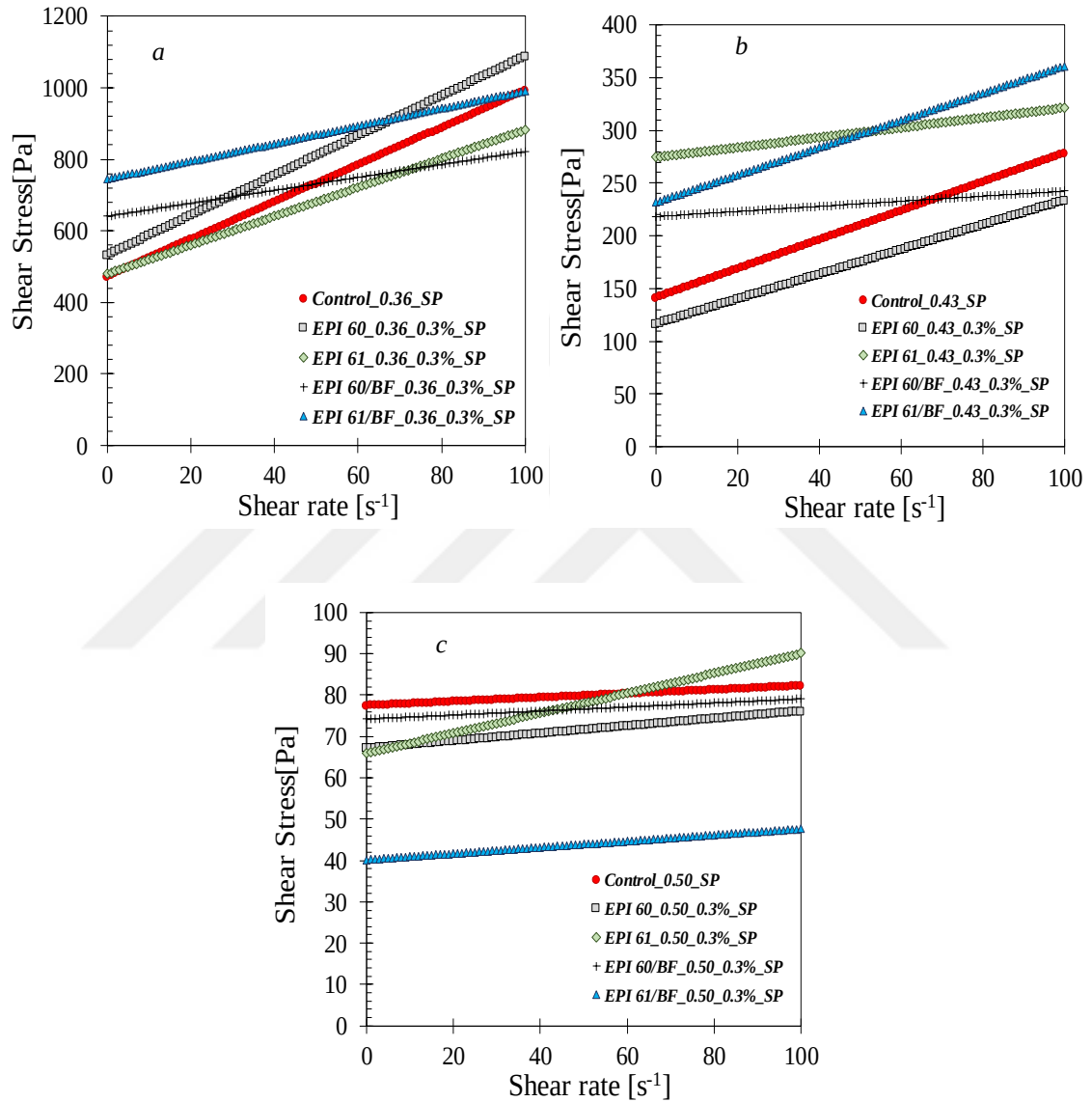


Figure 3.9: Rheological test results for cement paste samples. Control sample: Control SP: Cement paste containing 0.33% of superplasticizer; EPI 60: *B.amyloliquefaciens*; EPI 61: *P.polymyxa*; BF:biofilms ; VMA: 0.3% by cement weight; w/c: (a) 0.36, (b) 0.43 and (c) 0.50.

Table 3.6 Yield strength and average slump values of cement pastes with 0.3% of bio VMA addition by cement weight. Control sample: Contains 0.33% superplasticizer by cement weight; EPI 60: *B.amyloliquefaciens*; EPI 61: *P.polymyxa*; BF:biofilms ; w/c: 0.36, 0.43 and 0.50.

Sample Name	Mini Slump (cm)	Yield Stress [Pa]
Control_0.36_SP	4,5	471,3
EPI 60_0.36_0.3%_SP	4,5	530,7
EPI 61_0.36_0.3%_SP	5,0	480,2
EPI 60/BF_0.36_0.3%_SP	4,9	641,8
EPI 61/BF_0.36_0.3%_SP	5,5	745,9
Control_0.43_SP	8,5	140,9
EPI 60_0.43_0.3%_SP	9,0	116,1
EPI 61_0.43_0.3%_SP	5,9	274,6
EPI 60/BF_0.43_0.3%_SP	6,9	218,5
EPI 61/BF_0.43_0.3%_SP	7,1	232,1
Control_0.50_SP	9,9	77,4
EPI 60_0.50_0.3%_SP	10,5	67,1
EPI 61_0.50_0.3%_SP	9,1	66,0
EPI 60/BF_0.50_0.3%_SP	9,4	74,3
EPI 61/BF_0.50_0.3%_SP	11,2	40,2

As stated above, when the dosage of bio-VMAs increased to 0.3% by cement weight their viscosifying properties were less pronounced. This was also observed in terms of increasing the apparent viscosity at low shear rates. At a w/c of 0.36 incorporation of EPI 61 biofilms induced a slight increase in apparent viscosity at $17s^{-1}$. When the water content increased to 0.43, EPI 61 cell and biofilms as well as EPI 60 biofilm enhanced the apparent viscosity of cement pastes at both shear rates.

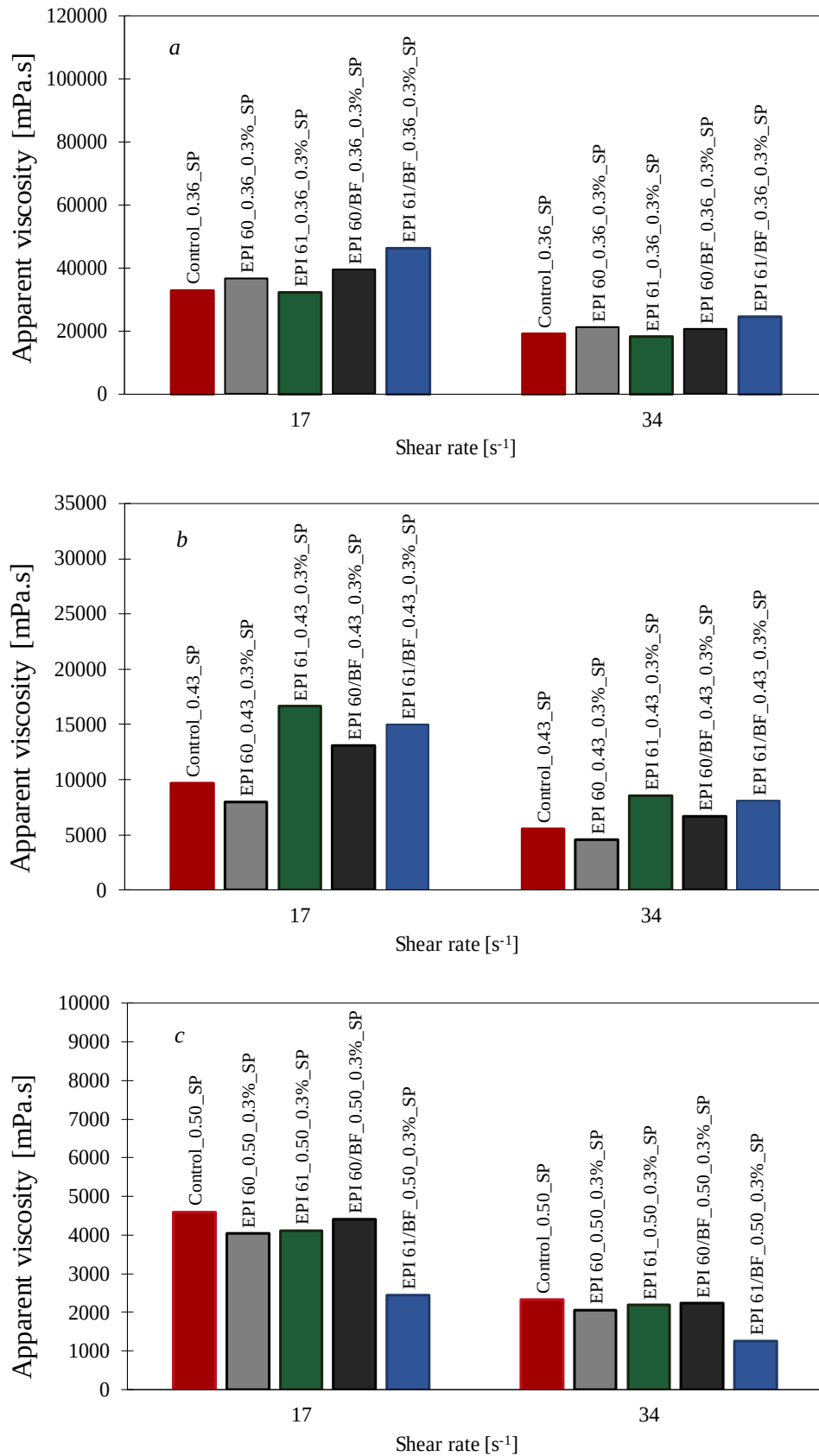


Figure 3.10: Apparent viscosity values for samples at shear rates of 17 and 34 s¹. Control SP:0.33% superplasticizer by cement weight. EPI 60: *B.amyloliquefaciens*; EPI 61: *P.polymyxa*; BF:biofilms; VMA: 0.3% by cement weight; w/c: (a)0.36, (b)0.43 and (c)0.50.

In addition, the viscosifying effect of those bio-VMAs was more pronounced in the presence of PCE superplasticizers when they were used at 0.1% of cement weight. Increasing bio-VMA dosage to 0.3% also affected the interaction between the PCE superplasticizer and bio-VMA. Table 3.7 summarizes the % increase in apparent viscosity for representative cement paste samples (in which an increase was observed) containing bio-VMA at 0.3%. While incorporation of superplasticizers increased the efficiency of bio-VMAs at low w/c, this effect was less pronounced with increasing w/c, particularly at w/c of 0.5. This was not observed when bio-VMAs were used at 0.1% of cement paste. Incorporation of EPI 61 cells at 0.1% led to almost 70% increase in the apparent viscosity at $17s^{-1}$ when they were incorporated with PCEs. But this effect was completely eliminated when the cell dosage was increased to 0.3%. This might be attributed the low molecular mass of bacterial cells and biofilms which reduces their affinity with multiple cement particles [2]. A polymer's (or biopolymer's) efficiency was found to be related to its ability to cover the particle surface and optimum flocculation could be attained when only half of the particle surfaces were covered [82], [83]. Another reason that causes this decreasing efficiency, could be enhanced anionicity due to the increased number of negatively surface charged cells and PCEs. This might increase the dispersion of the cement particles in the suspension. Thus, it might be concluded that it would be optimized to limit the dosage of bio-VMAs at 0.1% cement weight.

Table 3.7 The percent increase of apparent viscosity measurement for samples at shear rates at 17 and 34 s⁻¹ relative to control samples, Control sample: No additive/0.33% of SP by cement weight; EPI 60: *B.amyloliquefaciens*; EPI 61: *P.polymyxa*; SP: Superplasticizer; BF: biofilms; VMA: 0.3% by cement weight; w/c: 0.36, 0.43 and 0.50

Sample Name	% increase relative to control neat paste	
	17 s ⁻¹	34 s ⁻¹
EPI 60_0.36_0.3%	-	-
EPI 60_0.36_0.3%_SP	12%	11%
EPI 60/BF_0.36_0.3%	-	-
EPI 60/BF_0.36_0.3%_SP	20%	9%
EPI 61/BF_0.36_0.3%	35%	30%
EPI 61/BF_0.36_0.3%_SP	41%	28%
EPI 60_0.43_0.3%	63%	55%
EPI 60_0.43_0.3%_SP	-	-
EPI 61_0.43_0.3%	60%	45%
EPI 61_0.43_0.3%_SP	72%	55%
EPI 60/BF_0.43_0.3%	42%	25%
EPI 60/BF_0.43_0.3%_SP	36%	21%
EPI 61/BF_0.43_0.3%	62%	45%
EPI 61/BF_0.43_0.3%_SP	55%	47%
EPI 61_0.50_0.3%	21%	22%
EPI 61_0.50_0.3%_SP	-	-

3.3.3 Influence of Fly Ash on the Efficiency of Bio-based VMAs

Current technology in the field increased the demand for high performance concrete (HPC) mixes with enhanced workability and strength were maintained. In addition, possible applications of 3D printing and extrusion processes required design of HPC mixes in which bleeding and segregation is prevented. To achieve these properties, inorganic fines like fly ash, slag, or calcined clays are commonly used in mix design. The fundamental approach of using inorganic fines is to improve the strength and durability of concrete considering economic and environmental aspects [27]. Relatedly, these inorganic fines, i.e. mineral admixtures, will influence the rheology and particle packing in cement paste. Addition of mineral admixtures prone to create variations considering

the change in interparticle interactions. Both yield stress and plastic viscosity are generally affected by friction among solids size and improved packing efficiency which is attributed to surface roughness and shape of particles [4]. Park et al. [39] stated both yield stress and plastic viscosity to decrease with fly ash replacement due to spherical shape of fly ash particles which reduces the interparticle friction. Due to this effect, incorporation of fly ash decreases the water demand during mixing [27].

In most of the cases, chemical or biological additives might not be compatible with these inorganic fines. This section aims to evaluate the behavior of proposed bio-VMAs in fly-ash amended cement paste. Fly ash was used as a cement replacement by 15% of cement. Figure 3.11 summarize the shear stress profile for fly-ash amended cement paste samples containing 0.1% bio-VMAs.

Due to their spherical and fine particle size incorporation of fly ash decreased the yield stress and viscosity at any w/c compared to their counterpart cement samples. This decrease was much more pronounced at low w/c (0.36) where particle friction is the predominant mechanism for workability rather than water content. The strength of the particle network could directly affect the yield point of the material. The presence of spherical shaped fly ash inside the matrix leads to reduced friction among solid particles [59]. Thus, the motion of particles becomes smoother, and the fluidity of the matrix increase. As also stated by Sonebi [59], partial replacement of cement by fly ash, presented reduced yield stress values. However, it was possible to counter ball-bearing effect of fly ash, with the addition of bio-based VMAs.

Use of EPI 60 (*B.amyloliquefaciens*) and EPI 61 BF (*P.polymxa* biofilm) increased the yield strength without affecting the mini slump value (see Table 3.8). Incorporation of these 2 bio-VMAs improved the rheology of cement pastes without

sacrificing from workability. In fact, in all 3 mix designs EPI 60 cells were found to be effective $w/c < 0.43$ when they were used at 0.1% of cement weight. This is actually not the case for neither EPI 61 cells nor their biofilm.

The behavior of these bio-VMAs were slightly inconsistent with changing w/c and material composition. EPI 60 cells, i.e. *B.amyloliquefaciens* cells, has a cell structure resembling *B. subtilis*, which has negative surface charge and a relatively longer peptidoglycan chains (5 μm) compared to an average peptidoglycan chain in bacterial cells (3 μm) [51]. Peptidoglycan in these bacterial cells can adsorb water and form hydrogen bond between the water molecules. In addition, branches of peptidoglycan molecules could induce attractive forces that further restrict the motion of water leading to a further decrease in viscosity. As mentioned before these bacterial cells (also the peptidoglycan) are anionic thus they can bind positively charged calcium ions and decrease the fluidity by binding cement particles. Increasing particle density might enhance the interaction between the bacterial cells and positively charged particles.

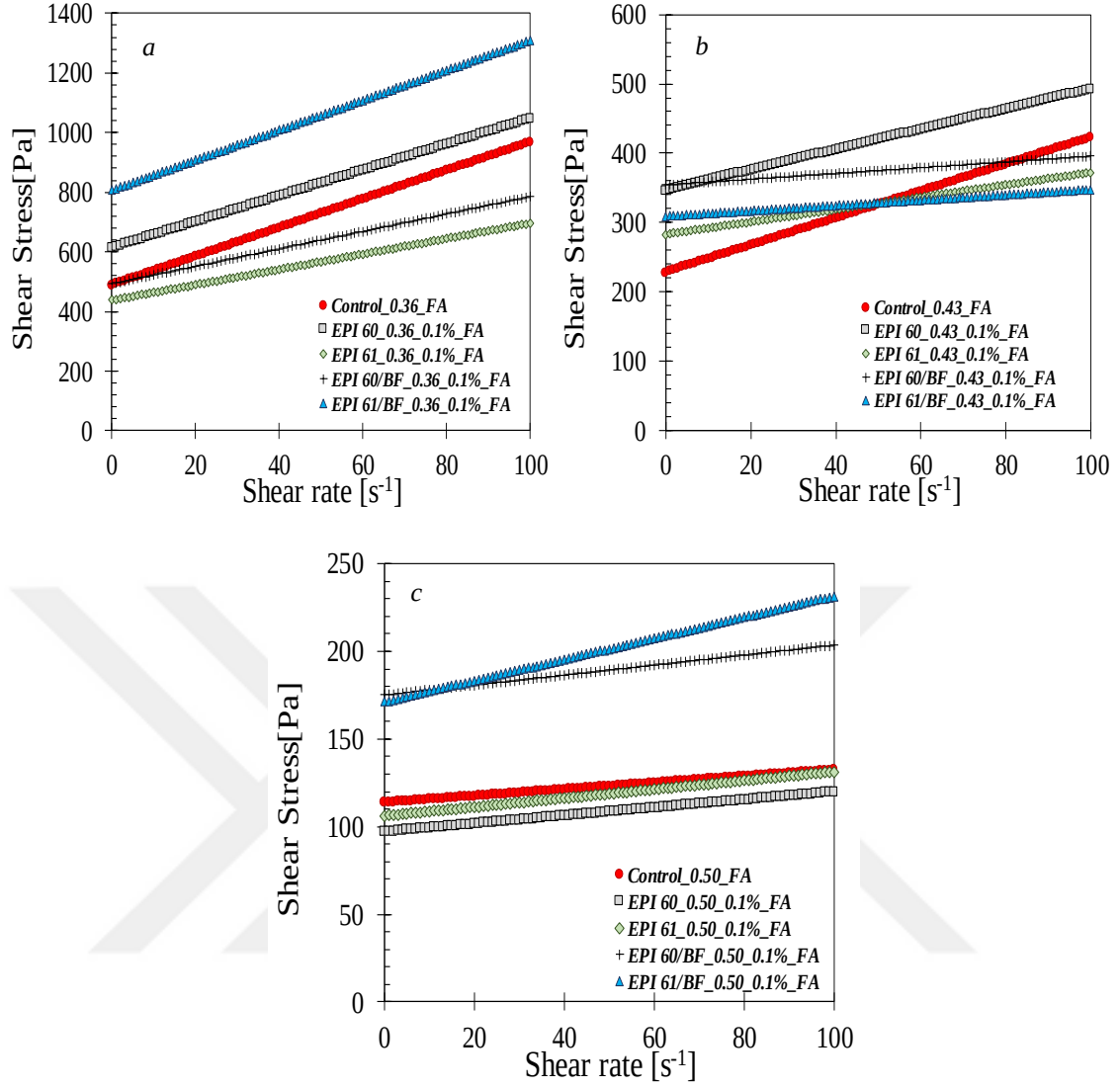


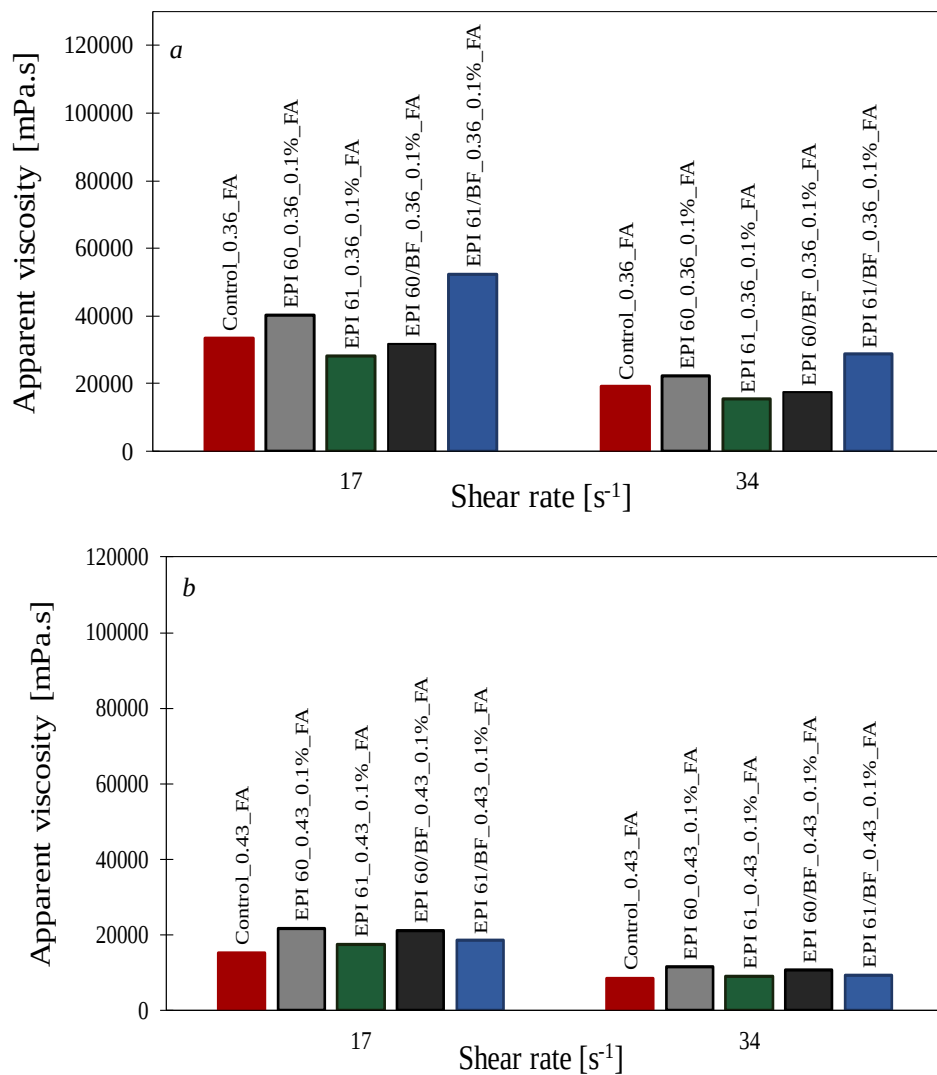
Figure 3.11: Rheological test results for cement paste samples. Control FA: Partial replacement of fly ash by 15% of cement weight. EPI 60: *B.amyloliquefaciens*; EPI 61: *P.polymyxa*; BF: biofilms ; VMA: 0.1% by cement weight; w/c: (a)0.36, (b)0.43 and (c) 0.50.

Table 3.8 Yield strength and average slump values of cement paste with 0.1% of bio VMA addition by cement weight. Control FA:15% replacement of cement by fly ash; EPI 60: *B.amyloliquefaciens*; EPI 61: *P.polymyxa*; BF:biofilms ; w/c: 0.36, 0.43 and 0.50.

Sample Name	Mini Slump (cm)	Yield Stress [Pa]
Control_0.36_FA	4,2	485,7
EPI 60_0.36_0.1%_FA	5,0	611,5
EPI 61_0.36_0.1%_FA	5,0	436,3
EPI 60/BF_0.36_0.1%_FA	4,4	491,6
EPI 61/BF_0.36_0.1%_FA	4,2	805,7
Control_0.43_FA	5,8	227,1
EPI 60_0.43_0.1%_FA	5,2	345,8
EPI 61_0.43_0.1%_FA	5,3	282,7
EPI 60/BF_0.43_0.1%_FA	5,7	353,8
EPI 61/BF_0.43_0.1%_FA	5,8	308,6
Control_0.50_FA	8,2	113,7
EPI 60_0.50_0.1%_FA	8,2	97,1
EPI 61_0.50_0.1%_FA	9,0	106,4
EPI 60/BF_0.50_0.1%_FA	8,6	175,3
EPI 61/BF_0.50_0.1%_FA	8,8	171,4

Previously, Pei et al.[51] observed an increase in apparent viscosity within a range of 20% at low shear rates (17 to 34 s⁻¹) when only *B. subtilis* cell walls (0.34% by weight of cement) were incorporated in cement paste (w/c=0.40). Figure 3.12 summarizes the apparent viscosity measurements at shear rates of 17 and 34 s⁻¹ for cement paste samples contain bio-VMAs (0.1% of cement weight) and 15% fly ash. While the performance of EPI 61 cells and biofilms were rather similar to *B. subtilis* cell walls, the change in apparent viscosity in cement paste containing EPI 60 cells and biofilm was significantly higher than the changed obtained with other approaches (see Table 3.9). Herein, active EPI 60 cells were directly added to the mix. One impact of using active cells compared to the incorporation of only bacterial cells walls could be explained by the theory of active suspensions [84]. Most microorganisms make use of their flagella to move in suspensions which could exert a net thrust on the surrounding fluid. This is the case of many types of bacteria such as the common *Escherichia coli* and *B. subtilis*, which use a bundle of

flagella for propulsion. Rafai et al.[85] showed that motile microalgae cells could increase the effective viscosity of a suspension. Moreover, the live cells showed a significantly higher efficiency than the dead cells, which suggested that the behavior was related to motility rather than cell structure. Even though, the theory was resolved through complex models, there is no evidence of these cells influencing complex suspensions like cement-paste. Further research has to be conducted to analyze the kinematics of vegetative gram-positive bacterial cells and how they actually influence the hydrodynamics of cement paste suspension.



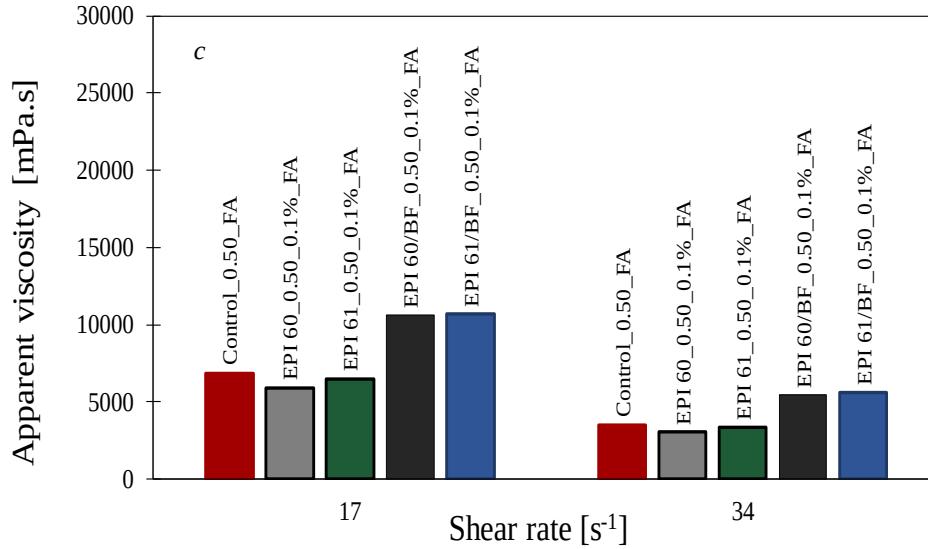


Figure 3.12: Apparent viscosity values for samples at shear rates of 17 and 34 s⁻¹. Control FA: 15% replacement of cement by fly ash; EPI 60: *B.amyloliquefaciens*; EPI 61: *P.polymyxa*; BF: Biofilms ; VMA: 0.1% by cement weight; w/c: (a) 0.36, (b) 0.43 and (c)0.50.

Table 3.9 The percent increase of apparent viscosity measurement for samples at shear rates of 17 and 34 s⁻¹ relative to control samples, Control FA: 15% replacement of cement by fly ash; EPI 60: *B.amyloliquefaciens*; EPI 61: *P.polymyxa*; BF:biofilms ; VMA: 0.1% by cement weight; w/c: 0.36, 0.43 and 0.50.

Sample Name	% increase relative to control neat paste	
	17 s ⁻¹	34 s ⁻¹
EPI 60_0.36_0.1%_FA	21%	17%
EPI 61/BF_0.36_0.1%_FA	57%	51%
EPI 60_0.43_0.1%_FA	42%	35%
EPI 61_0.43_0.1%_FA	14%	7%
EPI 60/BF_0.43_0.1%_FA	39%	25%
EPI 61/BF_0.43_0.1%_FA	21%	10%
EPI 60/BF_0.50_0.1%_FA	54%	54%
EPI 61/BF_0.50_0.1%_FA	55%	60%

As mentioned above, incorporation of fly ash decreases the yield strength and viscosity of cement paste suspensions due to their spherical particle size. As like in PCEs, there might be a need for increasing bio-VMA dosage to see an increase in rheological

properties. Figure 3.13 represents the shear profile for cement paste samples containing 15% fly ash and 0.3% bio-VMA by weight of cement.

As expected, all of the bio-VMAs were able to increase yield stress of cement-paste samples. Mini slump measurements were also in line with previous observations such that increasing w/c (see Table 3.10). While for low w/c, the mini slump readings were all almost the same even if the yield stress increases. But increasing w/c, the homogenous mixtures lead to slump measurements consistent with the change in yield stress. Increasing the dosage of bio-VMAs to 0.3%, enhanced the efficiency of both cells and biofilms compared to control sample at low w/c (0.36). Due to the spherical shape of fly ash, the particle packing density of matrix increases [59]. It might be possible for the trapped water inside the voids, replacing with fly ash, releasing water to the surface of solid particles, thus permitting bio-VMAs to initiate the water retention action [86], [87]. Similar to all mix compositions discussed so far, bio-VMAs were found to be more efficient when they were used at w/c of 0.43. Low w/c (0.36) samples might not have a homogeneous mix, rather the mix could be very stiff to take an accurate measurement. In contrast, high w/c (0.50) might lead separation of cement particles and only incorporation of biofilms can induce an increase in yield stress. A similar trend was also observed with samples containing fly ash and 0.3% bio-VMA at a w/c of 0.50. EPI 60 Biofilm resulted with a higher efficiency among the other alternatives at w/c 0.50, regardless of the dosage. In addition, the influence of EPI 61 cells was more pronounced at high w/c when they were used at 0.5%. Further investigations have to be done to understand the interaction of cells in cement-paste matrix

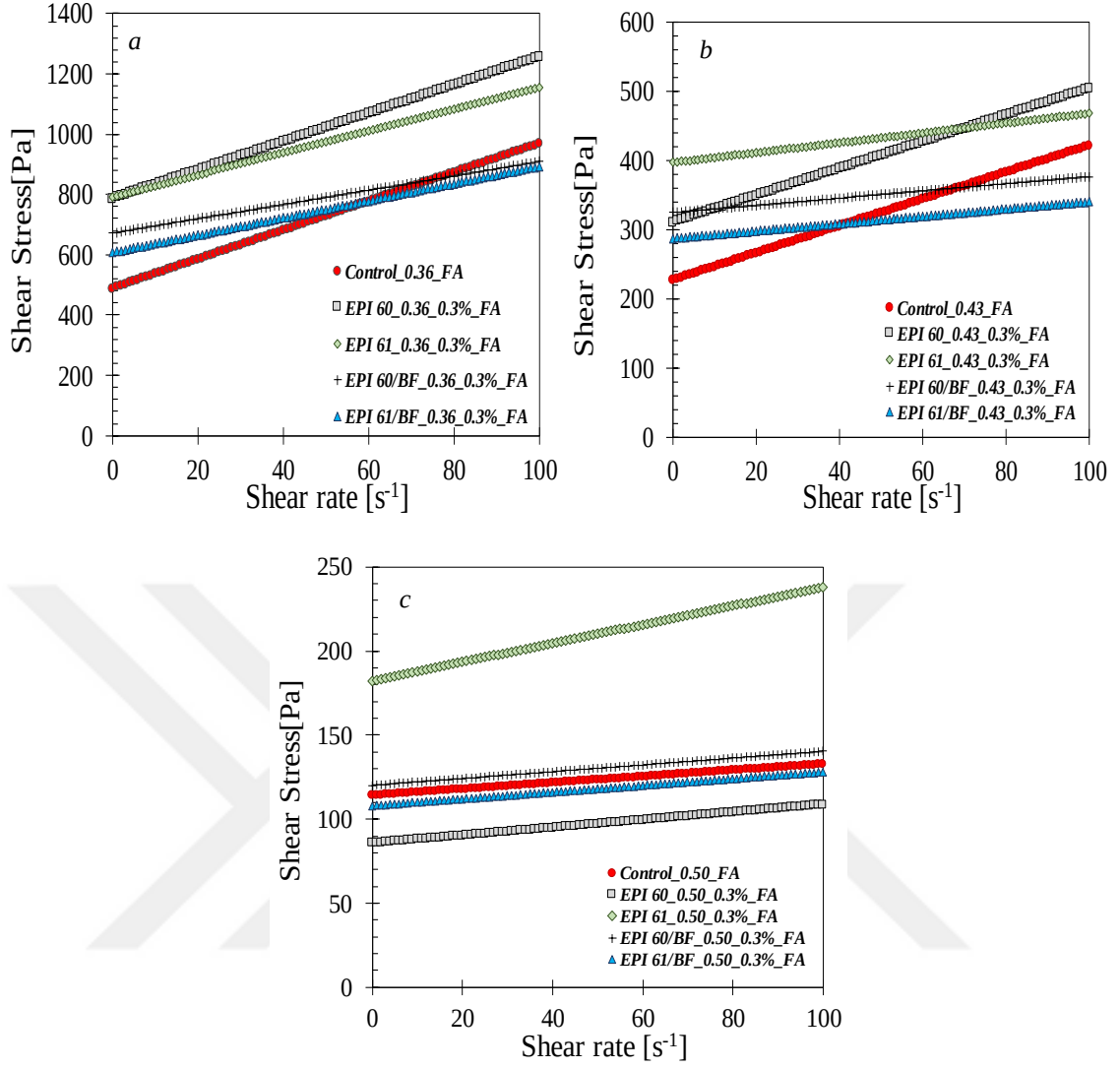


Figure 3.13: Rheological test results for cement paste samples. Control FA: Partial replacement of fly ash by 15% of cement weight. EPI 60: *B.amyloliquefaciens*; EPI 61: *P.polymyxa*; BF:biofilms ; VMA: 0.3% by cement weight; w/c: (a) 0.36, (b) 0.43 and (c) 0.50.

Table 3.10 Yield strength and average slump values of cement paste with 0.3% of bio VMA addition by cement weight. Control FA:15% replacement of cement by fly ash; EPI 60: *B.amyloliquefaciens*; EPI 61: *P.polymyxa*; BF:biofilms ; w/c: 0.36, 0.43 and 0.50.

Sample Name	Mini Slump (cm)	Yield Stress [Pa]
Control_0.36_FA	4,2	485,7
EPI 60_0.36_0.3%_FA	4,0	784,3
EPI 61_0.36_0.3%_FA	6,0	791,3
EPI 60/BF_0.36_0.3%_FA	4,3	670,3
EPI 61/BF_0.36_0.3%_FA	4,5	605,8
Control_0.43_FA	5,8	227,1
EPI 60_0.43_0.3%_FA	5,4	311,2
EPI 61_0.43_0.3%_FA	5,0	397,9
EPI 60/BF_0.43_0.3%_FA	5,7	325,5
EPI 61/BF_0.43_0.3%_FA	6,1	288,0
Control_0.50_FA	8,2	113,7
EPI 60_0.50_0.3%_FA	8,4	85,4
EPI 61_0.50_0.3%_FA	6,9	182,2
EPI 60/BF_0.50_0.3%_FA	7,9	119,9
EPI 61/BF_0.50_0.3%_FA	8,2	107,8

At last but not the least, Figure 3.14 represents the apparent viscosity measurements at shear rates of 17 and 34 s⁻¹ for cement paste samples containing 15% fly ash and 0.3% bio-VMAs. The change in apparent viscosities were at both shear rates was in line with the increase in yield stress. It should also be noted that regardless of the mixing composition, i.e. containing PCEs or fly ash or no additives, at high w/c (0.5) increasing dosage of bacterial cells increase their efficiency whereas an opposite trend was observed in biofilms. As mentioned before, the efficiency of bio-VMAs was attributed to their ability to cover the particle surface such that optimum flocculation could be attained when only half of the particle surfaces were covered. A similar behavior was also observed in EPI 61 cells, which has lower molecular weight compared to EPI 60. Based on the evaluations done, it could be concluded that EPI 60, *B.amyloliquefaciens*, cells were found to be more efficient among the rest of the proposed in terms of improving rheological properties (see Table 3.12).

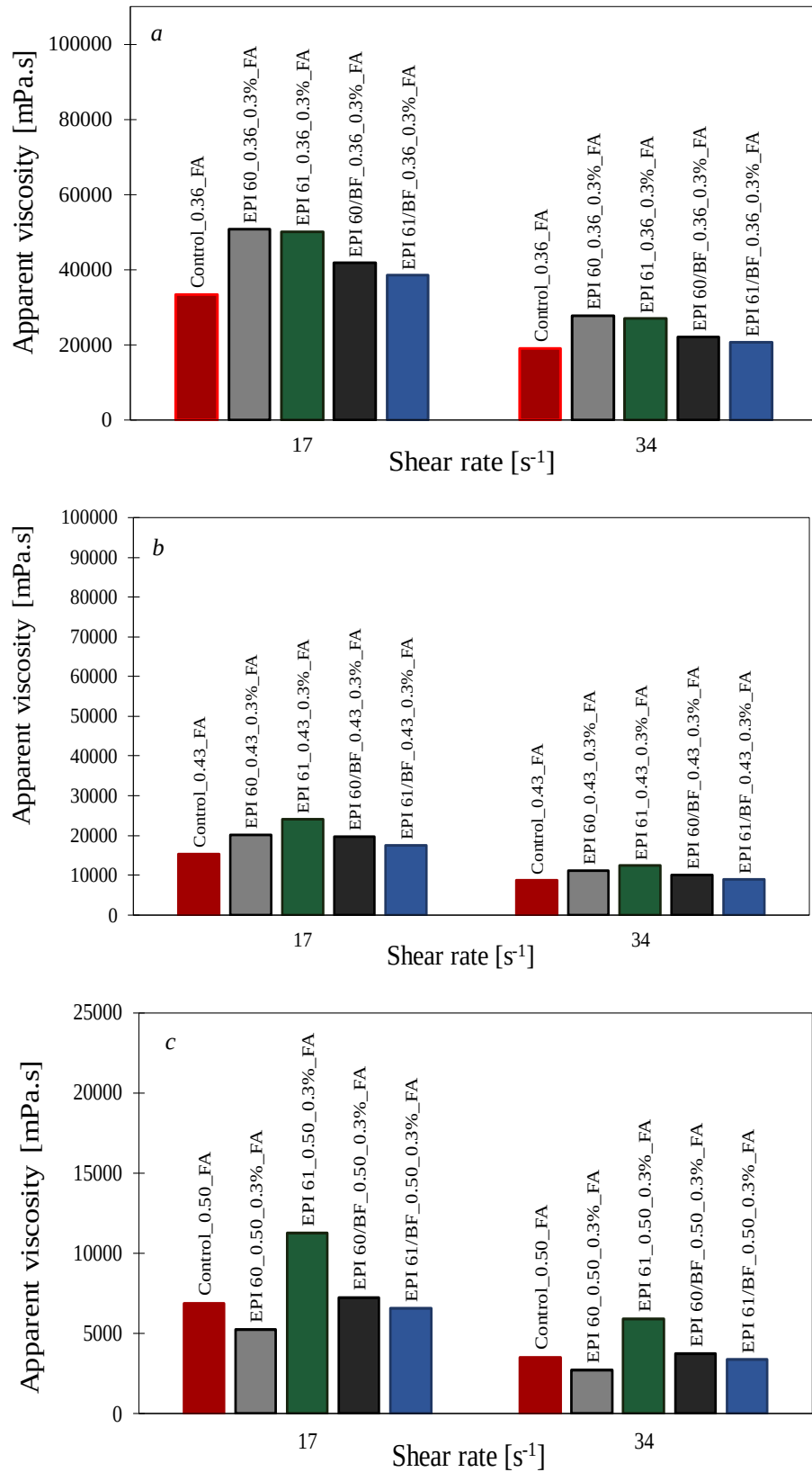


Figure 3.14: Apparent viscosity values for samples at shear rates of 17 and 34 s⁻¹. Control FA:15% replacement of cement by fly ash; EPI 60: *B.amyloliquefaciens*; EPI 61: *P.polymyxa*; BF:biofilms ; VMA: 0.3% by cement weight; w/c: (a)0.36, (b)0.43 and (c)0.50.

Table 3.11 The percent increase of apparent viscosity measurement for samples at shear rates of 17 and 34 s⁻¹ referenced to control samples, Control FA: 15% replacement of cement by fly ash; EPI 60: *B.amyloliquefaciens*; EPI 61: *P.polymyxa*; BF:biofilms ; VMA: 0.3% by cement weight; w/c:0.36, 0.43 and 0.50.

Sample Name	% increase relative to control neat paste	
	17 s ⁻¹	34 s ⁻¹
EPI 60_0.36_0.3%_FA	52%	46%
EPI 61_0.36_0.3%_FA	50%	41%
EPI 60/BF_0.36_0.3%_FA	25%	16%
EPI 61/BF_0.36_0.3%_FA	15%	8%
EPI 60_0.43_0.3%_FA	32%	29%
EPI 61_0.43_0.3%_FA	58%	44%
EPI 60/BF_0.43_0.3%_FA	28%	17%
EPI 61/BF_0.43_0.3%_FA	14%	4%
EPI 61_0.50_0.3%_FA	64%	67%
EPI 60/BF_0.50_0.3%_FA	6%	6%

Table 3.12 Summary of change in rheological properties compared to counterpart control samples, SP: 0.33% PCE addition; FA: 15% replacement of cement by fly ash; EPI 60: *B.amyloliquefaciens*; EPI 61: *P.polymyxa*; BF:biofilms

Sample Name	w/c=0.36		w/c=0.43		w/c=0.50	
	bio-VMA %					
	0.1%	0.3%	0.1%	0.3%	0.1%	0.3%
EPI 60	$\mu \uparrow \tau \uparrow$	$\mu \uparrow \tau \uparrow$	$\mu \Leftrightarrow \tau \Leftrightarrow$	$\mu \uparrow \tau \uparrow$	$\mu \Leftrightarrow \tau \downarrow$	$\mu \downarrow \tau \downarrow$
EPI 60+ SP	$\mu \Leftrightarrow \tau \Leftrightarrow$	$\mu \uparrow \tau \uparrow$	$\mu \uparrow \tau \uparrow$	$\mu \Leftrightarrow \tau \downarrow$	$\mu \uparrow \tau \uparrow$	$\mu \Leftrightarrow \tau \downarrow$
EPI 60+ FA	$\mu \uparrow \tau \uparrow$	$\mu \uparrow \tau \uparrow$	$\mu \uparrow \tau \uparrow$	$\mu \uparrow \tau \uparrow$	$\mu \Leftrightarrow \tau \Leftrightarrow$	$\mu \downarrow \tau \downarrow$
EPI 61	$\mu \uparrow \tau \uparrow$	$\mu \downarrow \tau \downarrow$	$\mu \uparrow \tau \uparrow$	$\mu \uparrow \tau \uparrow$	$\mu \Leftrightarrow \tau \Leftrightarrow$	$\mu \uparrow \tau \uparrow$
EPI 61+ SP	$\mu \Leftrightarrow \tau \downarrow$	$\mu \Leftrightarrow \tau \Leftrightarrow$	$\mu \uparrow \tau \Leftrightarrow$	$\mu \uparrow \tau \uparrow$	$\mu \Leftrightarrow \tau \downarrow$	$\mu \Leftrightarrow \tau \downarrow$
EPI 61+ FA	$\mu \downarrow \tau \downarrow$	$\mu \uparrow \tau \uparrow$	$\mu \uparrow \tau \uparrow$	$\mu \uparrow \tau \uparrow$	$\mu \Leftrightarrow \tau \Leftrightarrow$	$\mu \uparrow \tau \uparrow$
EPI 60/BF	$\mu \Leftrightarrow \tau \downarrow$	$\mu \Leftrightarrow \tau \downarrow$	$\mu \uparrow \tau \uparrow$	$\mu \uparrow \tau \uparrow$	$\mu \uparrow \tau \uparrow$	$\mu \Leftrightarrow \tau \downarrow$
EPI 60/BF+ SP	$\mu \Leftrightarrow \tau \downarrow$	$\mu \uparrow \tau \uparrow$	$\mu \uparrow \tau \uparrow$	$\mu \uparrow \tau \uparrow$	$\mu \downarrow \tau \downarrow$	$\mu \Leftrightarrow \tau \Leftrightarrow$
EPI 60/BF+ FA	$\mu \Leftrightarrow \tau \Leftrightarrow$	$\mu \uparrow \tau \uparrow$	$\mu \uparrow \tau \uparrow$	$\mu \uparrow \tau \uparrow$	$\mu \uparrow \tau \uparrow$	$\mu \Leftrightarrow \tau \Leftrightarrow$
EPI 61/BF	$\mu \uparrow \tau \uparrow$	$\mu \uparrow \tau \uparrow$	$\mu \Leftrightarrow \tau \Leftrightarrow$	$\mu \uparrow \tau \uparrow$	$\mu \Leftrightarrow \tau \Leftrightarrow$	$\mu \downarrow \tau \downarrow$
EPI 61/BF+ SP	$\mu \uparrow \tau \uparrow$	$\mu \uparrow \tau \uparrow$	$\mu \downarrow \tau \downarrow$	$\mu \uparrow \tau \uparrow$	$\mu \Leftrightarrow \tau \downarrow$	$\mu \downarrow \tau \downarrow$
EPI 61/BF+ FA	$\mu \uparrow \tau \uparrow$	$\mu \uparrow \tau \uparrow$	$\mu \uparrow \tau \uparrow$	$\mu \uparrow \tau \uparrow$	$\mu \uparrow \tau \uparrow$	$\mu \Leftrightarrow \tau \Leftrightarrow$

CHAPTER IV

4.1 Introduction

Besides chemical and biological VMAs, inorganic minerals, i.e., clays and pozzolans, have been widely used as rheology modifying additives for cement-based materials. Their strong surface charges and multi-layered structure of clays enable them to enhance rheological properties at low shear rates and results with a strong shear-thinning response with an increasing shear rate [2]. Among different clays, particularly bentonite, sepiolite, and kaolinite have been previously used as rheology modifiers. Two foremost approaches for sufficient flowability and stability are stated by increasing powder content and VMA which also decreases permeability and increases chemical resistance [88]. Like hydrophilic clays, they are capable to induce flocculation depending on their morphology and water absorption capacity [2]. In addition, incorporation of various inorganic materials in cement paste might affect particle interactions, total solid volume, packing density, setting time, and many other fresh state properties as well as the compressive strength. The second part of this thesis focuses on the development of a 2-part bio-VMA developed by immobilizing bacterial cells various inorganic minerals on diatomaceous earth (DE), sepiolite, metakaolin, and pumice.

With an acicular shape and fibrous particle morphology [89], sepiolite has a wide application as an absorbent, catalyst, and thickener [46], [90]. Whereas DE contains remains of diatom skeleton with a highly porous, hollow inner morphology as well as possessing high specific area and lightweight. Due to its unique morphology, DE is being used as a filtration agent, filler, absorbent, insulation material, etc.[91]. One of the most

impressive applications of DE is being and protective barrier for biological substances[91]. Besides, being a natural pozzolan, pumice has a volcanic origin with a porous particle morphology[92]. Pumice has a wide application as a lightweight aggregate due to its availability, resistance to high temperatures, and readymade structure [93]. Thereof they can be used in floor fills and a variety of insulation materials [93]. And lastly, metakaolin is thermally activated kaolinite clay with an irregular particle distribution, been stated to have a popular demand as cement substitute due to pozzolanic nature[42].

Previous studies have shown that *Sporosarcina pasteurii* (*S. pasteurii*) cells were effective in improving the rheological properties of cement-paste [31]. The bacteria cells have shown compatibility with PCE -based superplasticizers, as well as fly ash. It was found that the bacterial cells resembled VMA behavior by enhancing yield stress and viscosity which improves the stability of the cementitious mix. In this chapter, *S. pasteurii* cells were immobilized on DE, sepiolite, metakaolin, and pumice to develop a 2-part bio-VMA. The efficiency of the bio-VMA was evaluated through rheological analysis, mini-slump, and compressive strength tests.

4.2 Materials and Methods

4.2.1 Microorganism Selection and Growth

Leibniz Institute- German Collection of Microorganisms and Cell Cultures: *S. pasteurii* (DSMZ 33) cells were used in the experiments due to its high efficiency in previous studies [31], [94]. Gram-positive *S. pasteurii* cells were cultured in a medium including corn steep liqueur (CSL) (10 g), tris base (0.13 M), sodium acetate (10 g), and urea (20 g) per liter of distilled (DI) water. The pH of the medium was adjusted to 9. The solution cooled back to room temperature; the cells were added into the medium to be

incubated aerobically in sterilized liquid medium with shaking conditions (175 rpm) at 30°C until the stationary phase (10^9 CFU/mL) was reached. Then, the cells were collected from the culture by centrifuging at 6300g for 15 min. The cells were washed twice by Phosphate Buffer Solution (PBS) and stored at 4°C until immobilizing.

4.2.2 Inorganic Minerals and Material Compositions

To develop 2-part bio-VMA the bacterial cells were immobilized on DE, metakaolin, sepiolite, and pumice. These minerals were selected due to their porous structure and previously stated characteristics. In particular, the immobilization procedure was achieved by submerging the barriers to aqueous medium for 24 hours, thus absorption capacity of the minerals was specified as *24-hour absorption capacity*. The *24-hour absorption capacity* of the minerals and LWA are summarized in Table 1.

Table 4.1 Characteristics of the cement and mineral used in the study

SAMPLE	PARTICLE SIZE RANGE	AVERAGE PARTICE SIZE	ABSORPTION CAPACITY
CEMENT	5-90 μm	23.2 μm	-
METAKAOLIN (M) [95]	0.23-66 μm	9.95 μm	-
DIATOMACEOUS EARTH(DE)	0,375-90 μm	19.2 μm	110%
PUMICE (P) [96]	-	774 μm	45%
SEPIOLITE (S)	0.375-864 μm	277,8 μm	80%

The particle size was determined by a Mastersizer 2000 particle size analyzer with a Hydro MU 2000 wet dispersion unit (Malvern, Worcestershire, United Kingdom). To prevent the hydration of the cement particles, the cement particles were dispersed in ethanol. The average particle sizes of minerals and cement were also summarized in Table

1. Additionally, particle shape the minerals were evaluated through the Environmental Scanning Electron Microscope (ESEM) analysis. The samples were gold-coated prior to imaging with a FEI-Philips XL30 ESEM with Field Emission Gun (FEG) (Boğaziçi University, Istanbul). The accelerating voltage was kept at 10 kV while the working distance was held at 10 ± 1 mm at various magnifications. Figure 4.1 shows the SEM images of protection barriers before the immobilization process.

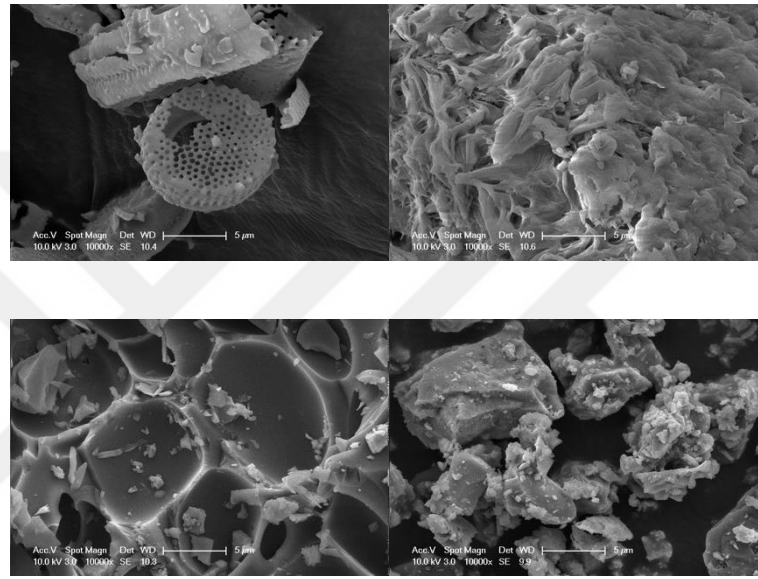


Figure 4. 1: ESEM images of the (a) diatomaceous earth, (b) sepiolite, (c) pumice and (d) CEM I 42.5 R cement.

The cement paste samples were prepared using OPC CEM I 42.5R and a PCE superplasticizer was used in the mixes. Mortar samples were prepared with standard sand accordingly to the norm EN 196-1.

4.2.3 Development of 2-part Bio-VMA

The proposed bio-VMA was developed by simply submerging the minerals to a bacterial suspension for 24 hours. To immobilize the cells on minerals, viable *S. pasteurii* cells were collected as it was stated in Section 4.2.1, and they were either resuspended in a sterilized PBS solution or nutrient medium. Two different dosages of bacterial cells

were used in the mix design as 1.33g and 2.66g (0.2 and 0.4% weight of cement). Then, the selected mineral was added to the suspension. Immobilization of minerals was achieved with shaking conditions (175 rpm) at 30°C for 24 hours. Then, the slurry was removed from incubation and kept at 40°C in an oven for an additional 12 hours. Excess PBS/nutrient medium content on the minerals was calculated by subtracting the known weight of minerals, bacterial cells, and theoretical absorbed aqueous solution from the final weight of the oven-dry slurry. This value was subtracted from the mixing water content.

Herein, 2 different types of bio-VMA were generated. The first type of bio-VMA was developed by immobilizing cells onto minerals with only PBS (bio-VMA-PBS/DE, S, M, and P). To produce bio-VMA-PBS additives, 2.6 grams of viable *S.pasteurii* cells were collected from the bacterial culture and resuspended in a sterilized 116.6 mL PBS solution. Then, the selected minerals (30 g) were simply added to this suspension. This suspension was then placed in an incubator with shaking conditions (175 rpm) at 30°C for 24 hours. Then the slurry was removed from incubation and kept at 40°C in an oven overnight.

The second type of additive referred to as “bio-VMA-NUT/DE, S, M, and P”, has a similar procedure but in this procedure, the total mineral content was divided into 2 as 15 g. Then, 1.3 grams of viable *S.pasteurii* cells were resuspended in a sterilized 58.3 mL PBS solution. The cell dosage was lowered to keep the cell g per mL of PBS constant. The first half of the mineral (15 g) was added to this suspension. The remaining half of the mineral is submerged in a 53.8 g nutrient medium with a pH value of 9, containing CSL (10g/L), urea (20g/L), and calcium acetate 10g/L. These slurries were placed in an

incubator with shaking conditions (175 rpm) at 30°C for 24 hours. Then the slurries were removed from incubation and kept at 40°C in an oven overnight.

In both cases, excess nutrient solution/PBS content was subtracted from the mixing water content. This value was calculated by subtracting the known weight of minerals, bacterial cells and theoretical absorbed PBS from the final weight of oven dry slurry. Figure 4.1 shows the ESEM images after immobilization.

The efficiency of immobilization was proven through scanning electron microscopy analysis (SEM) and the cells were successfully immobilized on the minerals [114].

4.2.4 Sample Preparation

Cement paste samples were prepared with Ordinary Portland Cement (OPC) CEM 42.5 R with a Blaine fineness of 3954 cm²/g conforming EN 197-1 norm. The w/c was kept at 0.50. The PCE superplasticizer was used as 0.5% of cement weight. A set of negative control samples were prepared by only tap water. Another set of control was prepared by only using bacterial cells (1.33g and 2.66g). In addition, a set of samples was prepared with only minerals. However, to see the impact of the saturation control set of samples were prepared with both air-dry minerals and mineral saturated with PBS. The proportions of the mixes used in the study are summarized in Table 4.2.

Table 4.2 Mix design of cement pastes. SP: Superplasticizer; DE: Diatomaceous earth; M: Metakaolin; S: Sepiolite; P: Pumice; PBS: Phosphate Buffer Solution

Sample Name	Ingredients of bio-VMA				Other Ingredients		
	Bacterial Cells (g)	Minerals (g)	PBS (mL)	Nutrient Medium(mL)	SP (g)	Water (g)	Cement (g)
Control Paste	0	-	-	-	3	300	600
Bacterial Paste_1.33	1,33	-	-	-			
Bacterial Paste_1.33	2,66	-	-	-			
Control_DE_D	-	30	-	-			
Control_M_D	-	30	-	-			
Control_S_D	-	30	-	-			
Control_P_D	-	30	-	-			
Control_DE	-	30	116,6	-			
Control_M	-	30	116,6	-			
Control_S	-	30	116,6	-			
Control_P	-	30	116,6	-			
bio-VMA-PBS/DE	2,66	30	116,6	-			
bio-VMA-PBS/M	2,66	30	116,6	-			
bio-VMA-PBS/S	2,66	30	116,6	-			
bio-VMA-PBS/P	2,66	30	116,6	-			
bio-VMA-NUT/DE	1,33	30	58,3	58,3			
bio-VMA-NUT/M	1,33	30	58,3	58,3			
bio-VMA-NUT/S	1,33	30	58,3	58,3			
bio-VMA-NUT/P	1,33	30	58,3	58,3			

Cement paste samples were prepared according to ASTM C305-14 Standard Practice for Mechanical Mixing of Hydraulic Cement Pastes and Mortars. For bacterial paste samples, the cells were added to mix water before mixing, homogenized with hand-mixing for the 30s, and then mixed with cement. The cells were added to the initial 2/3rd of the mix water and the superplasticizer was added to the remaining 1/3rd of the mix water; the last 1/3rd portion of the mix water including superplasticizer was added during the last 60s of the mixing for all samples.

For the preparation of samples with minerals (bio-VMAs), the same sequence of mixing process was followed. Both additives were introduced as in the same step with cement, to avoid creating a different shear history than samples containing solely *S. pasteurii* cells as bio-VMA. For all negative control samples and the samples with bio-

VMAs, the mixing procedure was completed within the 135s period defined by the ASTM C305-14 standard.

4.2.5 Rheological Measurements

The experiments were conducted using ANTON PAAR RheolabQC rotational rheometer (Anton Paar, Graz, Austria) at room temperature (23°C). Before the rheological measurement procedure, to ensure the homogeneity of cement pastes, a pre-shear stage applied where the shear rate was kept constant at 100s^{-1} for 60s. The measurements of rheological behavior were conducted at 3 intervals. The first interval analyzed by increasing the shear rate from 0.5 s^{-1} to 100s^{-1} for 120 s, the second interval had a constant applied shear rate of 100 s^{-1} for the 60s, and the third interval had a decreasing the shear rate from 100 s^{-1} to 0.5 s^{-1} for 120 s. The yield stresses and viscosity results are recorded where the rheological behavior of the cement paste was evaluated using the downward curve with the Bingham model (1) as it was explained in section 3.2.6.

4.2.6 Mini-Slump Test

For the measurements of the initial spread of cement pastes, a cone-shaped mold filled with sample, placed on smooth plexiglass. The dimensions of the mini-slump cone were explained in Section 3.2.7 After filling the samples into the cone placed on the center of plexiglass immediately after mixing, the cone was lifted to measure the flow. The diameters at four right-angle positions were measured and the average diameter was calculated.

4.2.7 Compressive Strength Testing

Flow table and compressive strength tests were conducted to determine the performance of cement-based mixes containing 2-part bio-VMAs. The preparation process of additives was stated in section 4.2.3. Mortar mixes were prepared by adding standard sand to the cement paste mixes listed in Table 4.2 with few modifications. The w/c was kept at 0.45. All mineral additions were calculated to be 5% by cement weight but herein pumice was used as a sand replacement 5% by sand weight. Cement to sand ratio was kept constant at 1:3. The workability of samples was evaluated according to ASTM C1437-15 Standard Test Method for Flow of Hydraulic Cement Mortar [97]. Herein, the flow diameters were kept at ± 1 cm range with respect to the control mortar for strength evaluation. The flow comparison was done based on the PCE superplasticizer demand to obtain the same flow diameter.

The mortars samples were then cast in 5x5x5 cm cubes. Samples were kept in a humid environment for 24 hours and then the molds were removed. Samples were further cured at room temperature (23°C) in a humid environment (100% relative humidity) until testing. Compressive strength test was done according to ASTM C109-13e1 Standard Test Method for Compressive Strength of Hydraulic Cement Mortars[98] at 3, 7, and 28 days after mixing.

4.3 Results and Discussion

This study was undertaken to evaluate the influence of a novel 2-part bio-VMA consisting of *S.pasteurii* cells immobilized on locally available minerals of diatomaceous earth, metakaolin, sepiolite, and pumice. The rheological analysis was done by viscosity and yield stress evaluation, followed by a mini-slump test. Our results showed that the

proposed bio-VMA significantly enhanced the yield stress and viscosity of cement pastes compared to control neat pastes.

4.3.1 Influence of Mineral Saturation on Rheological Properties

To develop the 2-part bio-VMA, the minerals were submerged either in PBS or nutrient medium. The effect of minerals might depend if they were introduced dry or saturated. Thus, out the first set of the initial evaluation was based on the rheological behavior of cement-paste samples when the minerals were added either air dry (D) or saturated condition. Figure 4.3 presents the shear profile of cement paste samples containing air dry (a) and saturated minerals (b).

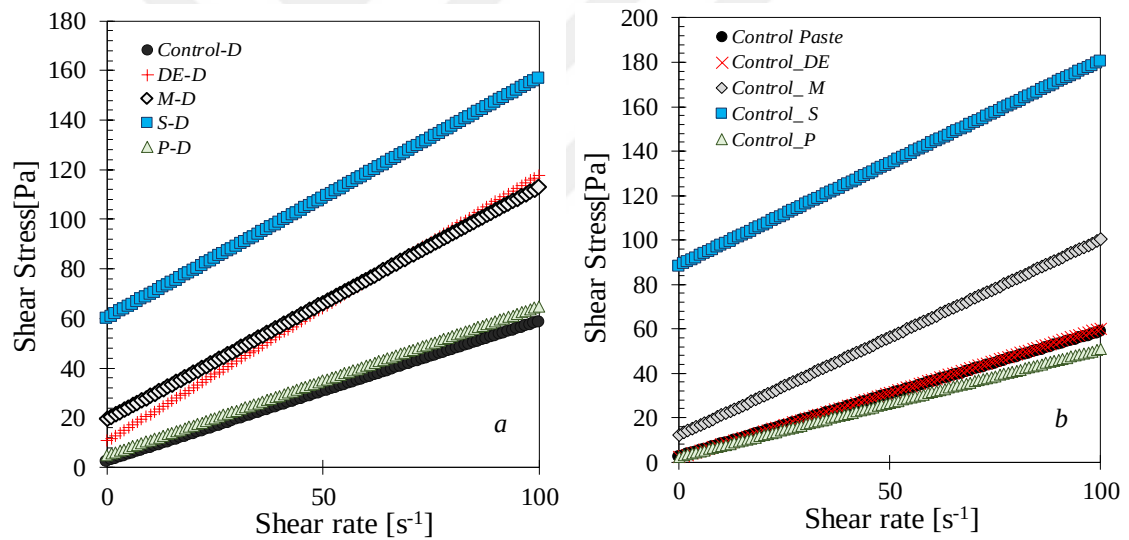


Figure 4.2: Rheological test results for cement pastes. w/c:0.50; All samples contain 0.5% of superplasticizer by cement weight. DE: Diatomaceous Earth; M: Metakaolin; S: Sepiolite; P: Pumice. Minerals (a) D: Air dry addition, (b) saturated addition. Control paste: No additive.

As mentioned before the rheological evaluation was done based on Bingham analysis. The presented yield stress results were calculated from the regression line of the downward flow curve and the plastic viscosity was determined from the slope of the regression line. Incorporation of dry minerals increased the yield stress of cement paste

compared to the control neat paste (see Table 4.2). The increase in yield stress was more pronounced in samples containing sepiolite and partially in samples containing metakaolin. Relatedly, mini-slump diameters of these mineral incorporated samples were reduced compared to control neat paste. Both metakaolin and sepiolite were classified as clays and particularly metakaolin was previously used as a rheological modifier in the literature [99]. Melo et al. [46], studied the effect of metakaolin and sepiolite addition on the performance of cement paste rheology and presented improved yield stress and water retention capacity of cement pastes. Clays are known to be hydrophilic materials which ease their incorporation in concrete, but they also reduce the workability of the mix due to their high-water absorption capacity and flocculated structure. Their adaptive agglomeration in the mix effect the rheology by increasing yield stress and viscosity. Researches also showed that these clays might induce thixotropic affect by improving structural rebuilding [100]. The addition of saturated DE and pumice eliminated their impact on yield stress, which suggested that these minerals directly affected the rheology by simply absorbing the water phase. Previous studies on the effect of the use of DE by on rheological properties of cement paste also suggested that the change in yield stress and viscosity was caused by the increased rate of water absorption [49]. DE is highly porous having approximately 80% void content [101]. A similar behavior could also be expected from pumice due to a relatively large pore structure (see Figure 4.1). They tend to improve the rheological properties by only absorb the mix water with highly porous morphology and high specific surface characteristics [102]. Partially saturating these minerals reducing their capability to absorb water and increasing yield stress.

Table 4.3 Yield stress, average mini-slump, and plastic viscosity measurements of cement pastes. Control: No Additive; D: Air Dry added; Control Minerals: Saturated minerals. DE: Diatomaceous Earth; M: Metakaolin; S: Sepiolite; P: Pumice.

Sample Name	Mini Slump (cm)	Yield Stress [Pa]	Plastic Viscosity (Pa.s)
Control Paste	13,6	2,3	0,6
Control_DE	13,4	2,2	0,6
DE_D	11,9	10,7	1,1
Control_M	12,1	12,4	0,9
M_D	10,5	19,4	0,9
Control_S	4,5	88,3	0,9
S_D	9,2	59,9	1,0
Control_P	14,6	2,5	0,5
Control_P_D	13,7	4,9	0,6

Even though there was a partial reduction in yield stress increase when saturated metakaolin was added to mix, the yield stress was still 6 times larger than the control neat paste. This was due to its possible greater specific surface area enhancing the interaction of solid particles even they are saturated [99].

Interestingly, there was an opposite trend in samples containing saturated sepiolite. Such that, the relative yield stress increase in samples containing saturated sepiolite was even more pronounced than that of samples containing dry sepiolite (see Table 4.2). The addition of saturated sepiolite resulted in an approximately 50% increase in yield stress compared to the incorporation of dry sepiolite. Even though the exact reason for such an increasing behavior is not known this might be due to possible nucleation of crystalline materials on sepiolite. The addition of sepiolite can improve particle packing and increase the friction between the particles [102]. Moreover, the fibrous microstructure of sepiolite (see Figure 4.1) increases the torque implemented which further increases the rheological properties. Melo et al. [46], explained the increase in yield stress due to metakaolin and sepiolite addition, by the formation of gel-like crystalline on the particle surface, due to the dissolving calcium and hydroxyl ions of

mineral when introduced to water. Further research has to be done to understand the kinematic of sepiolite in pore solution as well as its interaction with cement particles.

The mini-slump diameters were also in-line with the yield stress measurements such that as the yield stress increases mini-slump values were also reduced (see Table 4.2). This also suggests that incorporation minerals resulted in homogenous mixes in which the rheological behavior could be correlated with the workability.

Along with the yield stress, the flow curves suggested that the incorporation of dry minerals increased the plastic viscosity compared to the control neat sample. The increase in viscosity for DE and pumice was consistent with the increase in yield stress. A further analysis was done on the apparent viscosity of samples at low shear rates resembling pumping (see Figure 4.3). As expected, the highest apparent viscosity values were obtained with the incorporation of sepiolite regardless of its moisture state. Herein, it could be concluded that sepiolite and metakaolin could be incorporated in cement-based materials as VMAs (or rheology modifiers) regardless of their moisture state. Whereas the influence of DE and pumice on rheological properties was much less pronounced, even they were added air dry.

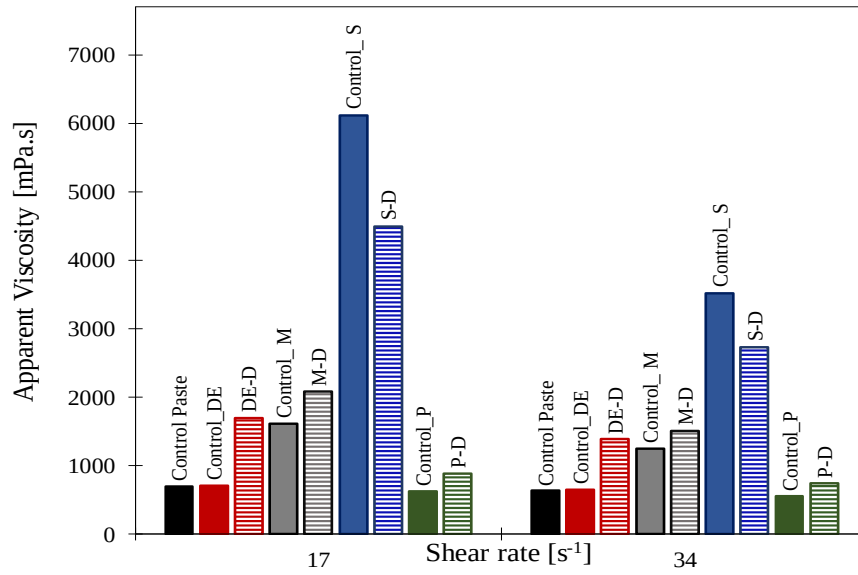


Figure 4.3: Apparent viscosity values for samples at shear rates of 17 and 34 s^{-1} . Control paste: No Additive; D: Air dry added; Control Minerals: Saturated mineral addition. DE: Diatomaceous Earth; M: Metakaolin; S: Sepiolite; P: Pumice.

4.3.2 Influence of 2-part Bio-VMAs on Rheological Properties: Without Nutrients

Previously, it was found that direct incorporation of vegetative *S.pasteurii* cells increased the yield stress and viscosity of cement paste having a w/c less than 0.45 [31]. However, the efficiency of cells on rheological properties was less pronounced when they were incorporated at higher w/c (0.50) and with high-range water reducers (HRWR). In particular, minerals, sepiolite, and metakaolin found to be effective in terms of increasing rheological properties at a w/c of 0.50 in cement paste samples containing PCE superplasticizers. Thus, it might be possible to develop a 2-part bio-VMA with improved efficiency by combining these 2 VMAs. Figure 4.4 show the shear profiles and viscosity curves for cement pastes samples containing bacterial cells. Remaining viscosity curves of cement pastes regarding chapter 4 presented in Appendix B. Figure 4.5 exhibits the shear profiles for cement paste samples containing 2-part bio-VMA. Incorporation of only

bacterial cells at a lower dosage (1.33 g) leads to higher yield stress and plastic viscosity compared to the sample containing 2.66 g of bacterial cells. This perfectly matched to our previous findings [31]. As explained in Section 3.3 such a trend could be related to the action mechanism of bacterial cells can such that peptidoglycans and polysaccharides in the cell structure restrict the motion of water through constituted gel structure due to intertwining of polysaccharides and peptidoglycans chains and resulting in a thickening behavior. This is much more predominant at low shear rates. While the increasing cell dosage led to higher plastic viscosity and yield stress at $w/c < 0.40$, the relationship between cell dosage and rheological performance was rather inconsistent when $w/c > 0.45$ [31]. It was found that for cement paste with higher w/c , the optimum cell dosage should be around 0.1%. Herein, we also observed a similar behavior, and incorporating *S. pasteurii* cells at 0.2% yielded higher rheological values compared to the addition of cells 0.4% of cement weight.

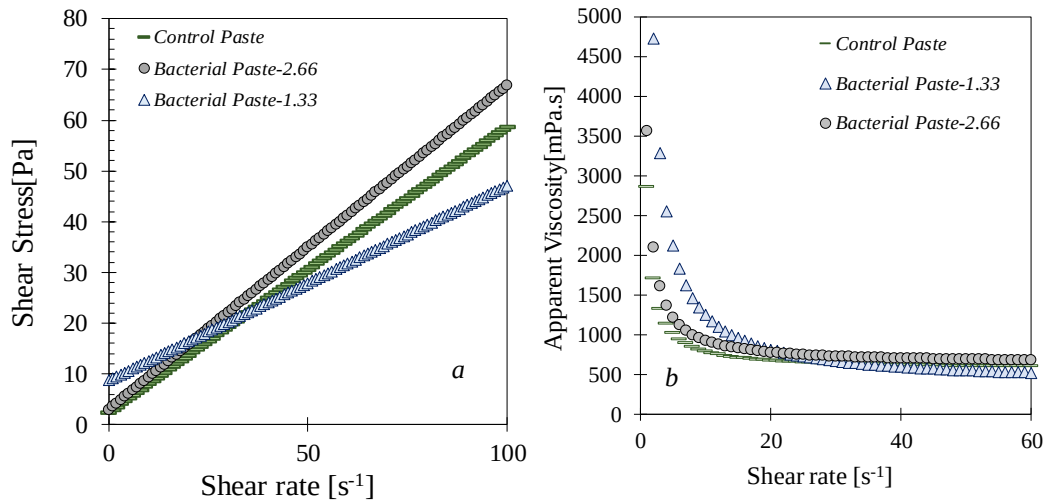


Figure 4.4: Rheological test results for cement pastes. (a) shear stress response with increasing shear rate (b) change in viscosity with increasing shear rate. $w/c:0.50$; All samples contain 0.5% of superplasticizer by cement weight. Bacterial Paste: Addition of vegetative *S.pasteurii* cells by 2.33 g and 1.33 g.

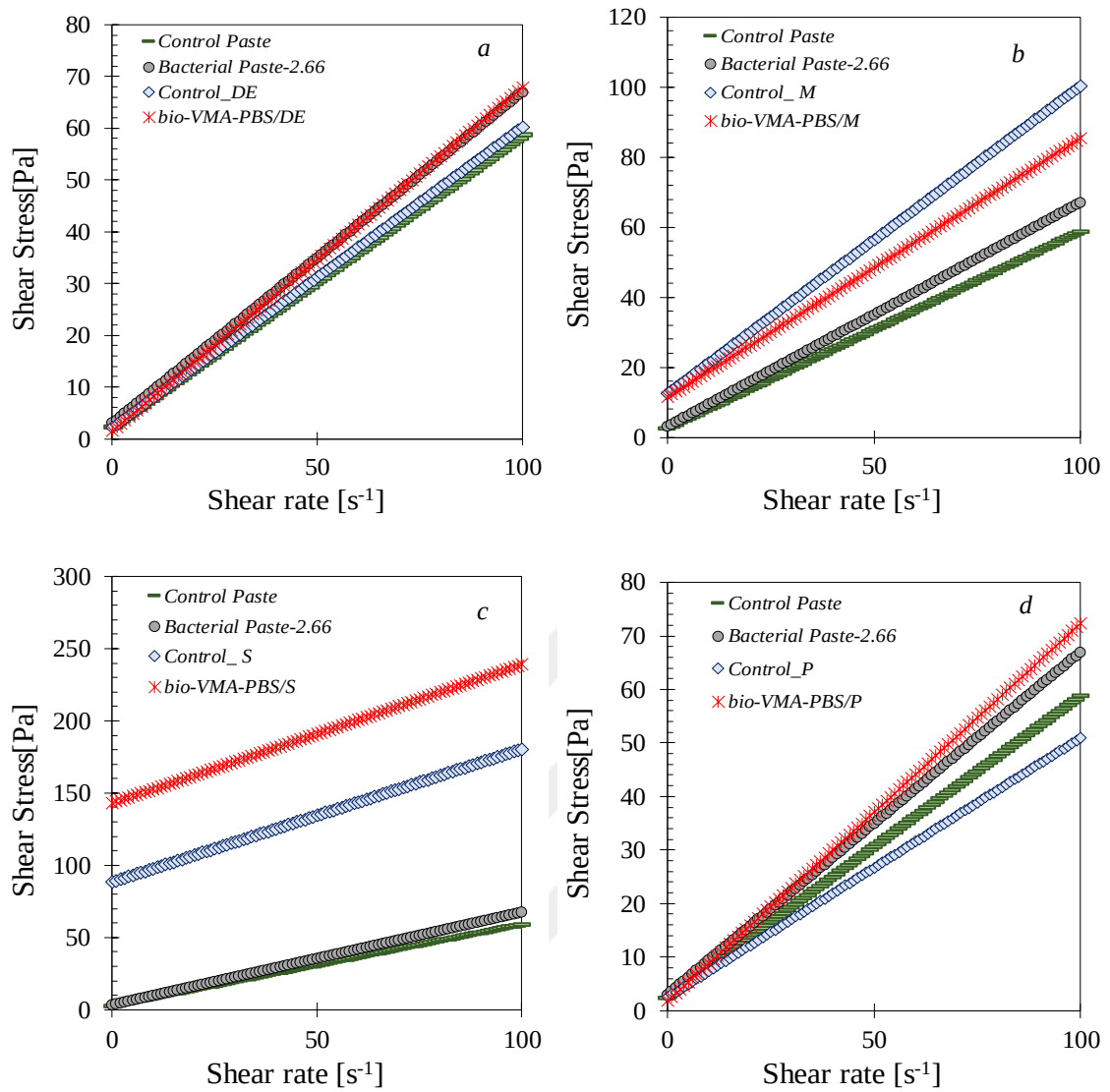


Figure 4.5: Rheological test results for cement pastes. w/c:0.50; All samples contain 0.5% of superplasticizer by cement weight. Control Paste: No Additive; Bacterial Paste: 2.66g *S.pasteurii* cells added paste; Control_Minerals: Saturated mineral added samples; *bio-VMA-PBS/Mineral*: 2.66 g *S.pasteurii* immobilized mineral samples.(a) DE: Diatomaceous Earth; (b)M: Metakaolin; (c) S: Sepiolite; (d) P: Pumice.

In terms of materials, VMA behavior was only observed in metakaolin and sepiolite. As expected, incorporation of neither DE nor pumice resulted in an increase in yield stress (see Table 4.3). It should also be noted that the control samples herein were also fully saturated. Immobilization of bacterial cells on DE and pumice resulted in lower yield stress even compared to their counterpart saturated minerals. The mini-slump

diameters were also decreased in these samples even though the yield stress values. But these changes are rather within 5% range, which could be neglected.

Table 4.4 Yield stress, average mini-slump, plastic viscosity measurements of cement pastes. Control Paste: No Additive; Bacterial Paste: 2.66g *S.pasteurii* cells used as bio-VMA; *bio-VMA-PBS/Minerals*: 2.66g *S.pasteurii* immobilized on minerals; DE: Diatomaceous Earth; M: Metakaolin; S: Sepiolite; P: Pumice.

Sample Name	Mini Slump (cm)	Yield Stress [Pa]	Plastic Viscosity (Pa.s)
Control Paste	13,6	2,3	0,6
Bacterial Paste-2.66	13,7	2,9	0,6
Control_DE	13,4	2,2	0,6
<i>bio-VMA-PBS/DE</i>	12,9	1,5	0,7
Control_M	12,1	12,4	0,9
<i>bio-VMA-PBS/M</i>	10,5	11,4	0,7
Control_S	4,5	88,3	0,9
<i>bio-VMA-PBS/S</i>	4,2	142,9	1,0
Control_P	14,6	2,5	0,5
<i>bio-VMA-PBS/P</i>	14,4	1,8	0,7

Both metakaolin and sepiolite are well-known rheology modifiers [46], [103], [104]. There are various studies evaluating the performance of metakaolin in increasing viscosity and yield stress. As mentioned in Section 4.3.1 incorporation of dry or partially saturated metakaolin increased the yield stress of cement paste compared to the control paste. Incorporation of metakaolin with bacterial cells resulted with a 17% decrease in mini-slump diameter and an 8% decrease in yield stress compared to the addition of partially saturated metakaolin. This was a similar trend of decrease when saturated metakaolin was used instead of dry metakaolin. However, even in this case, the yield stress was almost 6 times larger than control cement paste. As stated, before metakaolin is a calcined clay that influences the rheology by absorbing mix water and triggering flocculation in the mix. Saturating metakaolin decreases its absorption capacity during mixing thus reducing its efficiency as a rheology modifier due to increasing moisture state

of the mineral leading to a decrease in absorbed mixing water. Metakaolin is a calcined clay with low surface area and permanent negative charges in the faces [105]. Metakaolin has a relatively smaller particle size compared to ($d_{50}=12-13\text{ }\mu\text{m}$)[46] which might lead to smaller pores compared to other studies minerals. Hence, the efficiency of bacterial cell immobilization might be relatively lower among the rest of the minerals (ESEM data could not be provided due to COVID-19 lockdown). Thus, cells immobilized on metakaolin could not influence the yield stress as expected.

However, a totally different behavior was observed when sepiolite was incorporated with bacterial cells. Incorporation of saturated sepiolite resulted with a 50% increase in yield stress compared to the addition of dry sepiolite. Similarly, incorporation of 2-part bio-VMA containing bacterial cells immobilized on sepiolite lead to a 62% increase in yield stress compared to samples containing only saturated sepiolite. It should also be noted that the only difference between the Control-S sample and bio- VMA/PBS-S sample is the presence of cells. Other than this, the immobilization method, carrier PBS solution, and volume were constant. So even though the bacterial cells alone did not yield any increase in yield stress in a highly flowable mix, incorporation of cells with sepiolite significantly improved the yield stress. Sepiolite is a hydrated magnesium silicate clay with high porosity and fibrous structure (see Figure 4.1). The chemical composition and relatively large surface area of sepiolite made the mineral a suitable host for bacterial cells. Our previous studies showed that *S. pasteurii* cells immobilized on sepiolite can self-heal the microcracks in 28-day-old mortars and kept the cells viable for a prolonged time period when nutrients were provided [114]. Even though there is no direct evidence of strong cell attachment to sepiolite, these results might indicate that sepiolite with its fibrous shape might enable a strong cell attachment without caging the cells so that they could also bond with water molecules. Thus, intercellular peptidoglycans and

polysaccharides could also embed mix water due to the intertwining. The porous, fibrous, sharp edges of sepiolite particles[47], might be creating a strong solid particle network and further advancing its capability with *S.pasteurii* cells.

The addition of bio-VMAs obtained by metakaolin and sepiolite (*bio-VMA/PBS-M* and *bio-VMA/PBS-S*) have increased the plastic viscosity. Figure 4.6 summarizes the detailed apparent viscosity evaluation was done at low shear rates for samples containing bio-VMA. As expected, the highest increase was again recorded in *bio-VMA/PBS-S* sample which was in line with what was observed with the yield stress.

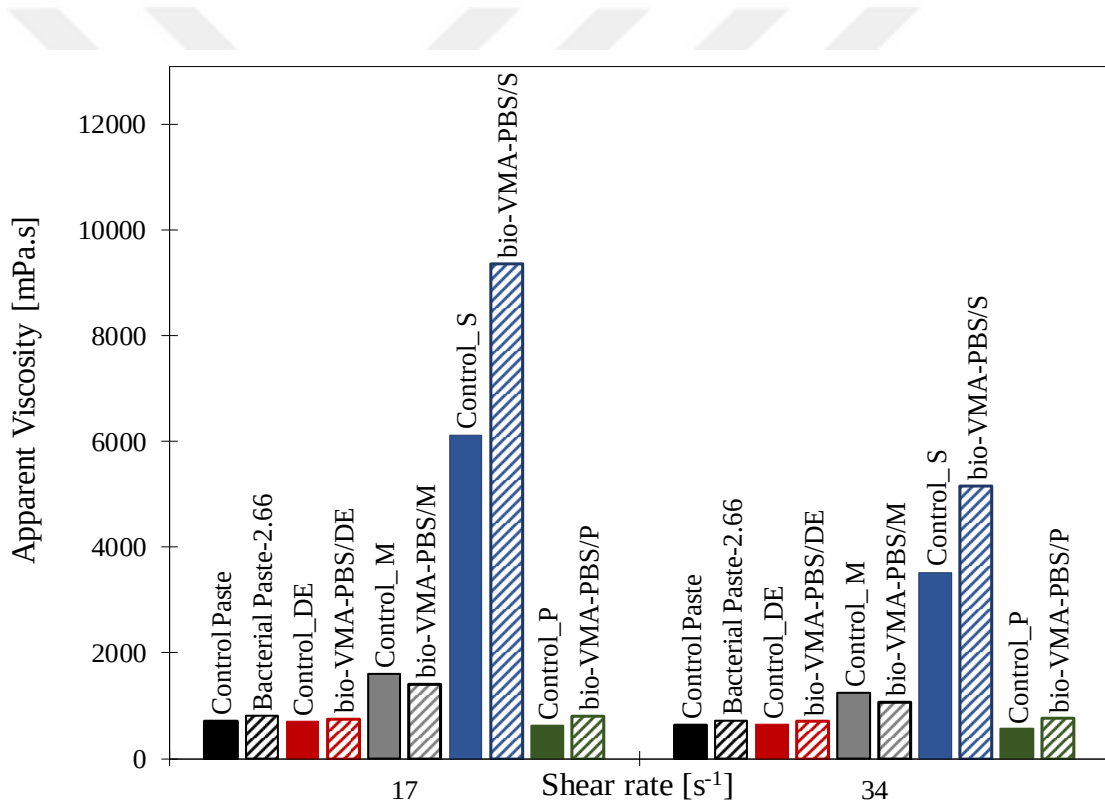


Figure 4.6: Apparent viscosity results for cement pastes. All samples contain 0.5% of superplasticizer by cement weight. Control: No Additive; Bacterial Paste: 2.66 g *S.pasteurii* addition; *bio-VMA-PBS/Minerals*: 2.66g *S.pasteurii* immobilized mineral addition. DE: Diatomaceous Earth; M: Metakaolin; S: Sepiolite; P: Pumice.

The apparent viscosity of the *bio-VMA/PBS-S* sample at $17s^{-1}$ was 65% than the apparent viscosity of *Control-S* sample at the same shear rate. Relatedly, the apparent

viscosity of *bio-VMA/PBS-M* sample at 17s^{-1} was almost on par with the apparent viscosity of *Control-M* sample at the same shear rate. As mentioned before both metakaolin and sepiolite were previously used as a rheology modifier in cement-based materials [46], [104], [106]. Saleh Ahari et al.[99] evaluated binary, ternary, and quaternary use of various supplementary cementitious materials (SCMs) on the rheology of cement paste. The study showed that the incorporation of metakaolin increased the plastic viscosity at a w/c of 0.50 when 36% of cement content was replaced with the mineral. Herein, all minerals were used as an addition in this study and they were incorporated as 5% of the cement weight. Similarly, Saleh Ahari et al.[99] also observed almost no change in viscosity with 4% metakaolin replacement and only a 5% increase with 8% metakaolin replacement. A similar trend was also observed in yield stress values at these dosages. We also observed a limited increase in rheological parameters with addition of 5% of metakaolin by weight of cement. Thus, it could be possible that rheology modifying effects of metakaolin could be observed when the mineral is used more than 30% of the binder weight. Based on these results *bio-VMA* containing sepiolite and bacterial yielded with promising rheological parameters that could be used in applications like 3-D printing. Further investigations should be done simply to understand the interaction of the microorganisms with sepiolite.

4.3.3 Influence of 2-part Bio-VMAs on Rheological Properties: With Nutrients

Previously, our results showed that bacterial cells immobilized on these minerals triggered self-healing in mortars mostly when nutrients were provided either in the mix or in curing solution [114]. In fact, incorporating the cells with nutrients might provide not only a cement-based mix with improved rheology but also enabling self-healing

mortar. The effects of introducing nutrients along with bio-VMAs were also evaluated. Figure 4.7 represents the shear profile of cement paste samples containing bacterial cells immobilized on minerals with their nutrients (bio-VMA/NUT). It should be noted that half of the mineral was partially saturated with nutrients while the other half was partially saturated with bacterial cell suspension in PBS. For this approach, 1.33 g of cells were incorporated along with 58.3 mL PBS to keep the cell g per mL of PBS constant.

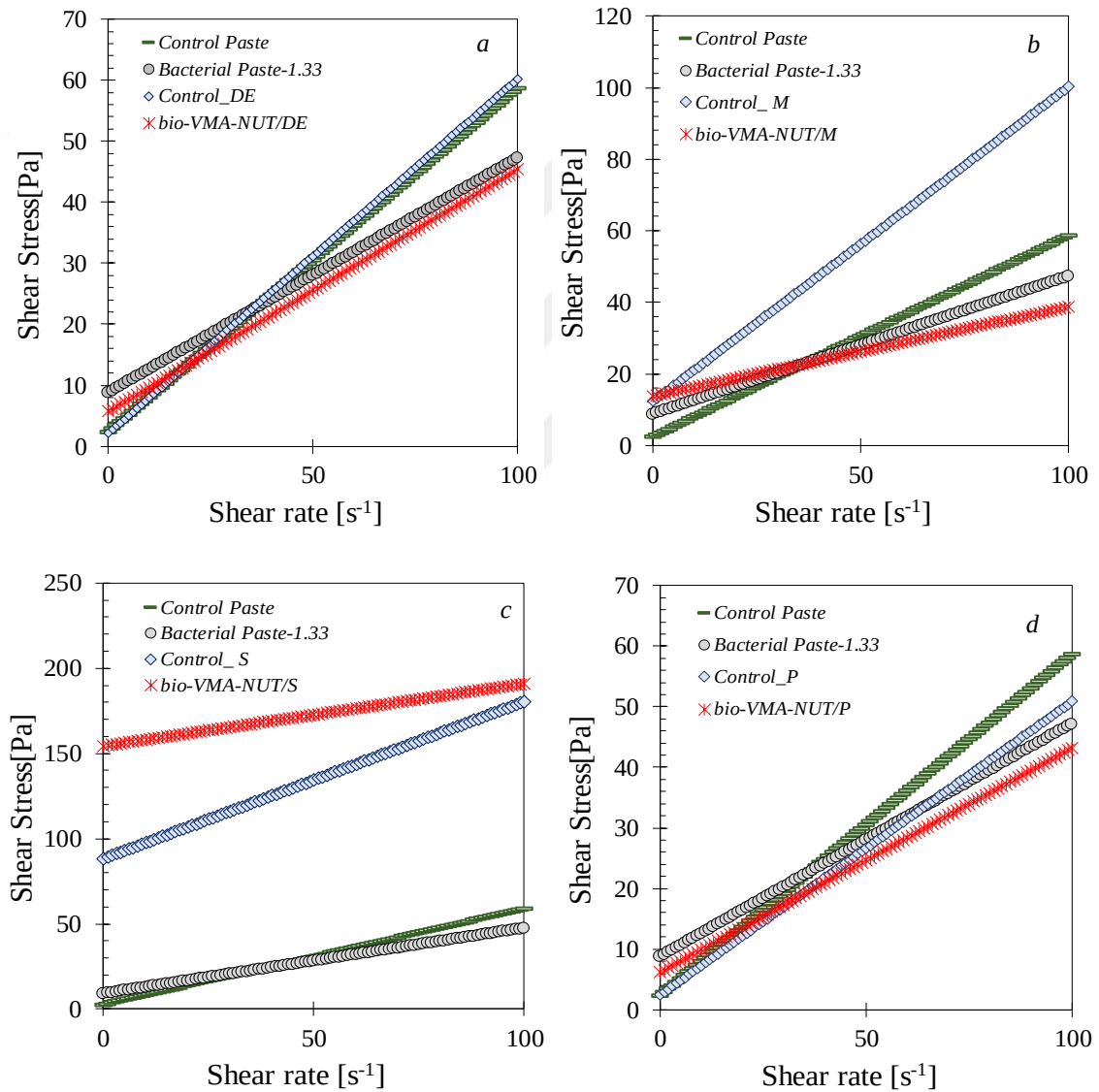


Figure 4.7: Rheological test results for cement pastes. w/c:0.50; All samples contain 0.5% of superplasticizer by cement weight. Control Paste: No Additive; Bacterial Paste: 1.33 g *S.pasteurii* cells added paste; Control_Minerals: Saturated mineral added samples; bio-VMA-NUT/Mineral: 1.33 g *S.pasteurii* immobilized with nutrients on minerals. (a) DE: Diatomaceous Earth; (b)M: Metakaolin; (c) S: Sepiolite; (d)P: Pumice.

Interestingly, incorporation of bacterial cells along with their nutrients resulted in improved yield stress for all cement-paste samples. There was not any yield stress increase in neither partially PBS saturated Control -DE sample nor bio- VMA/PBS-DE sample. But the yield stress was doubled compared to control cement pastes with incorporation cells immobilized on DE along with its nutrients (see *bio-VMA/NUT-DE* sample in Table 4.4). Similar behavior was also observed when bacterial cells were incorporated on pumices with their nutrients (see *bio-VMA/NUT-P* sample in Table 4.4). Herein, only 58.3 mL of the nutrient solution was used in the mix which contained 1% of CSL, 1% Na-Acetate, 1.5% tris base, and 2% urea by weight of the solution. So, 95% of the nutrient medium is DI water. The amount of chemicals (nutrients) presented in the mix made up ~0.6% of cement weight. But they significantly affected the yield stress values in those minerals, DE and pumice, which did not yield any impact previously.

Table 4.5 Yield stress, average mini slump and plastic viscosity measurements of cement pastes. Control: No Additive; *bio-VMA-PBS/Minerals*: 2.66g *S.pasteurii* immobilized minerals; *bio-VMA-NUT/Mineral*: 1.33 g *S.pasteurii* immobilized with nutrients on minerals. DE: Diatomaceous Earth; M: Metakaolin; S: Sepiolite; P: Pumice

Sample Name	Mini Slump (cm)	Yield Stress [Pa]	Plastic Viscosity (Pa.s)
Control Paste	13,6	2,3	0,6
Bacterial Paste-1.33	12,8	8,7	0,4
Bacterial Paste-2.66	13,7	2,9	0,6
Control_DE	13,4	2,2	0,6
<i>bio-VMA-NUT/DE</i>	14,1	5,7	0,4
<i>bio-VMA-PBS/DE</i>	12,9	1,5	0,7
Control_M	12,1	12,4	0,9
<i>bio-VMA-NUT/M</i>	13,4	13,8	0,3
<i>bio-VMA-PBS/M</i>	10,5	11,4	0,7
Control_S	4,5	88,3	0,9
<i>bio-VMA-NUT/S</i>	4,8	154,3	0,4
<i>bio-VMA-PBS/S</i>	4,2	142,9	1,0
Control_P	14,6	2,5	0,5
<i>bio-VMA-NUT/P</i>	14,0	6,2	0,4
<i>bio-VMA-PBS/P</i>	14,4	1,8	0,7

As mentioned above, the negatively charged *S. pasteurii* cells have the capacity to bond with cement and water molecules, as well as increasing the weight of flocs when immobilized onto mineral. As mentioned in Section 4.3.2, the influence of cells was much more pronounced when they were incorporated at a lower dosage (1.33 g). In addition, the presence of neither DE nor pumice induced a change in yield value in the previous analysis. Lowering the cell dosage and incorporating the nutrients also affected the efficiency of bio-VMA prepared by metakaolin. While the incorporation of bacterial cells without nutrients (*bio-VMA/PBS-M* sample) resulted in a slight decrease in yields stress compared to the incorporation of metakaolin without bacterial cells (*Control-M* sample). In fact, providing nutrients along with bio-VMA (*bio-VMA/NUT-M* sample) resulted with an increase in yield stress compared to both *Control-M* and *bio-VMA/PBS-M* samples. Interestingly, the addition of nutrients along with bio-VMA containing sepiolite resulted with an 8% increase compared to *bio-VMA/PBS-s* sample, which was a less pronounced impact compared to the other immobilization minerals. However, as a general trend, *bio-VMA/NUT* samples showed a higher performance compared to *bio-VMA/PBS* samples in terms of improving rheology. The increase in yield stress *bio-VMA/NUT* samples could be solely due to the incorporation of bacterial cells at optimum dosage. As stated in Section 4.3.2, the incorporation of bacterial cells at lower dosage (1.33g) resulted with a higher yield value compared to a higher dosage (2.66 g). While *bio-VMA/PBS* samples contain 2.66 g of bacterial cells, *bio-VMA/NUT* samples contain 1.33g samples. This decrease in yield stress with increasing cell dosage might be attributed the low molecular mass of bacterial cells which reduces their affinity with cement particles [2]. Increasing anionicity due to a higher dosage of bacterial cells and PCEs might reduce the flocculation of particles. In addition, incorporation of nutrients ions might interfere with surface charges in the bio-VMA and improving flocculation in cement paste [47].

The impact of *bio-VMA/NUT* samples on plastic viscosity was rather different than *bio-VMA/PBS* and *Control* samples. Such that incorporation of *bio-VMA/NUT* samples resulted with an increase in yield stress compared to *bio-VMA/PBS* samples. However, incorporation of low dosage bacterial cells with their nutrients did not affect the plastic viscosity, except for samples containing sepiolite (see Table 4.4). To understand the influence of cells on viscosity, further analysis was done at low shear rates and Figure 4.8 summarized the apparent viscosity of samples at 17 and 34 s⁻¹.

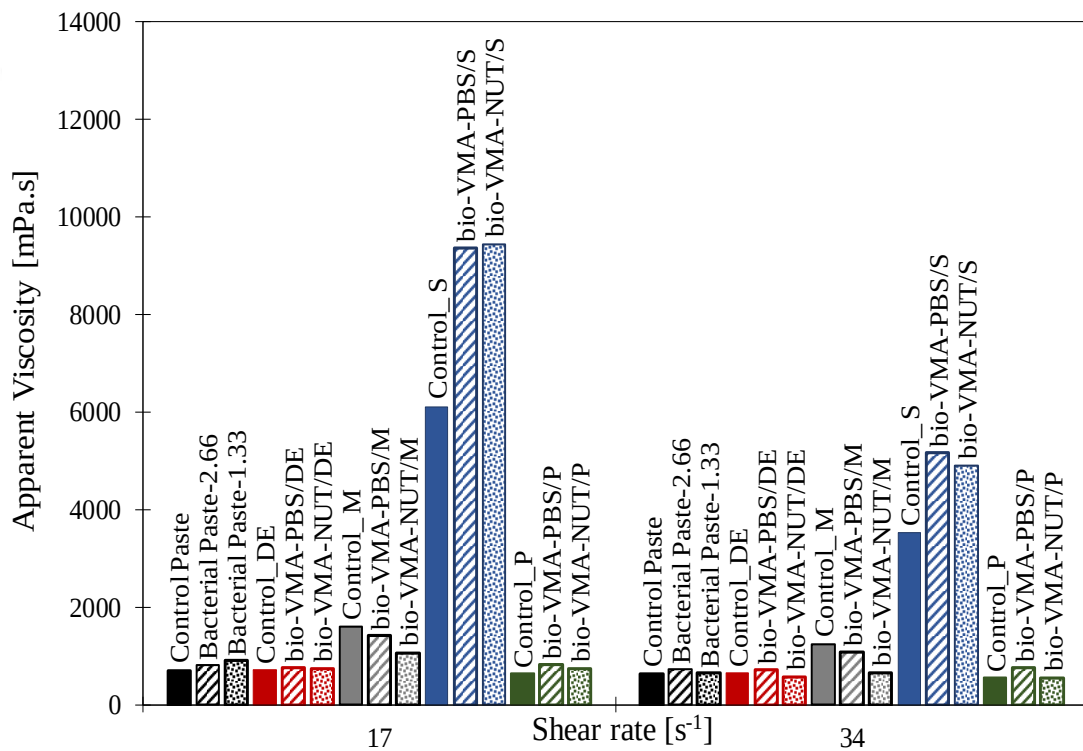


Figure 4.8: Apparent viscosity results for cement pastes. All samples contain 0.5% of superplasticizer by cement weight. Control: No Additive; bio-VMA-PBS/Mineral: 2.66 g *S.pasteurii* immobilized on minerals; bio-VMA-NUT/Mineral: 1.33g *S.pasteurii* immobilized with nutrients on minerals. DE: Diatomaceous Earth; M: Metakaolin; S: Sepiolite; P: Pumice.

While the change in yield stress and viscosity in *Control* and *bio-VMA/PBS* samples were relatively correlated. Incorporation 2-part bio-additives were prepared with nutrients and lower cell dosage led to higher yield stress but lower apparent viscosity

values even at a very low shear rate (17s^{-1}). As expected, the only viscosity increase was observed in samples with sepiolite (*bio-VMA/NUT-S*) and it was on par with *bio-VMA/PBS-S* sample. Increasing the shear rate to 34s^{-1} , resulted with a 40% decrease in viscosity, which is also an indicative of strong shear-thinning behavior. Bio-VMAs containing a lower cell dosage and nutrients lead to increased yield stress while both apparent and plastic viscosity changes were limited compared to sample containing the use of higher cell dosage without nutrients. This behavior could be attributed to the change in material with applied torque. Yield stress is a parameter reflecting the workability of the mix, but the actual flow behavior is governed by viscosity [79]. Incorporation of efficient bio-VMAs absorbs mixing water and increase particle flocculation. This interaction results with a more cohesive and stiff mixes which require a higher torque and shear stress to initiate flow. Thus, corresponding yield stress increases. However, once this high torque is applied and overcomes the yield stress the material starts to flow. Flow behavior is governed by viscosity. While 2-part bio-VMAs containing DE, pumice, and MK influences the rheology by improving only yield stress containing admixture resulted with a significant increase in both rheological parameters. Further research has to be done to evaluate the possible applications of sepiolite containing bio-VMA in applications like additive manufacturing or grouting.

4.3.4 Performance of Mortars Containing Bio-VMAs

Previously, the minerals used in the development of bio-VMAs found to be effective in increasing the compressive strength of concrete when they were used as a cement replacement [101], [107]–[112]. Thus, it is important the evaluate the workability and strength of mortars prepared with the proposed bio-VMAs.

Table 4.6 summarizes the average flow table diameters for mortar samples containing bio-VMAs. To provide the same consistency in the mixes for strength assessment, the flow diameters were kept in a range of ± 1 cm with respect to the control mortar. Herein, samples containing sepiolite, which again shows that incorporation of sepiolite reduces the workability by increasing consistency and stiffness. It should be noted that pumice used as an aggregate here, thus it would not be correct to directly correlate the rheology to the workability of the mortar. However, incorporation of bacterial cells immobilized in pumice aggregates ($< 2\text{mm}$) led to the highest flow diameters which could be expected bacterial cells immobilized on fine pumice particle provided the least efficient bio-VMA performance.

Another point that should be mentioned is the incorporation of bacterial cells immobilized on metakaolin did not require any PCE superplasticizers to maintain the same slump with the control mortar, while the rheological analysis showed that the yield strength of metakaolin containing samples was almost 6 times larger than the control paste. An opposite trend was also observed in DE containing samples such that while the yield stress of *Control DE* and *bio-VMA/PBS-DE* samples were on par with control paste, these samples required 3 g of PCE superplasticizers to maintain the same flow diameter. Except for *bio-VMA/NUT-S* sample incorporation of bacterial cells did not increase the stiffness of the mix. There is no correlation between the workability of mortar mixes with the rheology of cement paste. However, since the same type and content was the same for all samples (except samples containing pumice), it could be concluded that sepiolite minerals and bio-VMAs produced using sepiolite could be used as a rheology modifier both in cement paste and mortar. Further research has to be done to evaluate the rheology of mortar samples containing bio-VMA.

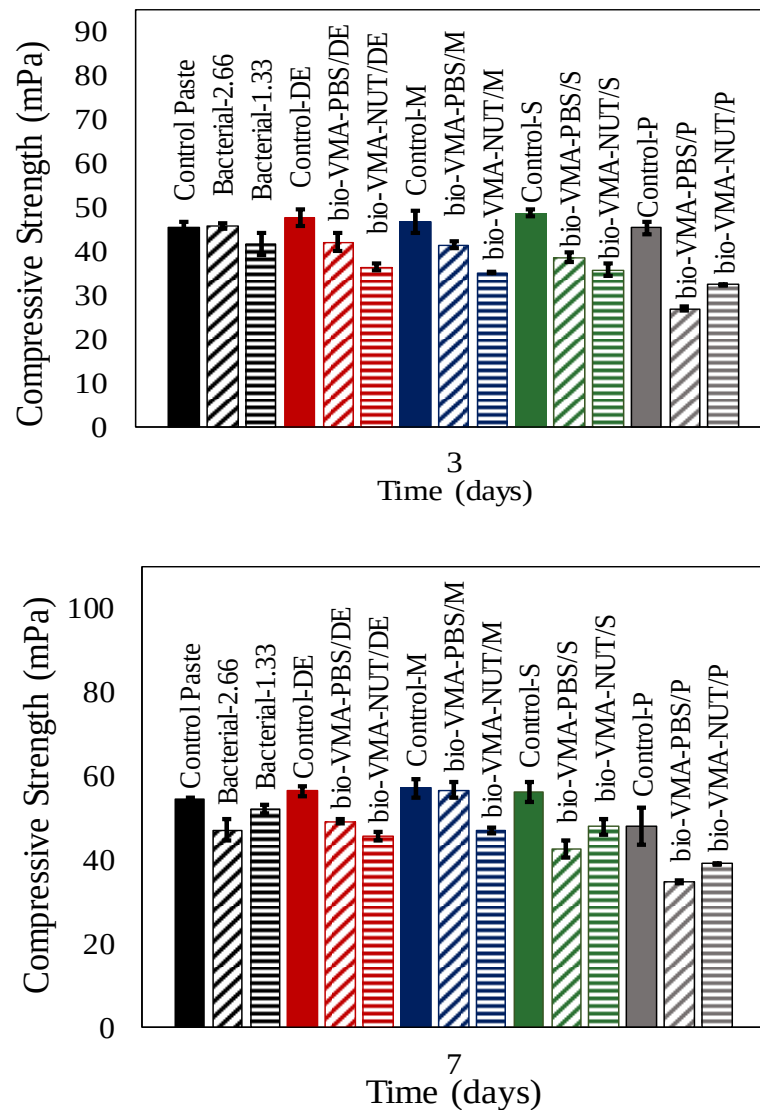
Table 4.6 Flow table diameters for mortar samples and dosages of admixtures used. Water: 202,5 g, Cement: 450 g, Sand: 1350 g.; DE, Metakaolin, and Sepiolite was used 5% cement addition and Pumice was used as 5% sand replacement.

Sample Name (w/c = 0.45)	Mineral (g)	<i>S.pasteurii</i> cells(g)	Superplasticizer (g)	Flow (cm)
Control Paste	-	-	-	13 ± 0,1
<i>Bacterial-2.66</i>	-	2	1	13,1 ± 0,2
<i>Bacterial-1.33</i>	-	1	1	13,1 ± 0,2
Control-DE	22,5	-	3	12,9 ± 0,1
<i>bio-VMA-PBS/DE</i>	22,5	2	3	13,4 ± 0,3
<i>bio-VMA-NUT/DE</i>	22,5	1	-	13,5 ± 0,3
Control-M	22,5	-	2,25	12,9 ± 0,3
<i>bio-VMA-PBS/M</i>	22,5	2	-	13,5 ± 0,1
<i>bio-VMA-NUT/M</i>	22,5	1	-	12,7 ± 0,1
Control-S	22,5	-	4,5	12 ± 0,3
<i>bio-VMA-PBS/S</i>	22,5	2	3	12 ± 0,1
<i>bio-VMA-NUT/S</i>	22,5	1	4,5	10,4 ± 0,1
Control-P	67,5	-	2,25	13 ± 0,2
<i>bio-VMA-PBS/P</i>	67,5	6	-	18,5 ± 0,4
<i>bio-VMA-NUT/P</i>	67,5	3	-	15,1 ± 0,4

Figure 4.9 represents the compressive strength test results for mortar samples. Incorporation of bacterial cells with, both *bio-VMA/PBS* and *bio-VMA/NUT* samples, slightly decreased the compressive strength of mortars compared to neat mortar and their counterpart control samples. This decrease was more pronounced, particularly at early ages. This could be expected in samples containing lightweight pumice as an aggregate replacement. However, this decrease was observed in all minerals used in the experiment. In fact, there was not any decrease in compressive strength in mortar samples containing only bacterial cells without minerals. Similarly, there was not any decrease in samples containing partially saturated minerals. strength decrease.

It should be noted that there was a slight decrease in compressive strength development (from 3 to 7 and from 7 to 28 days) in samples containing only bacterial cells. Herein, the bacterial cells could decompose due to lack of nutrients leaving air bubbles in the cement paste matrix, thus leading to a decrease in compressive strength. Such a decrease was not observed in samples where bacterial cells were incorporated with immobilization barriers, except when cells were carried by sepiolite. Actually, this could

be related to the poor compaction of the samples. Based on ASTM C109 standards compaction in these samples was provided as hand compaction. However, all the results indicated that incorporation of bacterial cells resulted with an increased rheological properties and reduced workability even if the superplasticizer content was doubled. Therefore, this might lead to poor compaction and decrease compressive strength. Samples containing bio-VMA with sepiolite might require mechanical compaction due to their high stiffness. Even with this possible experimental error, the decrease in mortar sample *bio-VMA/PBS-S* and *bio-VMA/NUT-S* samples were less than 15%.



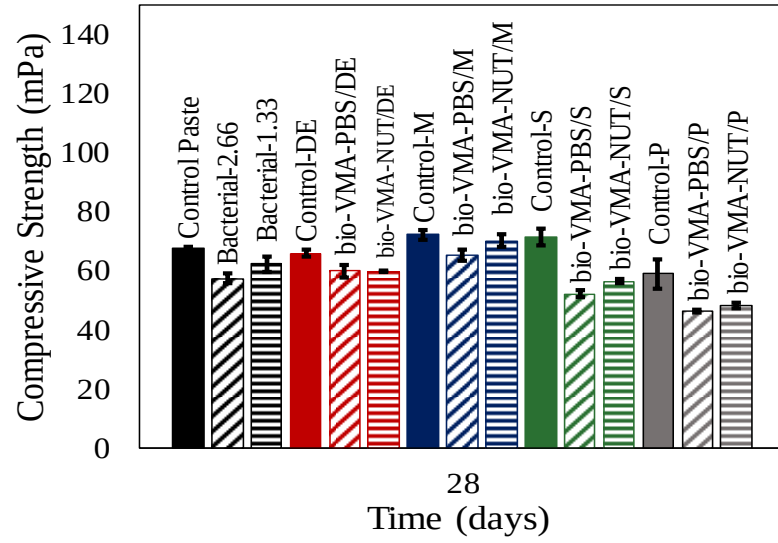


Figure 4.9: Influence of bacterial cells immobilized on minerals on the compressive strength at (a) 3 days (b) 7 days (c) 28 days; w/c =0.45; Error bars represent the standard deviation; Cement to sand ratio was 1:3.

CHAPTER V

CONCLUSION

The emerging concrete technology requires a robust mix design formulation which have sustainable system that meets the rheological and mechanical performance needs of additive manufacturing. Inorganic minerals such as clays and pozzolans can be used as rheology modifying additives for cement-based materials. An alternative sustainable additive is using bio-based admixtures to improve rheological performance of cement-based materials. This study presents the results of an extensive study to evaluate possible use of microorganism as rheology modifying agents. Thus, the proposed 2-part bio-based admixtures could be an alternative sustainable solution as well as enhancing the influence of microorganism on rheological performance of cement-based materials.

The flow behavior of cement pastes including *B.amyloliquefaciens* and *P.polymyxa* cells and biofilms were evaluated with rheological tests and mini slump tests. The influence of superplasticizer and fly ash was investigated with different dosages of bio-VMA and w/c's. Considering the effective results of *S.pasteurii* cells on improving rheological properties of cement paste, a novel 2-part additive was developed with immobilizing *S.pasteurii* cells on locally available minerals such as diatomaceous earth, metakaolin, sepiolite and pumice. The performance and influence of 2-part bio-VMAs were evaluated through rheological tests, mini slump tests and compressive strength tests.

5.1 Chapter III: Influence of *B.amyloliquefaciens* and *P.polymyxa* Cells and Biofilms on Rheology of Cement Pastes

- Incorporation of cells and biofilms influenced the rheological character (yield stress and apparent viscosity) of cement paste especially at lower w/c. Cement pastes exhibited shear thinning behavior regardless of bio-VMA type and dosage. The influence of bio-VMAs was attributed to the intertwining of chains increases the viscosity of paste yet increasing the shear rate might be breaking the entanglement of polysaccharide chains and releasing the trapped water.
- When the bio-VMA dosage was 0.1%, the effects of bacterial cells were more pronounced compared to biofilms at lower w/c. Due to their negative surface charge, the cells could bind $[Ca^{+2}]$ on the surface of cement particles. The efficiency of biofilms was more significant with the availability of water phase.
- All bio-VMAs showed efficient VMA action but the influence of bio-VMA dosage was more pronounced in bacterial cells rather than biofilms. Increasing the bio-VMA dosage to 0.3%, increased the efficiency of bacterial cells at high w/c's as well as shear-thinning response especially at w/c of 0.43. Increasing the cell dosage increases the surface charge attraction and intertwining of chains of cells. Whereas, increasing dosage of biofilms did not affect their performance as it was in cells.
- Incorporation of PCE superplasticizers interfered with working mechanism of cells which governed by surface attraction. However, increasing the bio-VMA dosage presented less pronounced viscosifying properties. Thus, the optimum dosage should be 0.1% when superplasticizer is incorporated.

- Regardless of the mixing composition, i.e. containing PCEs or fly ash or no additives, at high w/c (0.5) increasing dosage of bacterial cells increase their efficiency whereas an opposite trend was observed in biofilms.
- EPI 60, *B.amyloliquefaciens*, cells were found to be more efficient among the rest of the proposed in terms of improving rheological properties considering highly flowable cement pastes performance.

5.2 Chapter IV: Influence of 2-part Bio-VMAs on Rheology of Cement Pastes

- The proposed 2-part bio-VMAs significantly enhanced the yield stress and viscosity of cement pastes compared to control neat paste.
- The incorporation of minerals resulted with homogenized mixes. The mini-slump diameters were in line with yield stress measurements when minerals were incorporated.
- Regardless of the moisture state, the highest rheological performances (highest yield stress and apparent viscosity, lowest mini-slump diameters) were attained with sepiolite incorporation.
- Metakaolin increases the yield stress and apparent viscosity of cement pastes regardless of its moisture state. But saturating metakaolin decreases the absorption capacity, thus reduces its efficiency as a rheology modifier.
- Lower cell dosage (0.2% by cement weight) yielded higher rheological values compared to 0.4% cell addition by cement weight (2.66g). The optimum cell dosage should be evaluated.

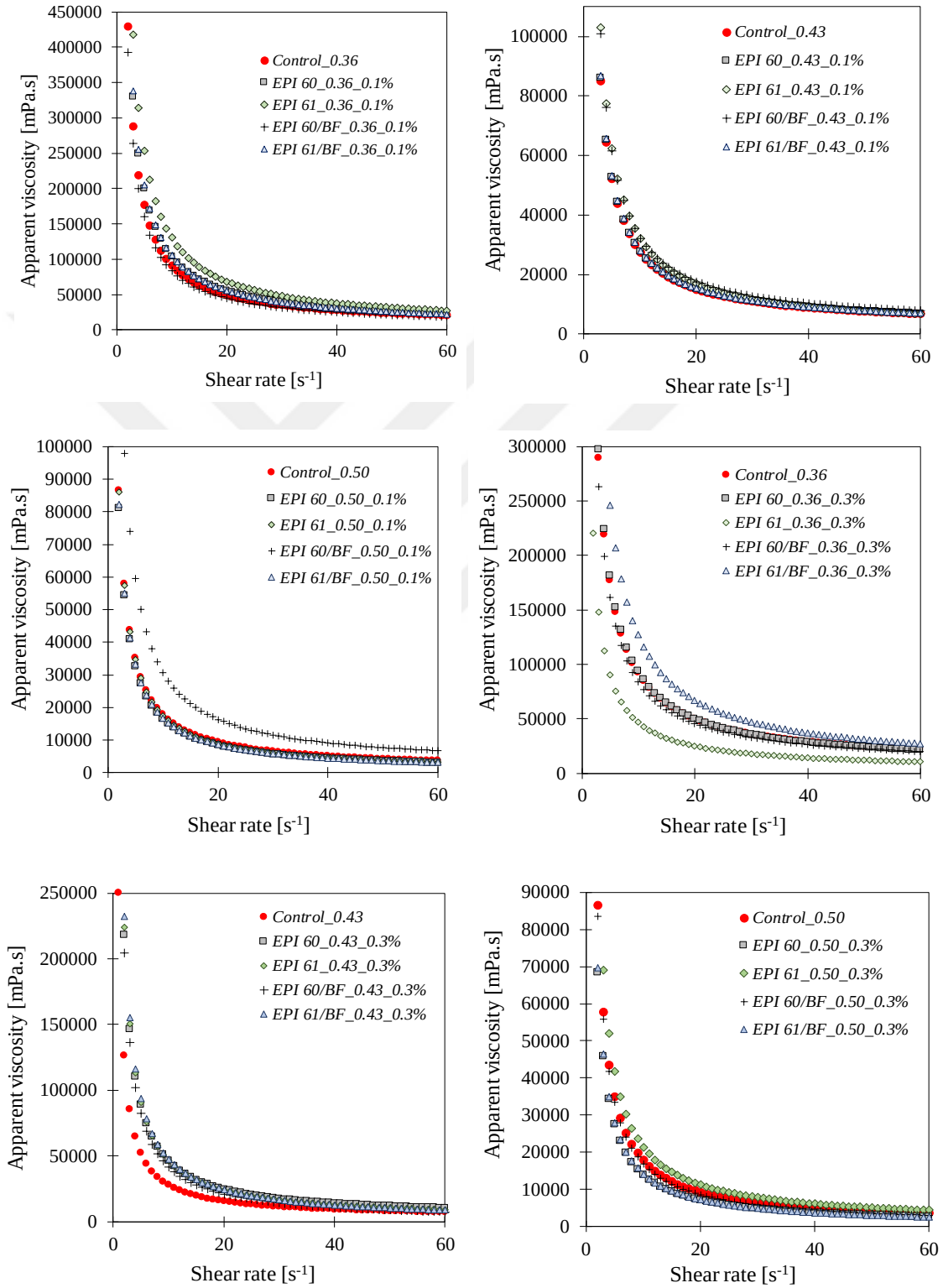
- Immobilization of cells on sepiolite significantly enhanced the rheological measurements. However, for DE and pumice, immobilization of bacterial cells resulted with lower yield stress compared to their counterpart minerals.
- Incorporation of bacterial cells along with their nutrients showed higher performance compared to bio-VMA-PBS/Mineral samples in terms of improving rheology due to optimum cell dosage.
- Incorporation of 2-part additives slightly decreased the compressive strength. Mechanical compaction can prevent the poor compaction occurring due to decreasing workability with 2-part bio-VMA addition.

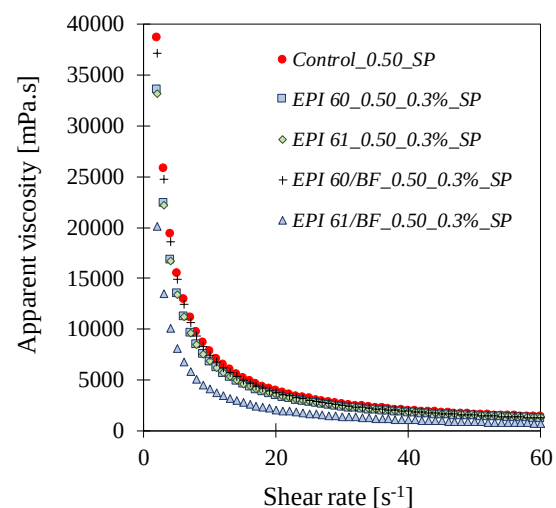
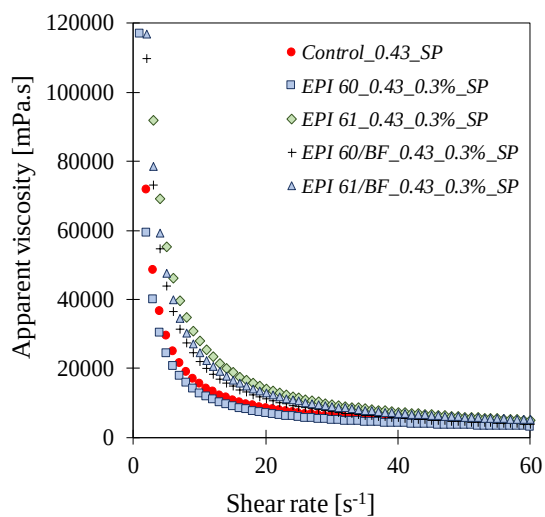
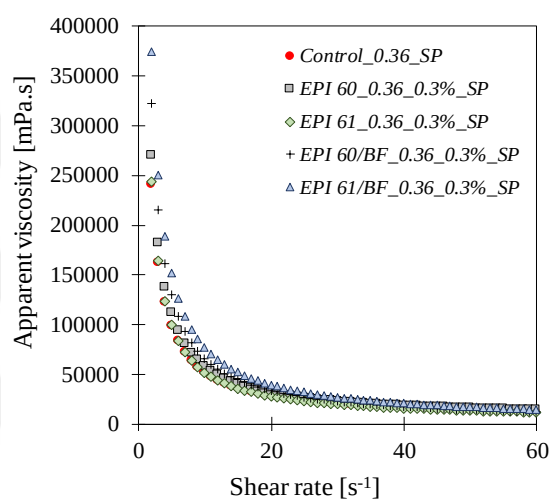
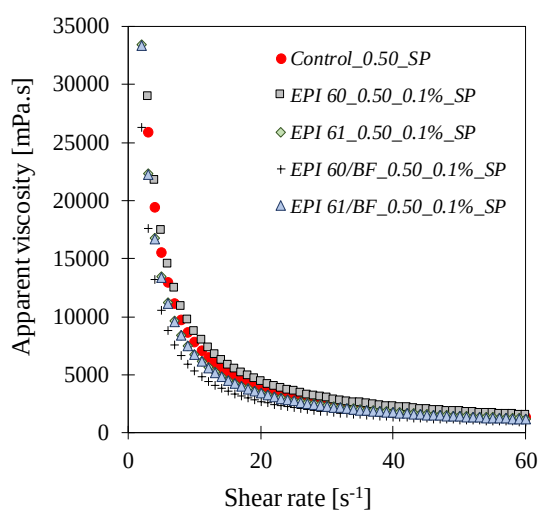
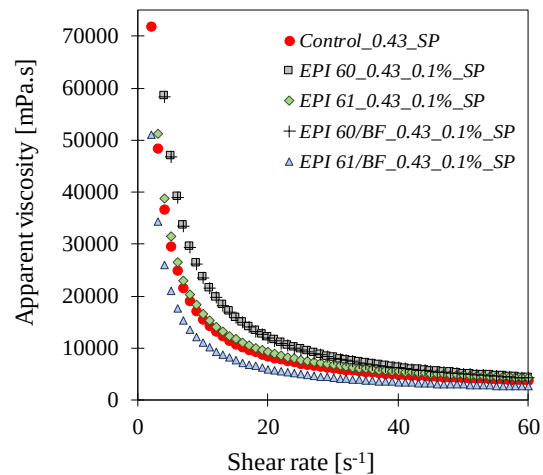
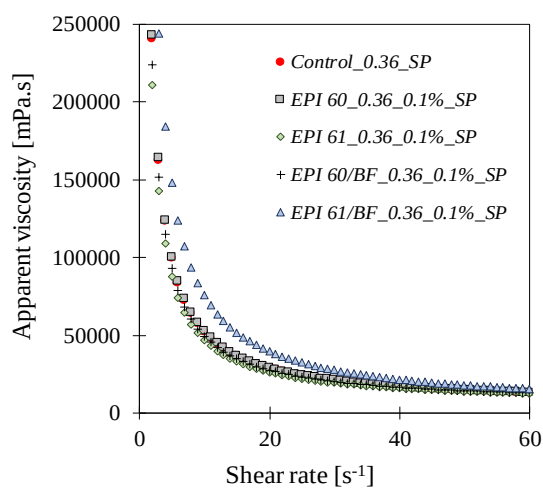
5.3 Future Work

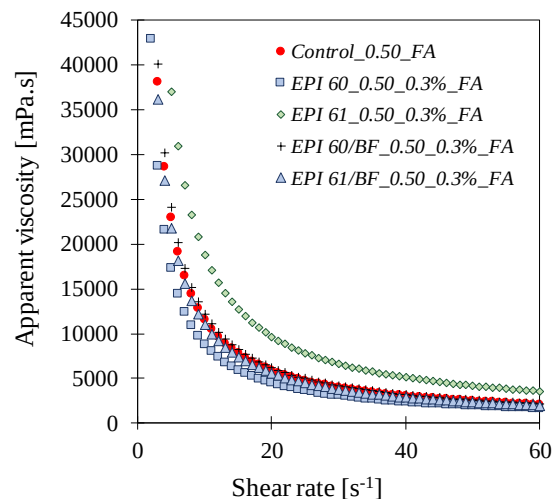
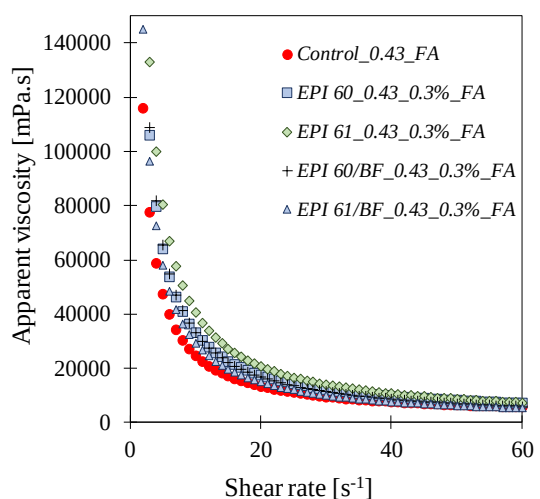
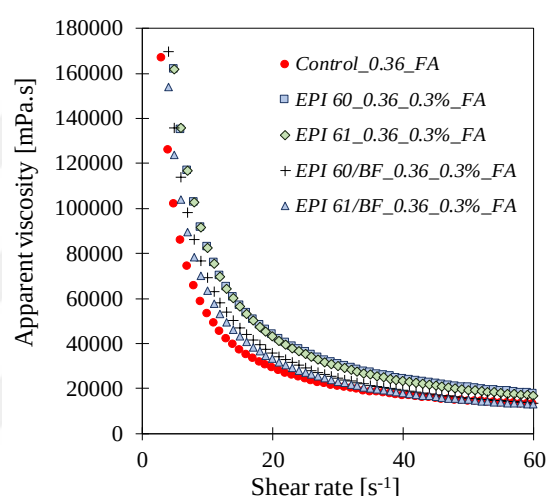
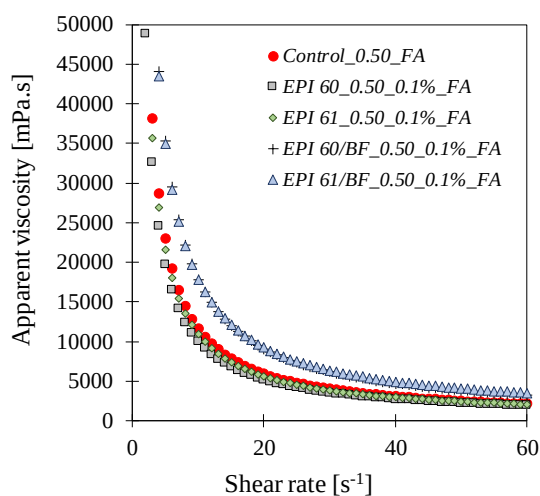
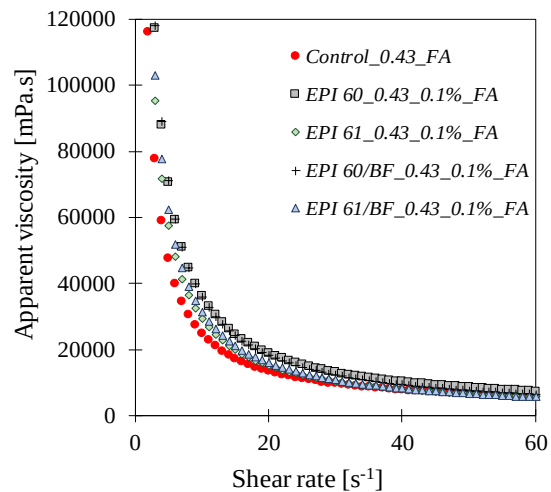
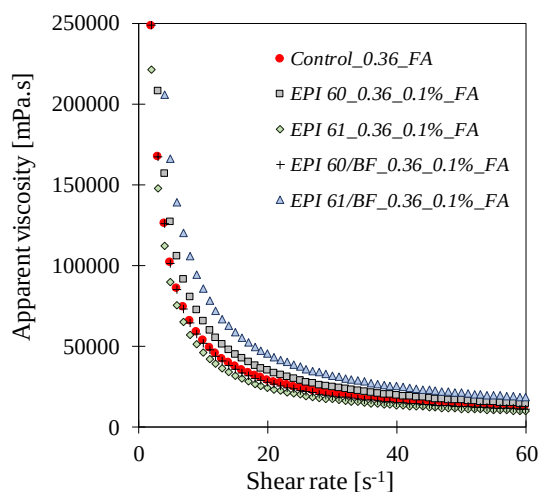
The interaction between cells and cement-based matrix needs further investigation. Such that cell-particle interaction and biofilm-water molecule interaction should be evaluated, so that the practical application of bio-VMAs can further advance. The rheological performance of 2-part-bio additives should be evaluated with different type and dosages of clays and pozzolans. Also, different type and dosages of HRWRs can be evaluated. The most significant enhancement of rheological performance was observed with sepiolite added samples. 2-part bio additive developed with sepiolite can be used in application like 3D printing. Further investigations should be conducted to understand the interaction of sepiolite with microorganisms, the kinematic of sepiolite in pore solutions as well as on cement particle interaction.

APPENDICES

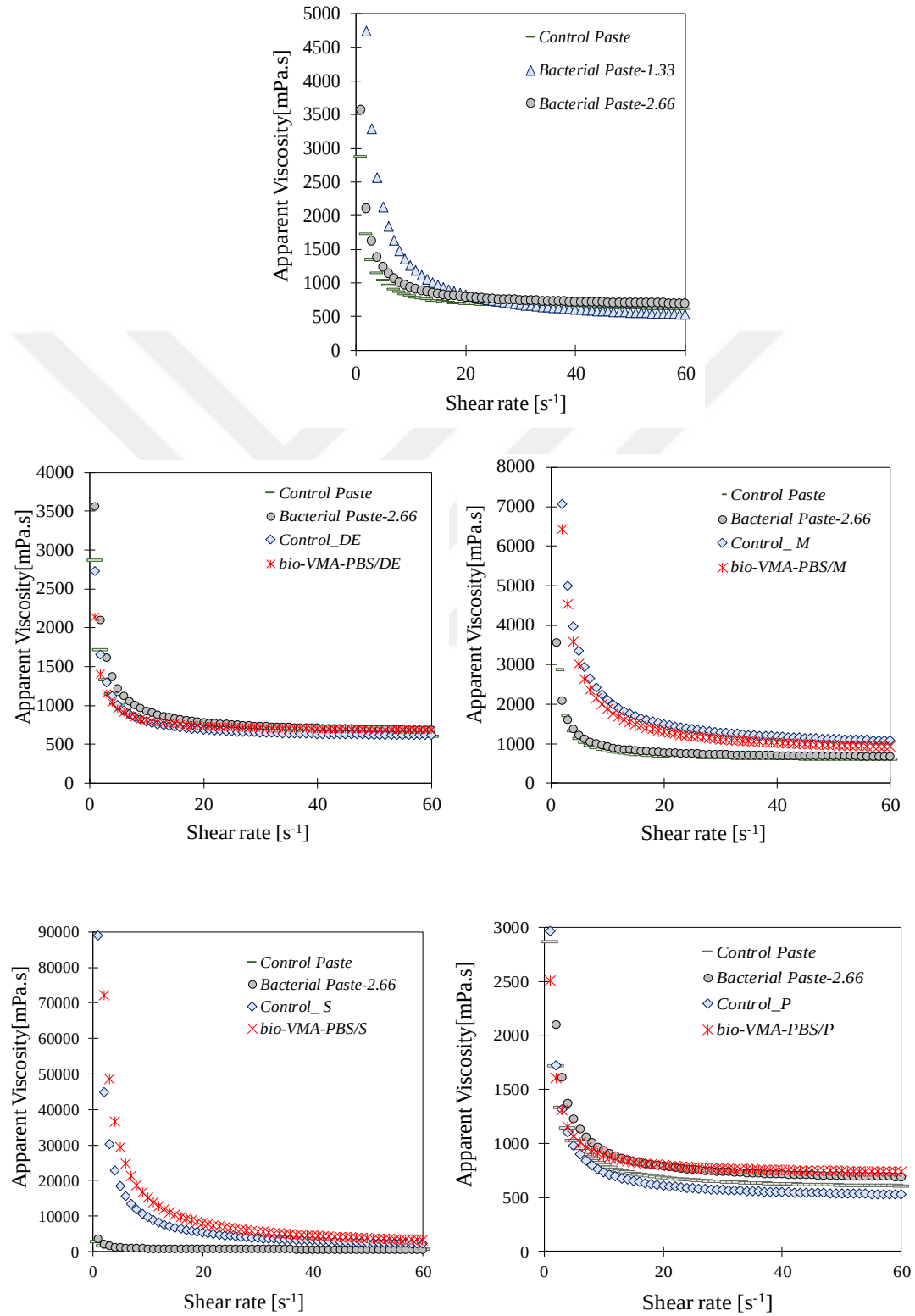
Appendix A CHAPTER III

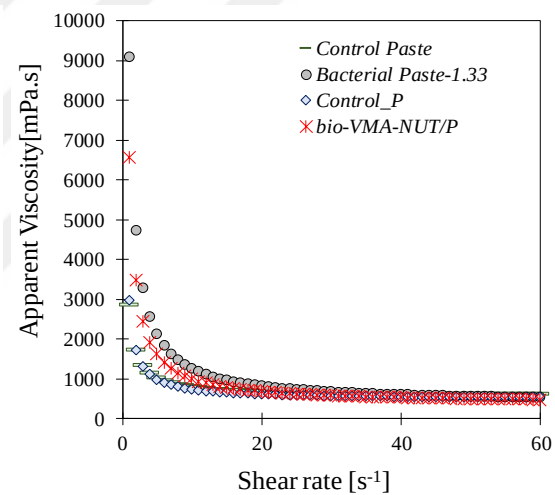
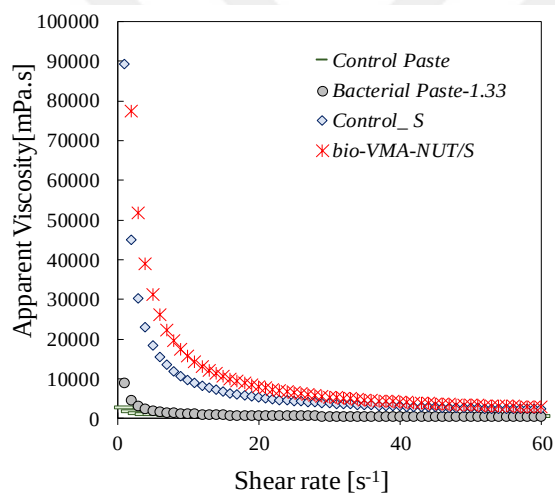
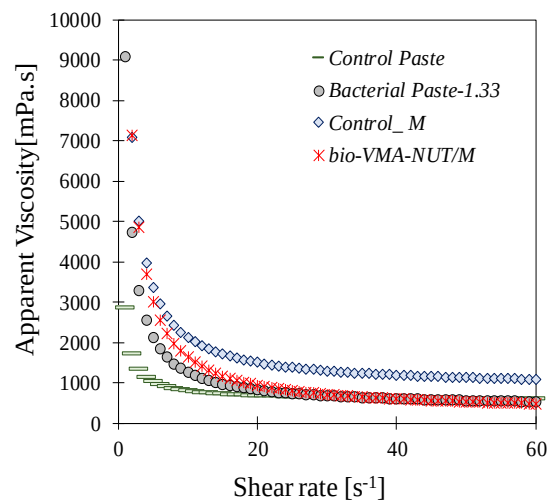
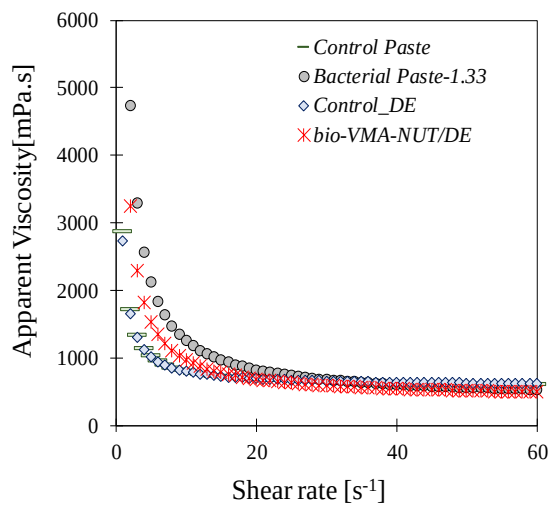






Appendix B CHAPTER IV





BIBLIOGRAPHY

- [1] L. Reiter, T. Wangler, N. Roussel, and R. J. Flatt, "The role of early age structural build-up in digital fabrication with concrete," *Cement and Concrete Research*. 2018.
- [2] D. Marchon, S. Kawashima, H. Bessaies-Bey, S. Mantellato, and S. Ng, "Hydration and rheology control of concrete for digital fabrication: Potential admixtures and cement chemistry," *Cement and Concrete Research*. 2018.
- [3] K. H. Khayat, "Viscosity-enhancing admixtures for cement-based materials - An overview," *Cem. Concr. Compos.*, 1998.
- [4] A. I. Laskar and S. Talukdar, "Rheological behavior of high performance concrete with mineral admixtures and their blending," *Constr. Build. Mater.*, 2008.
- [5] V. N. Nerella, M. Näther, A. Iqbal, M. Butler, and V. Mechtcherine, "Inline quantification of extrudability of cementitious materials for digital construction," *Cem. Concr. Compos.*, 2019.
- [6] J. T. Kolawole, R. Combrinck, and W. P. Boshoff, "Measuring the thixotropy of conventional concrete: The influence of viscosity modifying agent, superplasticiser and water," *Constr. Build. Mater.*, 2019.
- [7] P. Banfill, "The rheology of fresh cement and concrete-a review," in *Proceedings of the 11th International Cement Chemistry Congress*, 2003.
- [8] R. J. Flatt, "Dispersion forces in cement suspensions," *Cem. Concr. Res.*, 2004.
- [9] H. M. Jennings, A. W. Saak, and S. P. Shah, "New Methodology for Designing Self-Compacting Concrete," *Mater. J.*, vol. 98, no. 6.
- [10] J. H. Kim, M. Beacraft, and S. P. Shah, "Effect of mineral admixtures on formwork pressure of self-consolidating concrete," *Cem. Concr. Compos.*, 2010.
- [11] J. Di Filippo, J. Karpman, and J. R. DeShazo, "The impacts of policies to reduce CO₂ emissions within the concrete supply chain," *Cem. Concr. Compos.*, 2019.
- [12] A. P. Gursel, H. Maryman, and C. Ostertag, "A life-cycle approach to environmental, mechanical, and durability properties of 'green' concrete mixes with rice husk ash," *J. Clean. Prod.*, 2016.
- [13] K. L. Scrivener, V. M. John, and E. M. Gartner, "Eco-efficient cements: Potential economically viable solutions for a low-CO₂ cement-based materials industry," *Cem. Concr. Res.*, 2018.
- [14] S. Afroz, T. Manzur, and K. M. Anwar Hossain, "Arrowroot as bio-admixture for performance enhancement of concrete," *J. Build. Eng.*, 2020.
- [15] A. Hazarika *et al.*, "Use of a plant based polymeric material as a low cost chemical admixture in cement mortar and concrete preparations," *J. Build. Eng.*, 2018.
- [16] *Rheology of Fresh Cement and Concrete*. 1991.

- [17] A. W. Saak, H. M. Jennings, and S. P. Shah, "A generalized approach for the determination of yield stress by slump and slump flow," *Cem. Concr. Res.*, 2004.
- [18] N. Pashias, D. V. Boger, J. Summers, and D. J. Glenister, "A fifty cent rheometer for yield stress measurement," *J. Rheol. (N. Y. N. Y.)*, 1996.
- [19] L. Struble, R. Szecsy, W. G. Lei, and G. K. Sun, "Rheology of Cement Paste and Concrete," *Cem. Concr. Aggregates*, 1998.
- [20] G. Liu, W. Cheng, L. Chen, G. Pan, and Z. Liu, "Rheological properties of fresh concrete and its application on shotcrete," *Construction and Building Materials*. 2020.
- [21] J. Kruger, S. Zeranka, and G. van Zijl, "A rheology-based quasi-static shape retention model for digitally fabricated concrete," *Constr. Build. Mater.*, 2020.
- [22] V. N. Nerella and V. Mechtcherine, "Studying the Printability of Fresh Concrete for Formwork-Free Concrete Onsite 3D Printing Technology (CONPrint3D)," in *3D Concrete Printing Technology*, 2019.
- [23] M. Chen *et al.*, "Rheological parameters, thixotropy and creep of 3D-printed calcium sulfoaluminate cement composites modified by bentonite," *Compos. Part B Eng.*, 2020.
- [24] C. F. Ferraris and J. M. Gaidis, "Connection between the rheology of concrete and rheology of cement paste," *ACI Mater. J.*, 1992.
- [25] A. Papo, "Rheological models for cement pastes," *Mater. Struct.*, 1988.
- [26] A. Leemann and F. Winnefeld, "The effect of viscosity modifying agents on mortar and concrete," *Cem. Concr. Compos.*, 2007.
- [27] C. F. Ferraris, K. H. Obla, and R. Hill, "The influence of mineral admixtures on the rheology of cement paste and concrete," *Cem. Concr. Res.*, 2001.
- [28] D. Feys, R. Verhoeven, and G. De Schutter, "Fresh self compacting concrete, a shear thickening material," *Cem. Concr. Res.*, 2008.
- [29] H. A. Barnes and J. F. Hutton, "Chapter 2: Viscosity," in *An Introduction to Rheology*, 1989.
- [30] A. W. Saak, H. M. Jennings, and S. P. Shah, "The influence of wall slip on yield stress and viscoelastic measurements of cement paste," *Cem. Concr. Res.*, 2001.
- [31] M. Azima and Z. B. Bundur, "Influence of *Sporasarcina pasteurii* cells on rheological properties of cement paste," *Constr. Build. Mater.*, 2019.
- [32] J. Plank, "Applications of biopolymers and other biotechnological products in building materials," *Applied Microbiology and Biotechnology*. 2004.
- [33] H. F. W. Taylor, "Cement chemistry. 2nd ed.," *Acad. Press*, 1997.
- [34] H. Bessaies-Bey, R. Baumann, M. Schmitz, M. Radler, and N. Roussel, "Effect of polyacrylamide on rheology of fresh cement pastes," *Cem. Concr. Res.*, 2015.
- [35] C. Brumaud, R. Baumann, M. Schmitz, M. Radler, and N. Roussel, "Cellulose ethers and yield stress of cement pastes," *Cem. Concr. Res.*, 2014.
- [36] L. Patural, P. Marchal, A. Govin, P. Grosseau, B. Ruot, and O. Devès, "Cellulose

ethers influence on water retention and consistency in cement-based mortars,” *Cem. Concr. Res.*, 2011.

[37] N. A. Tregger, M. E. Pakula, and S. P. Shah, “Influence of clays on the rheology of cement pastes,” *Cem. Concr. Res.*, 2010.

[38] P. K. Mehta and P. J. M. Monteiro, *Concrete: Microstructure, Properties and Materials*. 2001.

[39] C. K. Park, M. H. Noh, and T. H. Park, “Rheological properties of cementitious materials containing mineral admixtures,” *Cem. Concr. Res.*, 2005.

[40] N. A. Libre, R. Khoshnazar, and M. Shekarchi, “Relationship between fluidity and stability of self-consolidating mortar incorporating chemical and mineral admixtures,” *Constr. Build. Mater.*, 2010.

[41] M. Sonebi, M. Lachemi, and K. M. A. Hossain, “Optimisation of rheological parameters and mechanical properties of superplasticised cement grouts containing metakaolin and viscosity modifying admixture,” *Constr. Build. Mater.*, 2013.

[42] S. M. Mansour, M. T. Abadlia, K. Bekkour, and I. Messaoudene, “Improvement of rheological behaviour of cement pastes by incorporating metakaolin,” *Eur. J. Sci. Res.*, 2010.

[43] N. Cruz, Y. Peng, S. Farrokhpay, and D. Bradshaw, “Interactions of clay minerals in copper-gold flotation: Part 1 - Rheological properties of clay mineral suspensions in the presence of flotation reagents,” *Miner. Eng.*, 2013.

[44] C. Viseras, C. Aguzzi, P. Cerezo, and A. Lopez-Galindo, “Uses of clay minerals in semisolid health care and therapeutic products,” *Appl. Clay Sci.*, 2007.

[45] S. Kawashima, M. Chaouche, D. J. Corr, and S. P. Shah, “Rate of thixotropic rebuilding of cement pastes modified with highly purified attapulgite clays,” *Cem. Concr. Res.*, 2013.

[46] J. Pinilla Melo, A. Sepulcre Aguilar, and F. Hernández Olivares, “Rheological properties of aerated cement pastes with fly ash, metakaolin and sepiolite additions,” *Constr. Build. Mater.*, 2014.

[47] R. Jarabo, E. Fuente, A. Moral, Á. Blanco, L. Izquierdo, and C. Negro, “Effect of sepiolite on the flocculation of suspensions of fibre-reinforced cement,” *Cem. Concr. Res.*, 2010.

[48] M. Şahmaran, N. Özkan, S. B. Keskin, B. Uzal, I. Ö. Yaman, and T. K. Erdem, “Evaluation of natural zeolite as a viscosity-modifying agent for cement-based grouts,” *Cem. Concr. Res.*, 2008.

[49] B. Hasanzadeh and Z. Sun, “Impacts of Diatomaceous Earth on the Properties of Cement Pastes,” *J. Build. Mater. Struct.*, vol. 5, no. October 2018, pp. 197–211, 2018.

[50] M. Saric-Coric, K. H. Khayat, and A. Tagnit-Hamou, “Performance characteristics of cement grouts made with various combinations of high-range water reducer and cellulose-based viscosity modifier,” *Cem. Concr. Res.*, 2003.

[51] R. Pei, J. Liu, and S. Wang, “Use of bacterial cell walls as a viscosity-modifying admixture of concrete,” *Cem. Concr. Compos.*, 2015.

- [52] W. Schmidt, H. J. H. Brouwers, H. C. Kühne, and B. Meng, "The working mechanism of starch and diutan gum in cementitious and limestone dispersions in presence of polycarboxylate ether superplasticizers," *Appl. Rheol.*, 2013.
- [53] H. Tan, F. Zou, B. Ma, Y. Guo, X. Li, and J. Mei, "Effect of competitive adsorption between sodium gluconate and polycarboxylate superplasticizer on rheology of cement paste," *Constr. Build. Mater.*, 2017.
- [54] F. Joel Ogbonna, "The secondary effects of lignosulfonate cement retarder on cement slurry properties," *J. Eng. Appl. Sci.*, 2009.
- [55] A. Pierre, A. Perrot, V. Picandet, and Y. Guevel, "Cellulose ethers and cement paste permeability," *Cem. Concr. Res.*, 2015.
- [56] A. Zima, J. Czechowska, D. Siek, and A. Ślósarczyk, "Influence of magnesium and silver ions on rheological properties of hydroxyapatite/chitosan/calcium sulphate based bone cements," *Ceram. Int.*, 2017.
- [57] M. Lasheras-Zubiate, I. Navarro-Blasco, J. M. Fernández, and J. I. Alvarez, "Studies on chitosan as an admixture for cement-based materials: Assessment of its viscosity enhancing effect and complexing ability for heavy metals," *J. Appl. Polym. Sci.*, 2011.
- [58] L. Xu, H. Gong, M. Dong, and Y. Li, "Rheological properties and thickening mechanism of aqueous diutan gum solution: Effects of temperature and salts," *Carbohydr. Polym.*, 2015.
- [59] M. Sonebi, "Rheological properties of grouts with viscosity modifying agents as diutan gum and welan gum incorporating pulverised fly ash," *Cem. Concr. Res.*, 2006.
- [60] F. M. León-Martínez, P. F. D. J. Cano-Barrita, L. Lagunez-Rivera, and L. Medina-Torres, "Study of nopal mucilage and marine brown algae extract as viscosity-enhancing admixtures for cement based materials," *Constr. Build. Mater.*, 2014.
- [61] Z. Başaran Bundur and M. Azime, "A BIO-BASED RHEOLOGY MODIFYING AGENT INSPIRED FROM NATURE," *Eskişehir Tech. Univ. J. Sci. Technol. A - Appl. Sci. Eng.*, 2019.
- [62] M. Azima, A. G. Guldogan, and Z. B. Bundur, "BIO-BASED RHEOLOGY MODIFYING AGENTS," vol. 37, no. 2, pp. 142–148, 2019.
- [63] G. G. Kahng, S. H. Lim, H. D. Yun, and W. T. Seo, "Production of extracellular polysaccharide, EPS WN9, from *Paenibacillus* sp. WN9 KCTC 8951P and its usefulness as a cement mortar admixture," *Biotechnol. Bioprocess Eng.*, 2001.
- [64] P. A. Williams and G. O. Phillips, "Introduction to food hydrocolloids," in *Handbook of Hydrocolloids: Second Edition*, 2009.
- [65] C. Even, C. Marlière, J. M. Ghigo, J. M. Allain, A. Marcellan, and E. Raspaud, "Recent advances in studying single bacteria and biofilm mechanics," *Advances in Colloid and Interface Science*. 2017.
- [66] P. Lembre, C. Lorentz, and P. Di, "Exopolysaccharides of the Biofilm Matrix: A Complex Biophysical World," in *The Complex World of Polysaccharides*, 2012.
- [67] I. V. Yegorenkova, K. V. Tregubova, L. Y. Matora, G. L. Burygin, and V. V. Ignatov, "Biofilm formation by *Paenibacillus polymyxa* strains differing in the

production and rheological properties of their exopolysaccharides,” *Curr. Microbiol.*, 2011.

[68] F. G. Priest, M. Goodfellow, L. A. Shute, and R. C. W. Berkeley, “*Bacillus amyloliquefaciens* sp. nov., nom. rev.,” *Int. J. Syst. Bacteriol.*, 1987.

[69] P. Gupta and B. Diwan, “Bacterial Exopolysaccharide mediated heavy metal removal: A Review on biosynthesis, mechanism and remediation strategies,” *Biotechnology Reports*. 2017.

[70] Y. Han *et al.*, “Rheological, emulsifying and thermostability properties of two exopolysaccharides produced by *Bacillus amyloliquefaciens* LPL061,” *Carbohydr. Polym.*, 2015.

[71] H. Koo, M. L. Falsetta, and M. I. Klein, “The Exopolysaccharide Matrix: A Virulence Determinant of Cariogenic Biofilm,” *J. Dent. Res.*, 2013.

[72] S. Timmusk, P. Van West, N. A. R. Gow, and R. Paul Huffstutler, “*Paenibacillus polymyxa* antagonizes oomycete plant pathogens *phytophthora palmivora* and *pythium aphanidermatum*,” *J. Appl. Microbiol.*, 2009.

[73] O. Fysun, T. Anzmann, P. Gschwind, J. Rauschnabel, R. Kohlus, and H. C. Langowski, “Biofilm and dairy fouling detection in flexible tubing using low-field NMR,” *Eur. Food Res. Technol.*, 2019.

[74] K. H. Khayat and A. Yahia, “Simple Field Tests to Characterize Fluidity and Washout Resistance of Structural Cement Grout,” *Cem. Concr. Aggregates*, 1998.

[75] M. Lachemi, K. M. A. Hossain, V. Lambros, P. C. Nkinamubanzi, and N. Bouzoubaâ, “Self-consolidating concrete incorporating new viscosity modifying admixtures,” *Cem. Concr. Res.*, vol. 34, no. 6, pp. 917–926, 2004.

[76] O. Li *et al.*, “Increasing viscosity and yields of bacterial exopolysaccharides by repeatedly exposing strains to ampicillin,” *Carbohydr. Polym.*, 2014.

[77] S. Stocks-Fischer, J. K. Galinat, and S. S. Bang, “Microbiological precipitation of CaCO_3 ,” *Soil Biol. Biochem.*, 1999.

[78] M. Azima and Z. Başaran Bundur, “Bio-Derived Rheology Modifying Agents for Cement-Based Materials,” in *RILEM Bookseries*, 2020.

[79] G. H. Tattersall, *Workability and Quality Control of Concrete*. 1991.

[80] E. Üzer and J. Plank, “Impact of welan gum stabilizer on the dispersing performance of polycarboxylate superplasticizers,” *Cem. Concr. Res.*, 2016.

[81] J. Plank, “Applications of Biopolymers in Construction Engineering,” in *Biopolymers Online*, 2005.

[82] D. Sableviciene, R. Klimaviciute, J. Bendoraitiene, and A. Zemaitaitis, “Flocculation properties of high-substituted cationic starches,” *Colloids Surfaces A Physicochem. Eng. Asp.*, 2005.

[83] J. Gregory and S. Barany, “Adsorption and flocculation by polymers and polymer mixtures,” *Advances in Colloid and Interface Science*. 2011.

[84] D. Saintillan, “Kinetic Models for Biologically Active Suspensions,” 2012.

- [85] S. Rafai, L. Jibuti, and P. Peyla, "Effective viscosity of microswimmer suspensions," *Phys. Rev. Lett.*, 2010.
- [86] T. Yang, H. Zhu, Z. Zhang, X. Gao, C. Zhang, and Q. Wu, "Effect of fly ash microsphere on the rheology and microstructure of alkali-activated fly ash/slag pastes," *Cem. Concr. Res.*, 2018.
- [87] S. Bendapudi and P. Saha, "Contribution of fly ash to the properties of mortar and concrete," *Int J Earth Sci Eng*, 2011.
- [88] B. Nécira, A. Guettala, and S. Guettala, "Effect of the viscosity modifying agent and the different mineral additions on rheology and compressive strength of self-compacting concrete," *Asian J. Civ. Eng.*, 2015.
- [89] E. Fuente, R. Jarabo, A. Moral, Á. Blanco, L. Izquierdo, and C. Negro, "Effect of sepiolite on retention and drainage of suspensions of fiber-reinforced cement," *Constr. Build. Mater.*, 2010.
- [90] J. A. Carmona, P. Ramírez, L. A. Trujillo-Cayado, A. Caro, and J. Muñoz, "Rheological and microstructural properties of sepiolite gels. Influence of the addition of ionic surfactants," *J. Ind. Eng. Chem.*, 2018.
- [91] J. Y. Wang, N. De Belie, and W. Verstraete, "Diatomaceous earth as a protective vehicle for bacteria applied for self-healing concrete," *J. Ind. Microbiol. Biotechnol.*, 2012.
- [92] H. Ulusu, H. Y. Aruntas, and O. Gencil, "Investigation on characteristics of blended cements containing pumice," *Constr. Build. Mater.*, 2016.
- [93] S. M. A. El-Gamal and F. S. Hashem, "Enhancing the thermal resistance and mechanical properties of hardened Portland cement pastes by using pumice and Al₂O₃," *J. Therm. Anal. Calorim.*, 2017.
- [94] A. Amiri, M. Azima, and Z. B. Bundur, "Crack remediation in mortar via biomineralization: Effects of chemical admixtures on biogenic calcium carbonate," *Constr. Build. Mater.*, 2018.
- [95] J. N. Y. Djobo, L. N. Tchadjié, H. K. Tchakoute, B. B. D. Kenne, A. Elimbi, and D. Njopwouo, "Synthesis of geopolymer composites from a mixture of volcanic scoria and metakaolin," *J. Asian Ceram. Soc.*, 2014.
- [96] N. K. Pazarlioğlu and A. Telefoncu, "Biodegradation of phenol by *Pseudomonas putida* immobilized on activated pumice particles," *Process Biochem.*, 2005.
- [97] ASTM, "C1437 - Standard test method for flow of hydraulic cement mortar," 2013.
- [98] ASTM, "Standard Test Method for Compressive Strength of Hydraulic Cement Mortars (Using 2-in . or [50-mm] Cube Specimens) 1," *Chem. Anal.*, 2010.
- [99] R. Saleh Ahari, T. Kemal Erdem, and K. Ramyar, "Effect of various supplementary cementitious materials on rheological properties of self-consolidating concrete," *Constr. Build. Mater.*, 2015.
- [100] R. Saleh Ahari, T. K. Erdem, and K. Ramyar, "Time-dependent rheological characteristics of self-consolidating concrete containing various mineral admixtures," *Constr. Build. Mater.*, 2015.

- [101] J. Li, W. Zhang, C. Li, and P. J. M. Monteiro, "Green concrete containing diatomaceous earth and limestone: Workability, mechanical properties, and life-cycle assessment," *J. Clean. Prod.*, 2019.
- [102] C. Wu and S. Kou, "Effects of high-calcium sepiolite on the rheological behaviour and mechanical strength of cement pastes and mortars," *Constr. Build. Mater.*, 2019.
- [103] A. Sepulcre Aguilar, J. Pinilla Melo, and F. Hernández Olivares, "Microstructural analysis of aerated cement pastes with fly ash, Metakaolin and Sepiolite additions," *Constr. Build. Mater.*, 2013.
- [104] J. Pinilla Melo, N. Flores Medina, A. Sepulcre Aguilar, and F. Hernández Olivares, "Rheological and thermal properties of aerated sprayed mortar," *Constr. Build. Mater.*, 2017.
- [105] I. Navarro-Blasco, M. Pérez-Nicolás, J. M. Fernández, A. Duran, R. Sirera, and J. I. Alvarez, "Assessment of the interaction of polycarboxylate superplasticizers in hydrated lime pastes modified with nanosilica or metakaolin as pozzolanic reactives," *Constr. Build. Mater.*, 2014.
- [106] M. Chen *et al.*, "Yield stress and thixotropy control of 3D-printed calcium sulfoaluminate cement composites with metakaolin related to structural build-up," *Constr. Build. Mater.*, 2020.
- [107] J. M. Mejía, R. Mejía De Gutiérrez, and C. Montes, "Rice husk ash and spent diatomaceous earth as a source of silica to fabricate a geopolymeric binary binder," *J. Clean. Prod.*, 2016.
- [108] B. Mobasher, M. Shekarchi, A. Bonakdar, M. Bakhshi, and A. Mirdamadi, "Transport properties in metakaolin blended concrete," *Constr. Build. Mater.*, 2010.
- [109] R. Siddique and J. Klaus, "Influence of metakaolin on the properties of mortar and concrete: A review," *Applied Clay Science*. 2009.
- [110] T. Kavas, E. Sabah, and M. S. Çelik, "Structural properties of sepiolite-reinforced cement composite," *Cem. Concr. Res.*, 2004.
- [111] B. Yan, P. Duan, and D. Ren, "Mechanical strength, surface abrasion resistance and microstructure of fly ash-metakaolin-sepiolite geopolymer composites," *Ceram. Int.*, 2017.
- [112] R. Bani Ardalan, A. Joshaghani, and R. D. Hooton, "Workability retention and compressive strength of self-compacting concrete incorporating pumice powder and silica fume," *Constr. Build. Mater.*, 2017.
- [113] M. Azima and Z. B. Bundur, "Use of microorganisms to improve viscosity and rheology of cement-based materials," *13th International Congress on Advances in Civil Engineering*, Izmir/TURKEY, 2018.
- [114] M. M. Tezer, "Development of Two-Phase Biological Self-Healing Agents for Cement-Based Mortar" (Ozyegin University, 2020), M.Sc. Thesis, Istanbul, Turkey.

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