RHEOLOGY MODIFYING AGENTS: A KEY TECHNOLOGY DEVELOPED BY USING MICROORGANISMS

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RHEOLOGY MODIFYING AGENTS: A KEY TECHNOLOGY DEVELOPED BY USING MICROORGANISMS

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To my parents for their infinite support, sacrifice, and encouragement

ABSTRACT

Recent development in concrete technology enabled the design of highly flowable mixes with improved workability. These advanced mixes require incorporation of fine materials or viscosity modifying agents (VMA) to reduce the possible segregation and bleeding due to the use of high range water reducers (such as superplasticizers). The VMAs used in concrete production are generally produced from acrylic polymers and polysaccharide-based biopolymers obtained from cellulose, starch or bacterial fermentation.

Diutan gum, produced by fermentation of *Sphinogomonas sp*, and welan gum, which is a fermentation product of *Alcaligenes sp*, are the most commonly used polysaccharide VMAs. Similar polysaccharides can be obtained by fermentation of genetically modified bacteria or using plant cell walls. Most polysaccharide based VMAs are able to increase the viscosity of cement paste and exhibit shear thinning behavior such that increased shear rate results with a substantial decrease in apparent viscosity. This behavior is attributed to the long molecular structure of bio-based polysaccharides. Though highly effective bacterial fermentation products can resist the high PH environment of cement-paste, the ecological population of the species is not known. Thus, they are among the most expensive cement admixtures. Advances in construction technology and risen importance of sustainability initiatives reinforce the use of biological admixtures, however, their relatively high cost can be a major drawback in practical applications. Through the literature, nopal mucilage, brown algae, and bacterial cell walls were proposed as alternatives to these bacterial fermentation products. However, these alternatives also require extra processing which required bigger budget even compared to bacterial

fermentation products. This project aims to incorporate bacteria cells to the cement-based mix as VMAs without any extra intervention. To achieve this goal, *Sporosarcina pasteurii (S.pasteurii), Bacillus megaterium (B. megaterium), Bacillus subtilis (B. subtilis)* and *Paenibacillus polymyxa (P. polymyxa)* were selected as suitable due to their abundant resource in nature. These Gram-positive bacterial cells include peptidoglycans and polysaccharides in their cell wall structure, which resembles the molecular structure of commercially used VMAs. In addition, these cells, particularly *B. subtilis*, can influence the viscosity of a suspension due to its motility. Throughout the study, these cells were grown in specified nutrient media and then harvested from the inoculum by centrifuging. Then, these cells were suspended in mixing water and their influence on the rheology of cement paste was evaluated. In addition, the influence of water to cement ratio, the dosage of cells added was evaluated along with the impacts of superplasticizers and fly ash on the performance of bacteria cells as VMAs.

There are few established industrial and various small-scale companies that produce biological admixtures for cement-based materials. However, nationwide these biological admixtures (for instance chitosan) are only produced for the food industry. The product obtained by the end of this study is a novel and sustainable practice in Turkey, where the construction industry leads the economy.

ÖZET

Günümüzde gelişen beton teknolojisi, yeni kuşak çimento esaslı malzemelerin gelişiminin önünü açmıştır. Akışkanlığı ve işlenebilirliği yüksek harç ve betonların kullanımı gittikçe artmaktadır. Bu tür malzemelerde dağıtma gücü çok yüksek akışkanlaştırıcı katkıların neden olduğu ayrışmayı (segregasyon) ve terlemeyi önleyebilmek için daha ince tanecikli malzemeler veya viskozite düzenleyici katkıların (VDK) kullanımı artmaktadır. VDK, akışkanlığı yüksek olan beton ya da harçların kararlılığını (stabilizesini) arttırmakta ve taze çimento hamuru performansını yükseltmektedir. Günümüzde beton üretiminde kullanılan VDK'lar suda çözünen polivinil alkol veya polimerlerden oluşmaktadır. VDK'lar akrilik polimerlerden, selülozdan, nişastadan veya bakteri fermantasyonu gibi polisakkarit bazlı biyopolimerlerden elde edilmektedir.

Bakterilerden elde edilen polisakkaritlerden CP Kelco adlı şirketin Sphingomonas bakterisinin fermantasyonu ile elde ettiği diutan sakızı, Merck & CO adlı şirketin Alcaligenes bakterisini kullanarak ürettiği welan sakızı çimento esaslı malzemelerde VDK olarak sıkça kullanılmaktadır. Benzer şekilde piyasada bakterilerin genleri ile oynanarak ya da bitkilerin hücre duvarları kullanılarak farklı polisakkaritler elde edilebildiği bilinmektedir. Çimento esaslı malzemelerde VDK olarak kullanılan biyolojik polisakkaritler çimento hamurunun viskozitesini arttırdığı belirlenirken, yapılan testlerde kayma hızı arttırılırken malzemenin inceldiği (shear-thinning) gözlemlenmiştir. Bu özelliklerin biyolojik polisakkaritlerin uzun moleküler yapısı ile ilişkili olduğu bilinmektedir. Bu fermantasyonun ürünü olan polisakkaritler yüksek PH değerlerine dayanma özellikleriyle ön plana çıkarken, bunları üretecek mikroorganizmaların ekolojik olarak popülasyonu bilinmemektedir. Bu nedenle birim fiyat yükselmektedir. İlerleyen

teknoloji ve sürdürülebilirlik bilinci biyolojik katkı malzemelerinin inşaat sektöründe kullanımının artmasını teşvik ederken, maliyetlerinin yüksek olması bir dezavantaj yaratmaktadır. Uluslararası literatürde bu fermantasyon ürünlerine alternatif olarak göllerden toplanan yosunlar ve bakterilerin, sadece hücre duvarlarının ayrıştırılarak kullanılması incelenmiştir. Ancak bu iki ürünün elde edilmesinde özel işçilik gerektirecek farklı işlemlerin uygulanması, maliyetlerin yine yükselmesine sebep olmuştur.

Bu projenin amacı doğadan kolayca elde edilen mikroorganizmaları, hücre duvarını ayrıştırılması gibi özel işlemler gerektirmeden, çimento esaslı malzemelerde VDK olarak kullanarak reolojisi iyileştirilmiş bir çimento harcı (ürün) elde etmektir. Bu amaç doğrultusunda *Sporosarcina pasteurii, Bacillus magetrium, Bacillus subtilis* ve *Paenibacillus polymyxa* bakteri suşları seçilmiştir. Bu çalışma süresince, bakteriler besi yerlerinde büyütüldükten sonra santrifüj edilerek ortamdan ayrıştırılmıştır. Ardından bu hücreler çimento karışım suyuna eklenmiş ve çimento hamurunun reolojisine olan etkileri test edilmiştir. Ayrıca farklı su oranlarında ve bakteri dozajlarının test edileceği karışımlara, süperakışkanlaştırıcıların ve uçucu külün bakterilere olan etkisi incelenmiştir.

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LIST OF KEY SYMBOLS

w/c	Water-to-cement ratio
τ	Shear stress
τy	Yield stress
$\tau 0$	Resultant shear stress
γ	Strain
$\dot{\gamma}$	Shear rate
$\gamma 0$	Constant shear strain
η	Viscosity
μ	Viscosity (for the Newtonian fluid)
K	Power law consistency index (for Herschel-Bulkley model)
n	Power exponent (for Herschel-Bulkley model)

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CHAPTER I

INTRODUCTION

1.1 General

Concrete is the most used construction material and the second most used material on earth. Even though concrete is a high-performance material, it is not perfect. One of the most significant problems occurs in concrete design is its negative impact on sustainability. The increasing environmental concerns increased the attention to the research studies focusses more optimized concrete mixes, alternative binders, and recycling options, to decrease the CO_2 emission caused by concrete production.

As a result of recent developments in the construction industry, cement-based materials are no longer including only cement, water, and aggregates. Currently, most high-quality mortar or concrete mixes often contain chemical additives. The global warming effects of CO_2 emissions from cement production are a frequently discussed issue and it is thought that about 7% of CO_2 emissions come from cement production. Similarly, the chemical additives and the using fuel during the production process take the second place in the terms of pollution and CO_2 emissions. Choosing the material to satisfy the construction necessities, economic, sustainability and mechanical properties, led the researchers to consider the biological and other alternative materials, as an outcome of high requirements in construction and building materials.

Another challenge in concrete materials is its use through high rise building or any other complex structure. Generally, pumping concrete to higher levels and obtaining different shapes require highly flowable concrete that would keep its consistency upon pumping and also do not require vibration to be molded. The direction of the studies towards the finding a solution for vibration in concrete led to the success in producing self-compacting concrete (SCC) that can result in more durable structures with less vibration requirement which has a direct effect on time, cost and environment [1]. In general, the SCC mixes have low yield stress and relatively high viscosity, reduce the problems related to stability and hemoginity**[**2**]**. One of the most effective new techniques is to reduce the water to cement ratio, incorporate superplasticizers, increase the amount of fine powder material and use viscosity modifying agents (VMA). While incorporation of could be a remedy in term of reducing the amount of cement used, it is crucial to find sustainable alternatives for chemical admixtures.

The most common VMA used in construction is generally produced from polymers and bio-based polysaccharides (cellulose, chitosan etc.) which are highly effective to reduce the bleeding since the long chain molecules of VMA stick to the water molecules, decrease their relative motion and forms a gel, so increase the yield stress an plastic viscosity[3]. In presence of most VMAs, shear-thinning behavior has been observed which means the viscosity of the material decreases with increasing shear rate[4]. According to Planck [5], most of the bio-derived polysaccharide VMAs are not economically feasible during to the labor-intensive process and the significant part of a product cost is due to the polysaccharides like cellulose ethers or chitosan. Thus, researchers started to focus on alternative bio-based VMAs such as the use of algae, bacterial cell walls or fermentation products such as extracellular polysaccharides (EPS) or diutan gum [6]. These abovementioned polysaccharide based biopolymers contain monosaccharide side chains and anionic carboxylate groups linked to long polysaccharide chains[7].

To this extent, the bacterial cells could also be incorporated into the mix without any external intervention which would increase the labor work and the cost of the application. Not only the peptidoglycans and polysaccharides in cell structure could act as a VMAs, they can also influence the rheology due to their motility [8]. Thus, it might be more efficient to incorporate not only the EPS or cell walls but directly adding the bacterial cells into the mix water.

1.2 Goals and objectives

Previous studies in the literature showed that bio-derived polysaccharides as welan gum [9], diutan gum [9], EPS [10], algae [6]and bacterial cell walls [3]could be used as VMA in cement-based materials. Yet, it is not clear if a simple approach such as suspending cells to mix water without any extra intervention can be used as VMA. Incorporation of cells to mortar mixes was found to be efficient to induce biomineralization within cement paste and improve strength [11], remediate both internal and external microcracks[12]. Thus, it might be possible to obtain a mix with improved rheology and performance by only incorporating cells into the mix.

This project aims to improve the rheology of cement paste by incorporating *S. pasteurii, B. megaterium, B. subtilis,* and *P. polymyxa* cells as VMAs in cement-based materials. To achieve this goal the bacterial cells were grown in specified nutrient media until they reach to the stationary phase. Then, the cells were harvested from the bacterial culture by centrifuging. The collected cells were added to the mix water and the influence of the cells on fresh state properties was evaluated by rheological analysis, mini cone and air content tests.

The specific objectives of the project can be listed as follows:

I. To determine the suitable incubation conditions for *S. pasteurii, B. megaterium, B. subtilis,* and *P. polymyxa* cells

II. To evaluate the influence of cells on yield strength, viscosity and thixotropy of cement paste

III. Determine the influence of increasing cell dosage and w/c on the rheology of cement paste

IV. Investigate the compatibility of bacterial VMAs with superplasticizers and fly ash

V. Determine the effects of cells on fresh state properties such as air content.

1.3 Outline of the thesis

This thesis consists of five chapters. Chapter I provides an overview of the introduction to the necessity of studying the cement paste rheological behavior, motivations and goals of the research and the outline of the thesis. Chapter II reviews the introduction rheology of cement paste with the rheology parameters to be used for this research and concrete rheology and the limitations of concrete rheology, rheology of cement paste and the influence of the additives of rheology, bio-based VMAs and use of microorganisms as VMA. Chapter III includes the methods and materials for microorganism and nutrient media, cultivation of bacterial cells, zeta potential measurement, the mixing process of cement-paste and the concrete mixture is discussed. Furthermore, the series of test methods used in the research is reviewed. It shows how the rheology test is conducted with different mix properties. Chapter IV summarizes the results from Chapters III and suggests the microorganisms for improving rheology in cement-based materials and matches the relationship between bio-based VMAs and the rheology of cement paste. Finally, in Chapter V, key conclusions and future works are provided.

CHAPTER II

LITERATURE REVIEW

Rheology has been used to understand the behavior of fresh state properties of cement-based materials. To develop a new type of admixture to modify the fresh state properties of cement paste would definitely require a rheological evaluation. Thus, the first part of this chapter gives a brief overview of rheology and techniques used to measure the rheological properties. This is followed by a discussion of how different admixtures and fine materials would influence rheology. At last, current studies on bio-based VMAs is presented.

2.1 Introduction

Throughout the history, Romans used a specified concrete mix design in the 18th century, developing an appropriate mixture proportion for sand, gravel, water, and cement[13]. As a result of Abram 1920 studies, he found that increasing the water to cement ratio would improve the workability and flowability of the fresh concrete [13]. Workability of concrete could be simply explained as ease of mixing, pumping, placing, compacting and finishing. Evaluation of workability can be done both in the lab and in the field. A crude slump test in the field would actually give a brief idea for the workability of concrete. In laboratory conditions, workability could be studied by complex approached but basically, it would include[14]:

I. Comparing the properties and use subjects to describe the material

II. Measurements such slump on a numerical scale

III. Describing the material in terms of times, length and etc. which require the rheology science and analytical models.

The last step went through main failures and workability measurements characterized based on step I and II for many decades, until Tattersall described [15], the most reliable way to measure the workability of the mixture was a subjective description by the operator since the two mixture having identical slump values could have different behaviors.

The complex behavior of cement and water mixture was a discouragement to many researchers. Besides, studies conducted using practical equipment that established for other purposes until 1950, and most of the researchers left the cement-based materials to study easier materials. [16–18]. After understanding the importance of thixotropy and structural breakdown in the 1950s, rheological studies, models for yield stress of suspension and the microstructural breakdown was started [15,19–22].

Although new techniques were established for mixing and placing concrete before the 1930s (like pumping and vibration before even studying about this initiative), Abram was the first scientist who mentioned the fresh concrete properties like workability that should be controlled and used terms like consistency [20,23].

Starting 1970s, measurements with a rotating vane and coaxial cylinders were initiated [24,25]. Some difficulties with measuring the requirements were seen until Tattersall and Bandfill succeed in establishing the two-point method [14]. which led the researchers to understand the rheology of fresh concrete and properties that could be controlled.

Along with the findings in the science of rheology, development of new techniques in mixing and processing concrete increased the need to measure the rheology of cement-based materials [26–28]. With the aid of computer-based systems, rheometers having the ability to collect data and model the flowing of complex mixtures were developed [22]. Rheometers like ViscoCorder and Viscomat were used to evaluate the rheology of mortar (*see* Figure 2-1) [29].

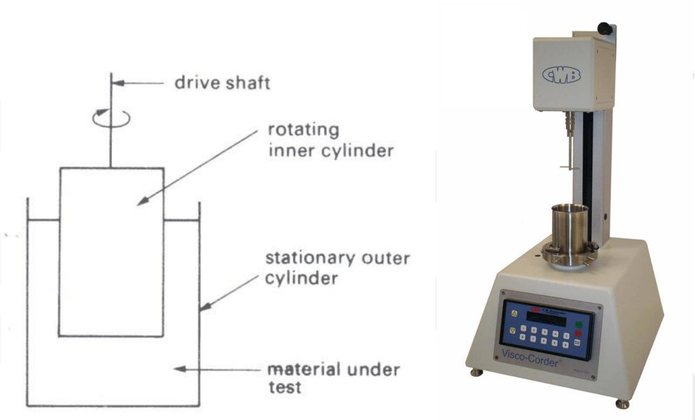

Figure 2-1 Coaxial cylinder viscometer(left) [29] and ViscoCorder(right) used to measure the rheological properties at first.

2.2 Introduction to Rheology

Rheology is the science of flow and deformation[30] and can be simply defined as a behavior of a fluid which depends on stress, strain, the rate of strain and time. The scope of rheology could be considered as understanding the behavior of complex fluids under different conditions.[31].

To understand the importance of rheology of fluids which have a complex behavior under shear and stress, it is useful to first know about the materials that have the simplest behavior. For instance, an ideal elastic material has a simple behavior in the appearance of load and when the load is removed. Moreover, the deformation in these materials is recovered after the loading process and the behavior is based on Hook's Law, thus the deformation can be measured regarded to the proportional spread of force or load[32].

The simplest case to understand the behavior of simple fluid rheology is also similar to an elastic material such that the fluid deforms no matter how small the applied stress is, so the shear strain will change by the time [32]. That would change the Hook's law to a little bit more complex equation which led to Newton's law of viscous flow that stated as in Equation 2-1:

$$\tau = \eta\dot{\gamma} \quad \text{Equation 2-1[33]}$$

Where τ is for Shear stress (Pa), $\dot{\gamma}$ is for shear strain(s^{-1}) and η is the coefficient of viscosity (Pa.s). Based on the Equation 2-1, it is seen that at a constant temperature, a signal-point experimental method could be done to find the shear stress. The materials where the rheology could be explained by this simple equation is known to be *Newtonian fluids* [33]. The Newtonian fluid was described with a plot of shear stress and shear rate, which the slope of the straight line going through the plot would be viscosity (η) as it is shown in Figure 2-2.

However, generally, the behavior of complex materials (suspensions) like cement paste could not be explained by the simple Newtonian equation. A great deal of studies has been conducted to determine the flow behavior of cementitious materials. The rheological properties of cement paste could differ by changing the dilution and concentration. This means that at high w/c, a Newtonian behavior could be seen where the material exhibits both viscous and elastic property. Thus, the relationship between shear stress and the shear rate could be demonstrated as a linear relation. However, at low w/c ratio, the cement paste mainly exhibits plastic behavior which was attributed to the increase in interparticle. Therefore, the Newtonian equation could not be used. Instead, the Bingham model is used to describe complex plastic materials like cement paste:

$$\tau = \tau + \mu\dot{\gamma} \quad \text{Equation 2-2[33]}$$

where τ is for Shear stress (Pa), $\dot{\gamma}$ is for Shear rate (s^{-1}), μ is for Viscosity (Pa.s) and τ_y is the yield stress (Pa). Where the shear rate is not constant, the Newtonian equation can be modified to an applicable equation.

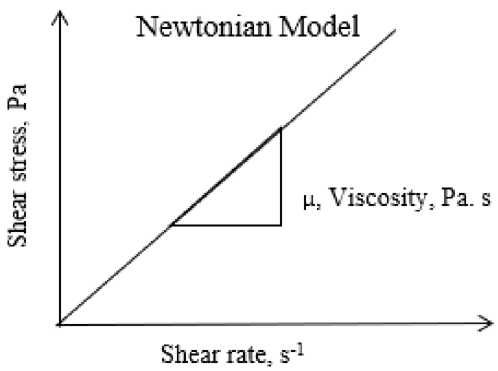

Figure 2-2 Newtonian Model for fluid[32]

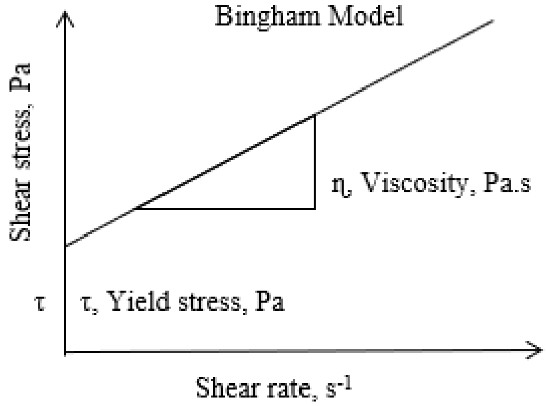

Figure 2-3 Bingham Model for fluid[32]

In case of a complex fluid like cement paste, the Bingham model could define the minimum energy required to initiate flow in the material, which is the yield strength. It is totally clear that having the same viscosity in two different materials does not mean that they have the same yield stress. Thus, measuring the rheological properties of the cement paste which includes both viscosity and yield stress is a valuable parameter to determine the workability and stability of the cement paste.

There might be cases where the complex material behavior could not even explain by the Bingham model. In some materials, the flow curve (shear rate versus shear stress) is not a straight line, which may or may not intersect the stress axis at a greater stress than zero[32]. As it is shown in Figure 2-4, when the flow curves concave towards the stress axis, it is said that the material showed a shear-thickening behavior. This behavior is a rheological response of condensed particles suspension to an increasing shear rate, which results in an increase in materials viscosity [34]. Thus, shear stress would go up greater than the shear rate. This would lead to an increase in fluids volume in high shear rates. On the other hand, when the flow curve in concave toward the shear rate axis, this shows the shear-thinning behavior of the studied material. In other words, in materials that showing shear thinning behavior, viscosity decreases with increasing shear rate.

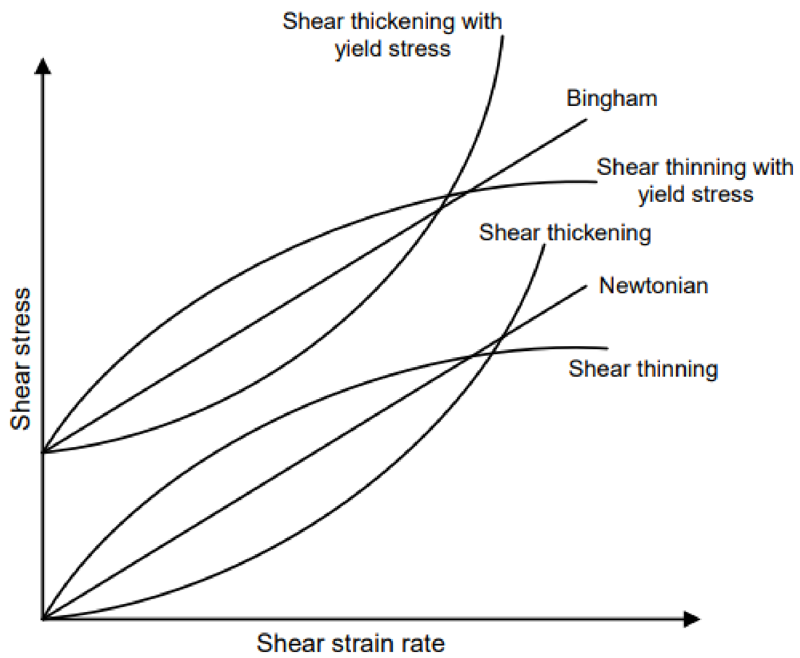

Figure 2-4 Shows a resulted shear stress versus shear rate idealized curves and a comparison of different studies through the modification made in studies[32]

During the past decades, many types of research conducted in order to explain the flow behavior of the cementitious materials. Other than two models for studying rheological properties that described before in rotational viscometers using coaxial cylinders, vane, and plate. Throughout the literature; there are also various models fitted to explain the rheological behavior of complex fluids. These equations can be lists as:

$\tau = \eta \dot{\gamma}$ Newtonian Equation 2-3[35]

$\tau = \tau_0 + \eta \dot{\gamma}$ Bingham Equation 2-4[15]

$\tau = \tau_0 + K \dot{\gamma}^n$ Herschel and Bulkley Equation 2-5[36]

$$\tau = A\,\dot{\gamma}^{\,n} \quad \text{Power} \quad \text{Equation 2-6 [36]}$$

n = 1 Newtonian flow

n > 1 shear thickening

n < 1 shear thinning

$$\tau = \tau_0 + ß \sinh^{-1} (\dot{\gamma}/C) \quad \text{Ostwald-deWaele} \quad \text{Equation 2-7[15]}$$

$$\tau = a\,\dot{\gamma} + ß \sinh{-1} (\dot{\gamma}/C) \quad \text{Eyring} \quad \text{Equation 2-8[36]}$$

$$\tau = a\,(\dot{\gamma} + C)^{\,b} \quad \text{Robertson-Stiff} \quad \text{Equation 2-9[36]}$$

$$\dot{\gamma} = a\,\tau^{\,2} + ß\tau + \delta \quad \text{Atzeni et al} \quad \text{Equation 2-10[36]}$$

Where τ is for Shear stress, η is for Viscosity τ_0 is for Yield stress and $\dot{\gamma}$ is for the Shear rate. Rest of the parameters are constants.

These different models had been used by researchers to determine the fundamental rheological properties of cement paste. The Power equations mentioned above (Herschel-Bulkley model, modified Bingham model) can be used to describe shear thinning (n<1) behavior. The Herschel-Bulkley model can be used for the case of both shear thinning and shear thickening material with yield stress[37]. Many researchers have focused their studies on comparing the equations describing the flow curves of cementitious materials [38,39].All of the relationships listed above used at least two parameters to describe cement paste flow. Those equations that have a term of yield stress (Bingham model, Herschel-Bulkley model) have a physical basis, this is while the other equations include more than two parameters without exact physical meanings [39].

It is undeniable that the different equations might be only suitable for limited ranges of material or measurement. For instance, it has been stated that the Herschel-Bulkley equation is more suitable than the Bingham model for concretes like self-consolidated concrete (SCC) [39]. Jones and Taylor mentioned in their study that the Robertson-Stiff model might be used to estimate the relationship a wide range of water to cement ratios (w/c) while the Herschel-Bulkley model can only estimate shear stress and shear rate data for a limited range of w/c[40]. Although rheological properties of cement paste are most commonly described using the Bingham model because the flow of most cement paste and concrete follows this equation fairly well [41] and also the two parameters in the Bingham model, yield stress and viscosity can be measured independently. Thus, the Bingham model was commonly used for rheological investigations on cement paste. The reason is in Bingham model the equation need two simple parameters and most of the studies show that this equation demonstrates the rheological properties of cement paste fairy well, nevertheless, in case of using superplasticizers for improving the workability, Herschel-Bulkley describes the behavior more sophisticated[42,43]. Since using the Bingham model could result in negative yield stress values in mixes containing superplasticizers.

As mentioned before a material is a Newtonian material if the proportional factor between the applied force and induced velocity gradient equals viscosity[44]. Equations 2-3 to 2-10 showed the equations applicable for cement paste since the suspension of cement particles in water is complex and commonly used equations relating viscosity is rendered caused of this structure.

2.2.1 Fundamental instruments for measuring rheology

Due to its complex nature, rheology of cement-based materials is still a field of research that needs development of new approaches [38]. Not only the equipment is complicated, the modeling and analysis of the behavior are still controversial due to its low repeatability. Thus, researchers working in this area not only focused on developing suitable equipment for testing, but also they tried to select the best approach to evaluation for selecting the best fit model to the behavior [35].

Through the literature, practical test methods of different types and quality have been developed and used to give some kind of rheological indication of the cement-based materials. The most famous, oldest and currently most used practical test is a slump or mini-cone test, which gives a single value. The test was developed in the USA 100 years ago[45]. The test apparatus is mainly associated with Abrams and it is believed that the first time of its using reported by Chapman [46]. Continuously, other different rheological tests have been developed like the flow table test, which was developed in Germany in by Graf [47], Other empirical test methods, the L-box and the V-funnel [48] (which generally are used for highly flowable concrete), have established by researchers since then.

The practical tests are very often operator-sensitive which means that with the sense that minor variations in the test, a completely different result would be given[15,30,48]. This is when the need for describing the rheological properties of fresh concrete in terms of physical quantities would sense. Regarded to this requirement much different class of rheological test equipment named viscometers has been established in the literature of concrete science.

The main purpose of the design of viscometers and advanced rheometers is to develop an operatively insensitive equipment, which means that meaning different carrying out techniques during the test, does not have any effect on the test results. An example of such viscometer is the coaxial cylinders viscometer.

A Plastometer which developed by Powers and Wiler used for the first time associating coaxial cylinders geometry to measure rheological properties of cement-based materials[46].

It was a closed inner cylinder suspended in the center of a larger bucket containing the fresh concrete. The bucket rotates back and forth, while torque is registered at the inner cylinder. The data was obtained consisted of stress-strain curves. After some time, it was found out that the using a smooth surface for both cylinders, could cause slippage and thus the angle of oscillation had to be smaller. That was when the first time the same device used with a protruding blade, to avoid possible slippage [30].

A fully rotational coaxial cylinders viscometer was not used for concrete until after about 1970[32]. After Tattersall was not successful with applying a coaxial cylinders geometry to measure the rheological properties, in 1973, he introduced the use of a modified food mixer [32] to extract the Bingham parameters, namely the plastic viscosity μ and the yield stress τ_0 (see section 2-2) which is known as the Mk I. A further development of the Mk I resulted in the famous Mk II and Mk III devices [19]. While the Mk II was established for highly workable concrete, the Mk III developed for lower workability concrete. These devices do not measure the yield stress τ_0 and the plastic viscosity μ directly, they first measure two values, designated as *g*- and *h*-values and then through a calibration technique, they convert them to yield stress and viscosity. The Mk systems are still being used and are continuously going through some developments[27]. The Mk systems have been commercially available and known as the “two-point” equipment.

To avoid the effect of shear stress τ generated from the lower part of the geometrical system in coaxial cylinders, a further improvement was done and as a result, the bottom part of the inner cylinder did not measure torque[49].

This system was further validated by numerical simulations which named the BML viscometer[50]. From the first day of this viscometer introduction, the system went through the more developments to improve instrumentation and software for controlling the viscometer. The successor of this viscometer is the ConTec BML Viscometer 3 and after that the ConTec Viscometer 5[46]. Another viscometer for fresh concrete, using parallel plates, named BTRHEOM designated in france[49].

The all Tattersall Mk system [43], ConTec BML Viscometer[50] 3 and the BTRHEOM [49] all measure the rheological properties of cement-based materials in terms yield stress τ_0 and plastic viscosity μ.

Figure 2-5 shows a comparison of devices was made for rheological properties measurements, the same batch of concrete used for all the devices and as it is represented in the figure 2-5, rheological values (τ_0, μ) measured by all devices are not the same[51].

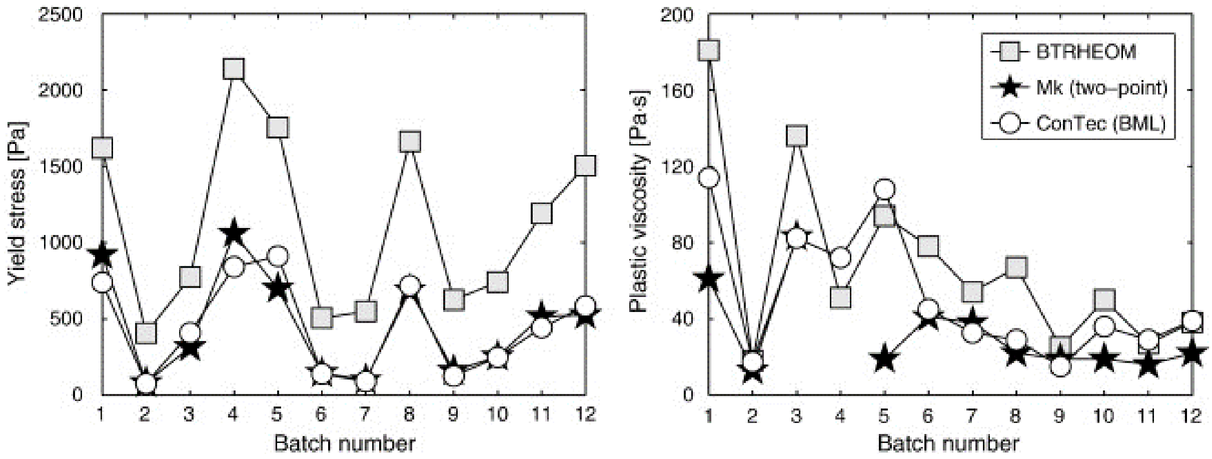

Figure 2-5 Comparison of results obtained for different concrete batches by the Mk II system, ConTec BML Viscometer 3 and the BTRHEOM [51].

2.3 Rheology of cement paste

Fresh cement-based materials are known as a highly concentrated suspension of solid particles in liquid [41]. Generally, the fresh state properties and particularly rheology of cement paste mainly influenced by the hydration process. The hydration process consists of four main stages [52–55] :

Stage I- Initial hydrolysis: A poorly crystalline skin which is a gelatinous skin over particles formed by the initial reaction between anhydrous minerals and water, that could be fully visible in a few minutes.

Stage II- Dormant period: The presence of the skin hinders the slow reaction after, that lasts few hours until the water becomes fully supersaturated with calcium hydroxide (the result of the slow reaction).

Stage III- Acceleration period: Calcium silicate hydrate and calcium hydroxide crystals interlocking process which is a fast and long reaction last between 6-12 hours.

Stage IV- Deceleration period: Deceleration reaction period, which at the last the latter is consumed and the remained space for other crystallization process and products is restricted.

The free water is used during hydration reaction resulting with an increase in an interlocking network of hydration products. This leads to a loss of workability and an increase in yield stress within the first few hours after mixing the paste, which generally occurs at the end of Stage 2. Thus, factors influencing the reaction rate such as w/c (or water to binder ratio), chemical admixtures, puzzolans, cement grain size, and composition will not only influence the reaction rate but also the rheology of cement paste.

As mentioned before Concrete is a complex composite material including aggregates (solid particle), cement and water (cement paste as viscous liquid) which makes the rheological measurements became harder with increasing grain sizes [42]. Cement paste itself is not a homogenous material since it contains cement grains and water.

In terms of cement grain size, the typical size ranges from 0.1 to 50 µm, and it can’t be considered as colloidal, but principles of collide science have been applied[56]. The particle size influences the rheology of cement paste such that as the particle size of cement decreases the particle to particle attraction increases, which results in thickening and fluctuations under shear stress. These properties that were induced by shear stress appearance are time-dependent, but there are still disagreements that could be explained by the thixotropic nature of the cement paste[57]. For satisfying the new properties expected from cement paste, investigating the effect of admixtures on rheology and microstructure was required, which started years ago and build up the body knowledge of rheology [51,52].

Another parameter influencing the rheology of cement-paste is the intrinsic property of called “thixotropy”. Technically thixotropy is defined as “the continuous decrease of viscosity with time when the flow is applied to a sample that has been previously at rest, and the subsequent recovery of viscosity when the flow is discontinued” [58]. Simply, the viscosity of a thixotropic material decreases under increasing shear stress and increases back again when the stress is removed at rest [29]. This condition results in a loop that explains the breaking down of the structure of the material and it's been called as “hysteresis loop”. The area between up and down the curve in shear stress versus shear rate figure describes the required energy to breaking down the structure or in other words the higher the degree of thixotropy. In terms of recovery, both hydration and thixotropy will affect structural rebuilding of cement paste. While the thixotropy is the reversible component that plays an influence on structural rebuilding at early ages (prior to set), hydration is not reversible and it continues throughout the lifetime of the composite (see Figure 2-6 Thixotropy and hysteresis loop. (a) Shear rate vs. time (b) Shear rate vs. shear stress[58]

The opposite behavior of thixotropy involving a gradual increase in viscosity under steady shear rate, followed by recovery is termed “negative thixotropy” or “anti-thixotropy”, which is normally resulted from the cement hydration during a prolonged rheology test[58]. Thixotropy usually happens in situations where the material is shear thinning however, anti-thixotropy generally occurs when a material is shear thickening[59]. Both types of behavior are reversible and happen over a much longer time than the effects associated with viscoelasticity.

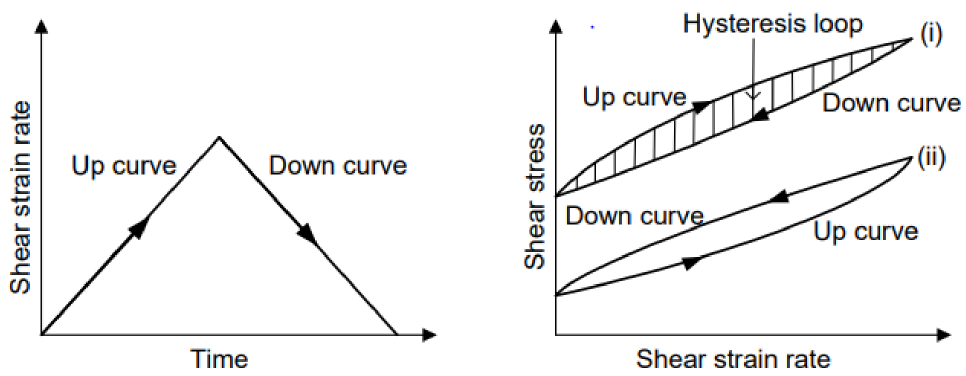

Figure 2-6 Thixotropy and hysteresis loop. (a) Shear rate vs. time (b) Shear rate vs. shear stress[58]

The shear history during mixing and the rotational shear in rheometers has a direct effect on particles interlocking and is a major interest for measuring the shear stress to initiate flow[60]. Up-to-date, rheology of cement paste was tested with several different testing equipments which would also consider the influence of thixotropy.:

i. Penetrometers and compressive testing, that measures the required force to insert a needle into the material or squeeze it [61,62].

ii. Vanes testing that measures the required shear stress to initiate the material to flow and overcome the internal forces in the structure of the material [63].

iii. The Capillary tube, Which measures the required shear stress to start flowing in the material[64–66].

iv. Raise-pipe method that the material rises up the pipe and stops when the wall shear stress reaches to the yield stress[67].

v. Oscillatory rotational and translational shear and stress relaxation methods had been all used to measuring the viscoelasticity which assisted the investigation of the elastic moduli [35,68–70].

A major drawback of these experimental procedures was, at high w/c the data was vague during to the segregation of the heavy particles. Thus, these test generally limits collecting data in mixes w/c higher than 0.45[29].

2.3.1 Influence of superplasticizers on the rheology of cement paste

Recently, the design of complex structures requires the high-performance concrete mixtures with improved workability, strength, and durability. These properties could only be achieved with low w/c and use of chemical additives to provide adequate workability. Since most of the time these additives influence the workability of concrete, they would also affect the rheology of the material. In general, the most commonly used admixture for high-performance concrete is high-range water reducers (HRWR) or superplasticizers [29].

The action mechanism of superplasticizers generally depends on the interparticle repulsion\attraction forces. The action mechanisms can be explained in 3 ways[27]:

i. The electrical layer around particles could either expanded or contracted.
ii. The adsorption of ions on cement particles could assemble a repulsive force interparticle.
iii. Protective barriers which could generate with superplasticizers and keep the particles from flocculation.

The adsorption of molecules on particles acts as a layer which restricts the physically approaching of particles. As soon as the adsorbed layers start to overlap the steric mechanism begins. This force increases continuously, strongly and reaches infinity[71]. This increase in the steric force in highly dependent on the adsorption mode of the polymer. As it was shown that as the surface charge of the polymer have a higher negative charge, the layer thickness increases[72]. With getting this barrier thicker than the distance to the potential energy, sticking particles would never happen which decrease the flocculation. The adsorption process and then barrier forming cause decreasing yield stress and viscosity, which are an operation by de-flocculation and the secondary electro-viscous effect respectively [31].

The typical amount for superplasticizer in laboratory rheological studies is known to be less than 1 percent by cement weight [15]. One of the early admixtures were lignosulfonates sault (LS) (a wood product) and synthetic resin superplasticizers such as synthetic sulfonated melamine- or naphthalene- formaldehyde polymers (SMFC and SNFC) that can reduce yield stress even to zero[73]. In consideration of the requirement to control the molecular size of superplasticizers, Polycarboxylate (PC) based superplasticizers recently are more attractive to engineers, on account of offering the optimization of the performance[41]. Superplasticizers are extraordinary additives in terms of flowing mixes with high workability and less water to cement ratio hence higher strength. However, their effect on rheology, the hydration process and the setting period resulted in problems in the field[74].

2.3.2 Influence of supplementary cementitious materials (SCM) on the rheology of cement paste

The aim of the use of SCMs in the mix design is to improve the properties such as strength, permeability, workability, and shrinkage. The most commonly used SCMs in the concrete mix design are fly ash, slag, and silica fume.

Fly ash which is also known as pulverized fuel ash (PFA), is an additional byproduct for blending in concrete[48]. The spherical shape and smooth texture of fly ash particles can lubricate the cement paste and decrease the friction between particles, thus it increases the workability in cementitious materials[75]. Addition of fly ash could decrease the yield stress and the viscosity of the cement paste. This was directly attributed to particle shape and size [76].

2.4 Influence of VMAs on the rheology of cement paste

2.4.1 Definition of VMAs

The use of superplasticizers in highly flowable mixes might also result with separation of heterogeneous phases, which is also known as segregation [77]. Segregation not only influences the physical appearance of the concrete member, it also decreases the strength, increases the permeability, thus leading to a shorter service life in structures. Incorporation of VMAs can provide stability against segregation without sacrificing the workability of the mix.

The major mechanism of water-soluble VMAs is their improvement on water retention of cement paste. VMAs also can improve the stability during transportation and pumping [5]. This improvement in stability results in a better bonding between aggregates and cement paste, which could indirectly improve the transition zone[5]. Addition of superplasticizer mainly results in reducing viscosity and yield stress. The combination of superplasticizer and VMA could improve workability, strength, stability and durable concrete[78]. It should be noted that high dosages of VMAs could cause to cohesiveness in concrete, which makes the molding process difficult[78].

The key benefits of VMAs are[78]:

I. Reducing the segregation caused by superplasticizers incorporation in HPC
II. Reducing washout in the application of underwater concretes
III. Reducing the pressure in the pump in pumping concrete
IV. Optimizing the poor grading (lack of fine aggregates)
V. Reducing powder content
VI. Reducing bleeding
VII. Improving the surface appearance
VIII. Reducing the effects of aggergates's moisture in the mixture

The dosage of using VMA in concrete differ based on the type of VMA and their application but it changes between 0.1- 1.5% by the weight of cement[77]. As for the types of VMAs, there are 5 major types of VMAs based on their action mechanism:[79]

A. *Water-soluble synthetic and natural organic polymers*: Increase the apparent viscosity of mixing water in concrete. This type consists of cellulose ethers, polyethylene oxides, polyacrylamide, polyvinyl alcohol, etc.
B. *Organic water-soluble flocculants:* Adsorbed on cement particles and increase the attraction force between cement particles, thus increasing the viscosity. This type

consists of styrene copolymers with carboxy1 groups, synthetic polyelectrolytes, and natural gums.

C. *Emulsions of various organic materials:* Increase the attraction force between cement particles and supply additional superfine particles in the cement paste. This class consists of acrylic emulsions and aqueous clay dispersions.

D. *Water-swellable inorganic materials:* Because of the high surface area, water retaining capacity of the paste would increase. It consists of bentonites, silica fume, and milled asbestos.

E. *Inorganic materials of the high surface area:* The number of fine particles would increase thus thixotropy would increase. Fly ash, hydrated lime, kaolin, various rock dust, and diatomaceous earth are some of the examples of these groups.

Class A water-soluble polymers are also classified into 3 groups[80]:

I. Semi-synthetic polymers

II. Synthetic polymers.

III. Natural polymers

Decomposed starch and its derivatives like cellulose-ether derivatives, hydroxypropyl methylcellulose (HPMC), hydroxyethyl cellulose (HEC), carboxymethylcellulose (CMC) and electrolytes, such as sodium alginate and propylene glycol alginate are the examples for semi-synthetic polymers. At last, there are synthetic polymers, which include polymers based on ethylene, such as polyethylene oxide, polyacrylamide, polyacrylate, and those based on vinyl, such as polyvinyl alcohol. Some examples for natural polymers are starch[81], guar gum[82], diutan gum[9], alginates, agar, gum arabic, welan gum[83], xanthan gum[84], rhamsan gum, and gellan gum and plant protein.

The other classification of VMAs that typically represented in literature is into two groups of the organic and inorganic group. To name the well-known inorganic VMAs, silica-based materials, such as nano-silica and colloidal silica [85]. This is while organic based VMAs are classified into three groups their self: natural (such as polysaccharides), semi-synthetic (such as cellulose ether derivatives) and synthetic polymers (such as polyethylene oxide and polyvinyl alcohol)[86].

Rols et al,[87] reported that the incorporation of starch and precipitated silica resulted in an improvement in segregation resistance and decrease bleeding SCC. Lachemi et al.[77] investigated the use of polysaccharide base VMA with water reducers. Another example of VMAs is the use of micro silica and nanosilica slurry [88]. This a high molecular weight ethylene oxide and natural polysaccharide derivative slurry increased the yield stress and viscosity

The performance of cement-based materials in addition of superplasticizers and VMAs got the most researchers interest and the improvement in rheological properties of the mixtures was reported through the literature [23,38,83,89,90]. A shear thinning behavior reported with the use of most of the VMAs, which means with increasing shear rate, viscosity would decrease [9]. The arrangement of the polymer chains of the VMAs along with the flow line causes this behavior[4]

2.4.2 Bio-based VMAs

Most commonly used water-soluble natural VMAs are polysaccharide based such as cellulose, ether fermentation and microbial-source polysaccharide-base such as welan gum. These natural VMAs have long polysaccharides backbones which could trigger 3 different action mechanisms [83]

I. Adsorption: The long-chain polymer molecules adhere to the periphery of water molecules, thus adsorbing and fixing part of the mix water and thereby expanding. This increases the viscosity of the mix water and that of the cement-based product.

II. Association: Molecules in adjacent polymer chains can develop attractive forces, thus further blocking the motion of water, causing a gel formation and an increase in viscosity.

III. Intertwining: At low rates of shear, and especially at high concentrations, the polymer chains can intertwine and entangle, resulting in an increase in the apparent viscosity. Such entanglement can disaggregate, and the polymer chains can align in the direction of the flow at high shear rates, thus resulting in shear-thinning.

Water adsorption on polysaccharides resulted in a higher entanglement after critical concentration and cause a significant increase in viscosity. The molecules are good at attracting particles and a shear-thinning behavior was observed at a high shear rate, which was attributed to the rearrangement of the molecules[91].

The high molecular weight and attraction to water properties of the VMAs, cause generating three-dimensional structures built up by the interaction between VMA molecules, cement particles, and water. This structure makes the breaking up process during a shear stress less, which result in higher yield stress and apparent viscosity[83].

The inorganic VMA particles are insoluble, amorphous and small-sized, which help them to suspend in water and form gels due to the ionic surface charge and the interaction of them with cement particles. This procedure makes the cement paste more homogenous which result in higher viscosity and thixotropy [37].

One of the well-known bio-based VMA is welan gum, which is a bacterial fermentation and has a really high molecular mass of approximately 2 million [4]. Xanthan gum is also a natural bio-based gum obtained from aerobic fermentation and the molecular mass is higher than 3 million which is causing higher water retention [92]. Both gums are resistant to high temperature and PH. In addition, most polysaccharides are incompatible with the hydration process of cement and increasing the environment pH up to 13 [7]. Starch include two polysaccharides named amylase and amylopectin. While amylase has short branching chain and thus less molecular mass, amylopectin includes long branch chains with a high molecular mass approximately 10^8 [92].

Chitosan used as an additive that improves the mechanical properties of many bio-cements, dental bio-cements, and cement for bone repair [93]. Chitosan improves the injectability of composites, the setting time (in a way similar to cellulose derivatives in cement–polymer systems) and acts as a VMA in calcium phosphate cement[94]. The addition of this polymer causes an increase in viscosity and a reduction in the fluidity of the mixture.

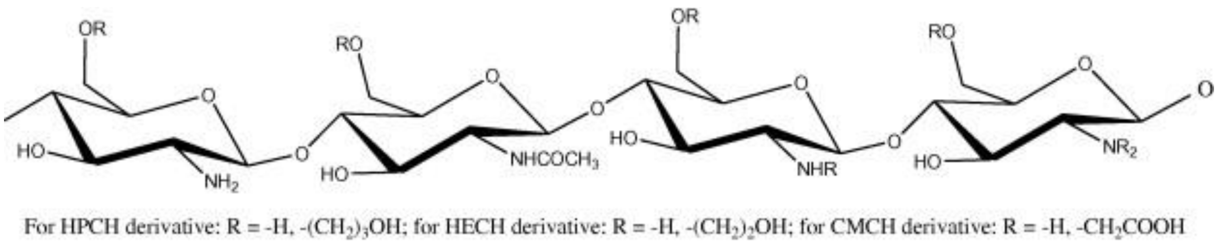

Figure 2-7 The chemical structures of chitosan derivatives are hydroxypropyl-chitosan (HPCH), hydroxyethyl chitosan (HECH) and carboxymethyl chitosan (CMCH) [95].

There are various examples for use of bio-based VMAs in cement-based materials. Sonobi [9] studied the use of diutan gum as a VMA in concrete and investigate the changes in rheology of cement paste containing fly ash. As a result of this study, it was determined that diutan gum exhibited a higher viscosity at a low shear rate than mixtures containing welan gum, which was attributed to the molecular structure of diutan gum. In addition, when fly ash was used, a lower decrease in yield stress was observed compared to mixtures containing only diutan gum without fly ash [9].

Besides welan and diutan gum, Kahng et al. [96] extracted the extracellular polysaccharides (EPS) from *Paenibacillus sp.* and incorporated in cement paste as a VMA. With this approach, the apparent viscosity of the paste was increased. However, there was also an increase in entraining air which might decrease the strength of the material.

Nopal mucilage and marine brown algae extract used as VMA in cement-based materials determining that while both showed shear thinning behavior, ALG had the highest influence on Herschel-Bulkley yield stress and the highest air- content compared to the MUC which might cause by the protein content in ALG and the interaction between polysaccharide chains[6].

Chitosans of different molecular weights were incorporated as VMA and also a heavy metal immobilizing agent. It was shown that Chitosan addition made the material thicker [97].

The different polysaccharides including welan gum, xanthan gum, and starch ether were used in different dosages with the addition of FA to the produce self-compacting concrete with less amount of fine material compare to the conventional SCC[98]

Wolfram Schmidt et al. 2013[7] Showed that in coarsely dispersed systems particle interactions contribute to the stabilizing mechanism of starch incorporation, however diutan gum functions by disabling the fluid phase between the particles.

2.5 Use of microorganisms as VMA

Through the development of bio-based admixtures, another possibility is to use cell structures as VMAs. The primary component of gram-positive bacteria cell walls (BCWs made up from dense peptidoglycan and polysaccharide backbones, which resembles the structure of a fermentation product like welan gum (*see* Figure 2-8) [3]. Moreover, the chemical structure of peptidoglycan includes long polysaccharide backbones and peptide side groups which consist of repeating units of the disaccharide, N-acetyl glucosamine-N-acetyl muramic acid and a short peptide composed of four amino acids respectively (*see* Figure 2-8)[99]. As long as the side groups are, as better they crosslink to form a three-dimensional peptidoglycan macromolecule and thus block the motion of water molecules. Additionally more anionic the structures showed higher performance in improving rheological properties at increasing pH [100].

(a) Peptidoglycan (b) Welan gum and diutan gum

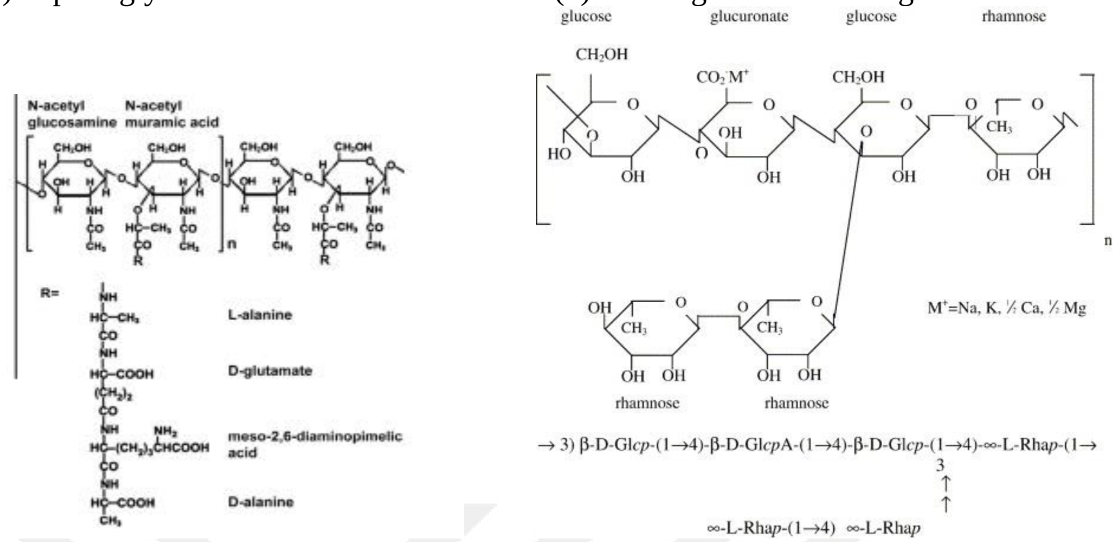

Figure 2-8 The chemical structures of (a) peptidoglycan, (b) welan or diutan gum, The structures are composed of the long-chain polysaccharide backbones and the short side groups, indicated as R [3]

Pei et al. [3] showed that the use of *Bacillus subtilis* cells walls could increase the viscosity of the cement paste. In this case, gram-positive *B. subitilis* cells have thick cell walls composed of peptidoglycans and polysaccharides which resembles the molecular structure of VMAs and led them to have a tendency to adsorb out on to the hydration of cement product, which is a preventable phenomenon by using superplasticizers [3,101]. However, extraction of cell walls again requires processing.

Yet, bacterial cells could be used as not VMAs not only their complicated cell wall structure, they can influence rheology as being micro-swimmers [8]. Micro swimming effect could be explained as microorganisms could influence the viscosity of a suspension as being a pusher (decreasing viscosity) or puller (increasing viscosity).

In nature, large organisms tend to move by using inertia forces in their motion, while smaller microorganisms such as bacteria cells or microalgae move at low Reynolds number, where viscous forces effects are over the influence of apathy [102].

Understanding the hydrodynamic forces developed with the motion of micro-swimmers in a fluid medium and the associated interactions was a matter of concern. It has been shown that micro-swimmer suspensions lead to complex dynamics called as weak turbulence or bioconvection phenomenon[103,104]. The fluids containing microorganisms made of suspension which generate a multiple force either at the front of the body (in this case they called pullers) or at the back (which they called pushers), thus they have the ability to be self-propelling[103]. The induced flow by the multiple forces can have an effect on hydrodynamic interaction which could have a result on the rheology of suspension[103]. Through the efforts have been made to model the effective viscosity suspensions containing microorganisms, Ishikawa et al.[105] show a difference in effective viscosity for suspensions of swimming spherical ‘‘squirmers’’ in a gravity field. In another study, it was shown that if the orientation distribution of swimmers assumed as anisotropic, micro-swimmers would result in a change in viscosity[106].

Saintillan [107] measured the rheology of a dilute suspension of self-propelled particles using a simple kinetic model, with applying a shear flow. Based on his study, pullers suspension exhibited a higher viscosity, while pusher suspensions exhibited a significantly lower viscosity compared to the control suspension without any cells. Hatwalne [108] showed that parameters such as swimmer types (puller or pusher), cell shape (spherical or ellipsoidal), and locomotion mechanisms were considered as factors affecting the rheology results. Sokolov et al.[109] studied the micro-rheology of suspensions of pusher-like bacteria, which led to the same results as pusher-like bacteria could cause a significant decrease in the viscosity of the material.

Based on the abovementioned parameters, it might be more efficient to incorporate not only the EPS or cell walls but directly adding the bacterial cells into the mix water. Thus, a VMA mechanism will be developed not only with the incorporation of polysaccharides but also by possibly inducing the micro-swimming effect.

2.5.1 Importance of bio-based VMAs to the concrete industry

As the benefits of using admixtures investigated in ancient time, recent development in the construction industry requires the various kinds of admixtures providing the additional properties. Base on the studies in 2003, while U.S. $ 15×10^{9} of the global market volume was related to chemical admixtures using in cementitious materials, U.S. $ 2×10^{9} was the relative market estimation for bio-based admixtures[110]. Because of the related environmental problems with synthetic chemical admixtures resulted with an increasing trend for use of bio-based admixtures. The term biobased admixture usually related to the product made in a fermentation process by employing bacteria or fungi [5]. Normally, bio-based admixtures are small molecules, such as tartaric acid or sodium gluconate, or macromolecular compounds, such as lignosulfonate, cellulose ether or Xanthan gum. The also included native products isolated from natural sources and used as is ("natural products"), or chemically modified by derivation from natural products [5]. Table 2-1 summarizes the products with significant volume in the market, the major impacts in the construction technology and the relative expenses[5].

Depending on the raw materials VMAs produced from, the prices could change. For instance, biopolymers or the mined admixtures are really costly during the labor-intensive process [5]. In fact, the high fuel consumption of such costly admixtures indicates that their beneficiation to the materials properties is tremendously high. It is well known that concrete is the most widely used material in construction and building materials. The high volume of admixture in concrete (15%) shows that bio-based admixtures could have a great impact on concrete technology[5].

New developments in concrete technology require improvements in an existing system such as improvement of cost-effectiveness, reduction of labor time and environmental effects. As the bio admixtures considered green or natural, their advantage over synthetic admixtures is inevitable. The sick house syndrome from the toxic emissions of building materials, proof the strong impact of synthetic materials in construction technology which leads to consideration of bio-based admixtures[110]. However, the demand for bio-based admixtures even with the high costs was acceptable, developing a less labor-intensive methodology is still a matter of concern.

This study conducted using microorganism without any extra intervention in order to reduce production process as possible as it is. It was hypothesized that considering the kinetic mechanism of cells in the aquatic environment and the long polysaccharide chains of it, it could also work as a VMA agent in the cement-based materials.

Table 2-1 Major bio-admixtures used in building materials[5]

Admixture	Product category	Source	Function	Major application (s)	Relative cost (%)
Tartaric acid	Natural product	Wine	Set retarder	Gypsum plaster	100
Sodium gluconate	Biotechnological product	Fungus	Retarder/plasticizer	Concrete	35
Lignosulfonate	Derivation product	Wood lignin	Dispersant/thinner	Concrete, plasterboards, oil well construction	15–65
Starch (ether)	Natural and derivation product	Corn, potato	Viscosifier	Grouts, plasters, oil well construction	20–50
Cellulose (ether)	Derivation product	Cotton, wood	Water retention	Grouts, plasters, oil well construction	200
Guar gum (ether)	Natural and derivation product	Guar plant	Viscosifier	Plaster, oil well construction	15–120
Vegetable oils (esters)	Natural and derivation product	Plants	Carrier fluid	Oil well construction; concrete	25–50
Waxes	Natural product	Plants, insects	Coating	Paints and coatings	60
Casein	Natural product	Milk	Dispersant/binder	Grouts, paints	100
Protein extract	Natural product	Animal hair, hides, hoofs	Air-entraining agent	Concrete, mortar	30
Humic acid	Natural product	Soil	Water retention	Oil well construction	10–35
Lignite	Natural product	Coal	Dispersant/thinner	Oil well construction	7
Bitumen	Natural product	Coal, petroleum	Coating	Road construction	5–10
Asphalt	Natural product	Petroleum	Coating	Road construction; oil well construction	5–15
Xanthan gum	Biotechnological product	Bacterium	Viscosifier	Oil well construction, floor screeds, paints	200
Welan gum	Biotechnological product	Bacterium	Viscosifier	Oil well construction, floor screeds, paints	600
Scleroglucan	Biotechnological product	Fungus	Viscosifier	Oil well construction	350
Succinoglycan	Biotechnological product	Bacterium	Viscosifier	Self-leveling compounds	500
Curdlan	Biotechnological product	Bacterium	Viscosifier	Concrete	350
Rhamsan	Biotechnological product	Bacterium	Viscosifier	Grouts	550
Polyaspartic acid	Biodegradable product	Synthetic	Dispersant	Gypsum retarder	30–65
Chitosan	Biodegradable product	Insect chitin	Viscosifier	Oil well construction	350

CHAPTER III

MATERIALS AND METHODS

This chapter includes a brief explanation of materials methods uses to measure evaluate the influence of microorganisms on the rheology of cement-based materials. The approach includes specification of growth media and conditions for each strain as well as the incorporation process. Then, the mix proportions for cement paste samples are defined. At last, the mini-con slump test, rheological measurements and air-content tests performed are described.

The goal of this study was to improve the rheology of cement paste by incorporating bacterial cells as VMAs in cement-based materials. To achieve this goal *S. pasteurii*, *B. megaterium*, *B. subtilis*, and *P. polymyxa* were selected. These microorganisms are known to be Gram-positive bacteria and include a thick cell wall containing peptidoglycan and polysaccharide. In addition, these bacteria are rod-shaped and have the ability to move. All of these strains, especially *P. polymyxa*, can also produce extracellular polysaccharides during growth. In literature, it was shown that *S. pasteurii* [111,112] and *B.megaterium*[112] could survive in high alkaline environments such as cement paste by making endospores.

3.1 Bacterial growth and characterization

As mentioned before there were 4 strains selected for this study: *S. pasteurii*, *B. megaterium*, *B. subtilis* and *P. polymyxa.* The first step of the cultivation process was to define the growth curve for each strain in specified nutrient media and conditions. Each stain was incubated until the cells reached stationary phase and then collected for mixing.

S. pasteurii (German Collection of Microorganisms and Cell Cultures-DMSZ 33) cells were cultured in a Urea-Corn Steep Liquor Medium (UCSL) which includes Tris base (15g), urea (10g) and Corn Steep Liquor (CSL) (15g) to a liter of deionized water (DI). The medium was adjusted to pH 9 by adding 0.1M HCl after the Tris base was added to 1L of DI water. CSL was provided in liquid form as a commercially available product from Sigma Aldrich and the chemical composition was not specified by the manufacturer. CSL was a suitable alternative to carbon sources such as yeast extract and peptone[113]. *P. polymyxa* strain was cultured in a nutrient medium including yeast extract (5g), NaCl (5g), and peptone (10g) to 1liter DI water and pH was adjusted to 7. Both *B. megaterium* (American Typical Cell Cultures- ATCC 14581) and *B.* subtilis (ATCC 6051) were grown in a medium containing Nutrient Broth (8g) per 1 liter DI water and pH was adjusted to 8. Twelve grams of agar was aged to these liquid culture media when solid media were required for plates.

After preparing the above-mentioned nutrient media, solutions were sterilized by using an autoclave (HIRAYAMA HV 25-L, Japan) at 121 ° C for 45 minutes. Then, the solutions were cooled down to the room temperature and the cells were added to them. The bacterial cultures were incubated aerobically with shaking conditions (210 rpm) at 30°C in an IKA KS 4000 model incubator. Sample aliquots were taken from these media periodically and plated on agar plates. Subsequently, samples were serially diluted (10^0-10^{-9}), and the cell concentration was obtained by viable plate counts and illustrated as colony forming units (CFU/mL). Bacterial growth curves were prepared in terms of CFU/mL vs.time. Growth experiments for each strain in special nutrient media were conducted as triplicates.

Once the cells reached their stationary phase, the cells were harvested from the culture by centrifuging at 6300g(800rpm) for 15 min. Afterward, the cells were washed twice by 15-20 mM Phosphate buffered solution (PBS) and kept at 4°C until testing.

3.2 Zeta potential measurements

To understand the action mechanism of bacterial VMAs, it is required to understand the particle to particle interaction. One of the parameters that might affect the action mechanism is the surface charge of the cells. To measure the surface charge, the bacterial cells were grown in abovementioned nutrient media until the stationary phase was reached. Then, cells were harvested by centrifugation at 6300 g for 15 min, washed with sterile DI water and resuspended in 4 different media:

I. Directly tested

II. Incubated in 100 ml DI water at shaking conditions (210 rpm) at 30°C till 8 hours.

III. Added to 100 ml DI water and 2g superplasticizer and incubated aerobically with shaking conditions (210 rpm) at 30°C till 8 hours.

IV. Added to 100 ml nutrient media (prepared for each strain as mentioned before) and 2g superplasticizer, incubated aerobically with shaking conditions (210 rpm) at 30°C till 8 hours.

Upon subsequent incubation, the cells were centrifuged again at 6300 g for 15 min and added to a sterilized PBS. A Malvern Zetasizer Nano ZS (Malvern, Worcestershire, United Kingdom) was used to measure the zeta potential of the cells. Afterward centrifuged again at 6300 g for 15 min and washed with PBS.

3.3 Preparation of cement paste samples

All mixtures were prepared using Ordinary Portland Cement (OPC) CEM I 42.5 R. To evaluate the influence of w/c ratio on the efficiency of cells, 4 different w/c ratios were used: 0.36, 0.40, 0.45 and 0.50. In case of superplasticizer addition, half of the mix water was added after waiting and the admixture was added to the last portion of the water. The weight of water content in bacterial cells was negligible so it did not affect the w/ c. To test the compatibility and the performance of the biological VMA with superplasticizers and fly ash, a polycarboxylate superplasticizer (by 1% of the cement weight), and an F-type fly ash (by 20% of the cement weight) were added to samples.

To investigate the effect of microorganisms on the rheology of the cement paste, cells were grown in the described medium until the concentration of cells was reached to 10^9-10^{10} CFU / mL. Then, the cells were collected from the nutrient medium by centrifuging and washed (*see* Section 3.1). Subsequently, these cells were directly added to the mixing water. The number of wet cells was adjusted in terms of percent weight of cement, such as 0.05%, 0.1%, and 0.5% of the cement weight. The cement paste samples were prepared according to ASTM C 305-11 standard [114] and the cells were added to mixing water prior, and then mixed with cement.

The standard mix procedure was done following these steps:

I. Cementitious materials and half of the water content were mixed together at 62.5 rev/min for 150 seconds.

II. The mix was left to rest for 75 seconds

III. Standard sand was added and mixed for 30 seconds at 62.5 rev/min and at 125 rev/min for another 30 seconds.

IV. The other half of the water content in addition to bacteria were added and mixed for 60 seconds at 125 rev/min for obtaining a homogeneous mixture

Table 3.1 describes the different cement paste mixtures prepared for this study, showing the different dosages of bacteria cells, w/c and amount of superplasticizers and fly ash that was added.

Table 3-1 Composition of each cement paste series for testing. SP: Superplasticizer; FA: fly ash.

w/c	Bacteria	Cell dosage per cement weight	FA per cement weight	SP per cement weight
0.36	*S. pasteurii* *P. polyxma* *B. subtilis* *B. megaterium*	0 0.05% 0.10% 0.50%	-	-
		0 0.05% 0.10% 0.50%	20%	-
		0 0.05% 0.10% 0.50%	-	1%
		0 0.05% 0.01% 0.50%	20%	1%
0.40	*S. pasteurii* *P. polyxma* *B. subtilis* *B. megaterium*	0 0.05% 0.10% 0.50%	-	-
		0 0.05% 0.10% 0.50%	20%	-
		0 0.05% 0.10% 0.50%	-	1%
		0 0.05% 0.10% 0.50%	20%	1%
0.45	*S. pasteurii* *P. polyxma* *B. subtilis* *B. megaterium*	0 0.05% 0.10% 0.50%	-	-
		0 0.05% 0.10% 0.50%	20%	-
		0 0.05% 0.10% 0.50%	-	1%
		0 0.05% 0.10% 0.50%	20%	1%
0.50	*S. pasteurii* *P. polyxma* *B. subtilis* *B. megaterium*	0 0.05% 0.10% 0.50%	-	-
		0 0.05% 0.10% 0.50%	20%	-
		0 0.05% 0.10% 0.50%	-	1%
		0 0.05% 0.10% 0.50%	20%	1%

3.4 Rheological measurements

3.4.1 Equipment and Geometry

Rheological measurements were made at room temperature (23 ºC) with ANTON PAAR RheolabQC rotational rheometer (Anton Paar, Graz, Austria Figure 3-2). More than 256 mixes were prepared at 4 different w/c ratios and 3 bacterial dosages, including the control samples without any microorganisms. For various rheology tests, the geometries were not changed. In this research, the smooth surface coaxial cylinder geometry for the flow curve test was used. The rheometer was connected to a computer and all experiments were controlled with the results plotted in the rheometer program in the computer. In the protocol specified in the equipment manual, was written that the re-calibration should be done every 6 months. It took 6 months finishing the rheological experiments and the rheometer was not re-calibrated during this period of time.

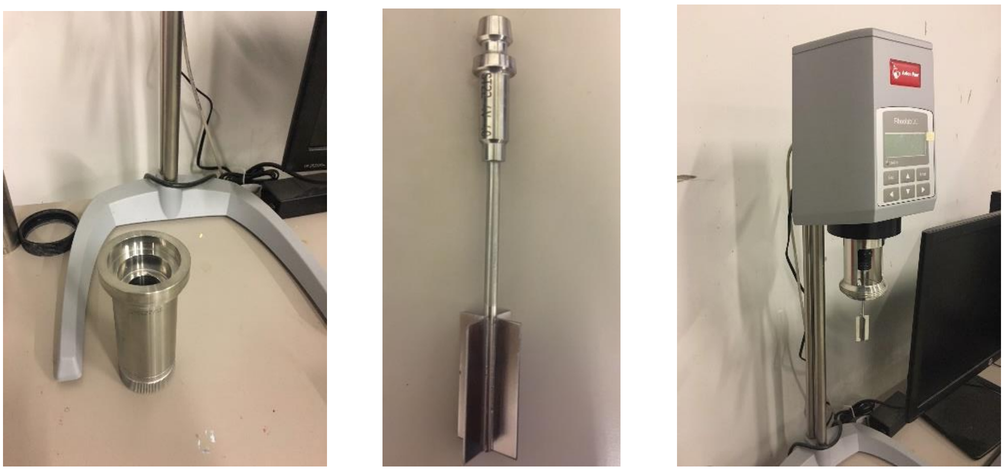

Figure 3-1 Image of the outer cylinder, the coaxial vane, and the rheometer device

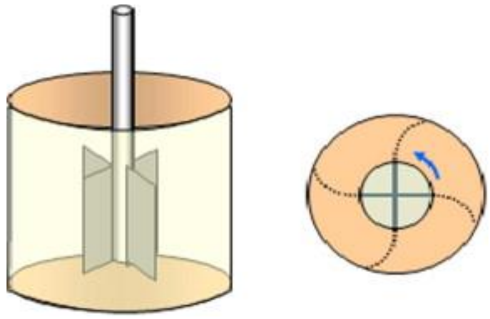

Figure 3-2 Geometry of vane spindle[115].

3.4.2 Pre-conditioning and flow-curve Protocols

Upon mixing, the samples were First, cement paste was mixed for 60 seconds to ensure the homogeneity of the mixtures. Then, a pre-shear stage where the shear rate was kept constant at $100s^{-1}$.

Following the pre-shear stage, the analysis was conducted by increasing the shear rate from $100s^{-1}$ to $1s^{-1}$ and the yield stresses and viscosity were recorded. The upcurve was chosen for evaluation of the rheological behavior of the samples.

The rheological behavior of the cement paste was evaluated using the Bingham model

$(\tau = \tau_0 + \mu\Upsilon)$.

$$\tau = \tau + \mu\dot{\gamma} \quad \text{Equation 3-1 [33]}$$

Where τ is for shear stress (Pa), $\dot{\gamma}$ is for Shear rate (s^{-1}), μ is for Viscosity (Pa.s) and τ_y is the yield stress (Pa)

An alternative model also should be considered, since the shear-thinning or shear thickening behavior of mixes were unknown. In case of a shear thickening behavior, the flow curve would not be simply explained by Bingham equation. Instead, a modified

Bingham equation (Equation 3-2) was used to analyze shear-thickening cement paste samples (3), which could also explain the shear thinning behavior well [116]:

$$\tau = \tau + \mu\gamma \dotplus c\dot{\gamma}^n \qquad \text{Equation 3-11}$$

where τ is the shear stress (Pa), τ_0 is the yield stress (Pa), μ is plastic viscosity, $\dot{\gamma}$ is the shear rate (s^{-1}) and c is second degree parameter ($Pa.s^2$) [116]. When c/ μ > 0 the materials is classified as shear-thickening and when c/ μ < 0, the materials shows shear thinning behavior. As expected, samples including superplasticizer resulted with a c/μ >0 and this was directly related to the type of the superplasticizer used in the mix[116].

To evaluate the time-dependent behavior of cement-paste, the samples were re-tested by increasing the shear rate from $100s^{-1}$ to $1s^{-1}$ without premixing after 10 minutes of waiting and the change in viscosity and yield strength were recorded.

3.5 Mini-slump flow test

One of the most common and easy tests for evaluating the workability of cement paste is the mini-slump flow test [117]. The mini-slump test was conducted based on the measurement of the spread of cement paste placed into a cone-shaped mold on a smooth plexiglass. For this approach, a specially manufactured stainless-steel cone with a height of 57.3 mm, an upper diameter of 19 mm and a lower diameter of 38 mm was used[9]. The cone was placed on the center of the plexiglass, and the sample was filled into the cone immediately after mixing. The cone was then lifted to let the cement paste flow. Then, the diameters at four right-angle positions were measured and the average diameter was calculated.

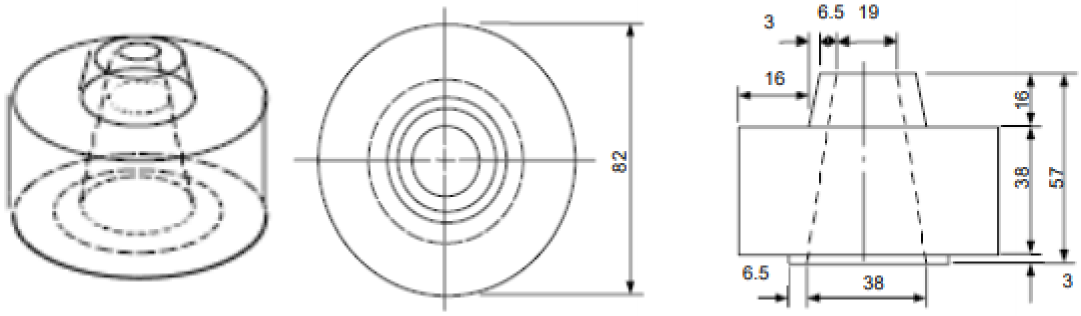

Figure 3-3 schematic illustration of mini-slump cone [118]

Figure 3-4 Mini-cone slump

3.6 Air content test

The objective of this test was to determine the influence of microorganisms on air-content in cementitious materials. The test applied mortar same as described in Section 3.3. The Air Content test conducted according to the ASTM C185[119]. According to this test method, a flow table test was applied to the cement paste and the next air content of the mixture was evaluated. With the standard procedure according to ASTM C1437 – 15[120]and following the flow table test, the mass per 400 mL of the mortar was determined. To get 400 ml mortar, the mortar was compacted as follows; the mortar was filled in the container in 3 layers by tamping 20 times around the surface of each layer. Finally, the weight of the mortar grout was measured and the air content calculated using the equation below:

$$\text{Air content, volume } \% = 100 - W\left(\frac{182.7 - P}{2000 + 4P}\right) \qquad \text{Equation 3-3}$$

Where W is the weight of sample compressed to a volume of 400 ml, and P is w/c of the mix.

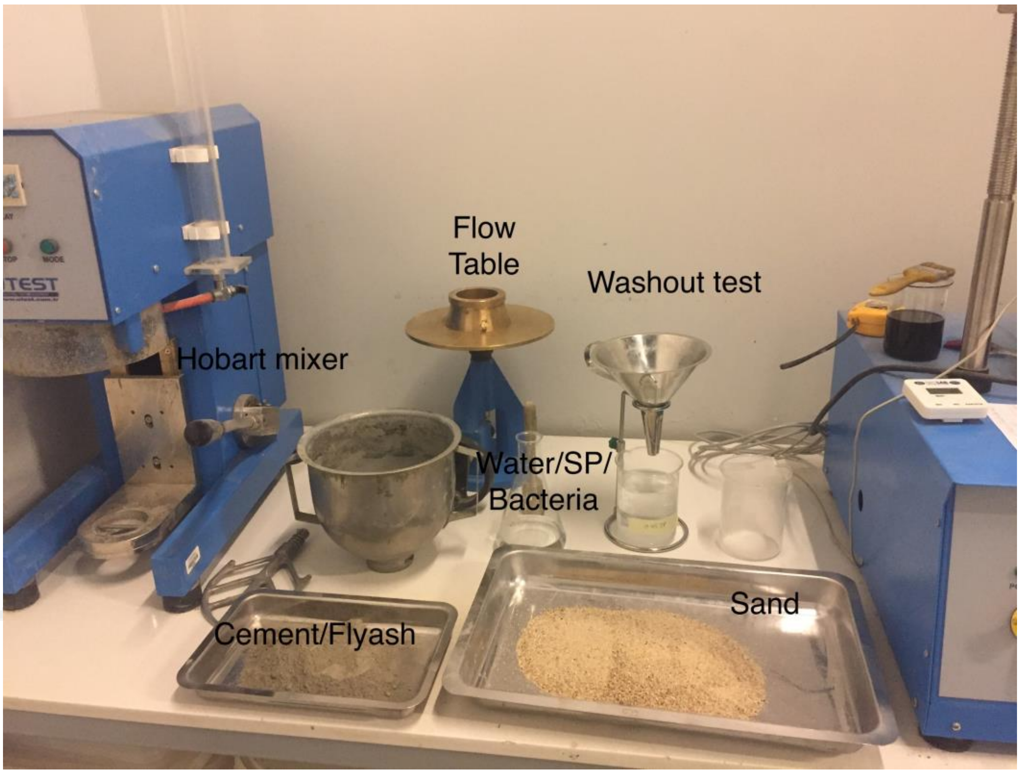

Figure 3-5 Hobart mixer wash-out test and flow table typical set up for mixing and testing various mortar samples

CHAPTER IV

RESULT AND DISCUSSION

The objective of this chapter is to investigate and discuss how different types of strains and dosages will affect the rheological properties of cement paste with and without incorporation of fly ash and superplasticizer. It also describes the effect of biological VMAs on time-dependent behavior of cement-paste and segregation resistance. The rheology measurements and mini cone test were analyzed to describe the workability, viscosity and yield stress of cement paste. Since the surface charge of cells might also interfere with their performance as VMA the growth curves and zeta potential of cells were also presented herein.

The results are presented in the following order:

I. Microorganism growth curves and zeta potential measurements for 4 different strains kept at different conditions.
II. Rheological parameters that were determined both by the *Bingham model* and *Herschel-Buckley model*.
III. A comparative assessment was made to verify whether the identification of the incompatible mixtures based on rheology method was done by mini cone slump and air content tests.

4.1 Microorganisms growth and zeta potential measurements

The different nutrient media for each bacteria were prepared and *S. pasteurii*, *B.megaterium*, *B. subtilis* and *P.polymyxa* were grown in 300 mL at 30^0c (*see* section 3.1) in their identified media. The growth profiles for each cell in each media averaged from triplicates of samples are shown in Figure 4-1 to Figure 4-4.

As represented in figures, it was possible to obtain the desired bacterial concentration and quantity with selected strains in specified nutrient media. However, by the time of cells added to the cement paste, instead of cell number, bacterial weight by the cement weight (600 g) was reported. *S.pasteurii*, *B. megaterium,* and *B. subtilis* required less labor (by the means of centrifuging cells out of the culture) and time to obtain the demanded weight of cells compared to *P.polymyxa.*

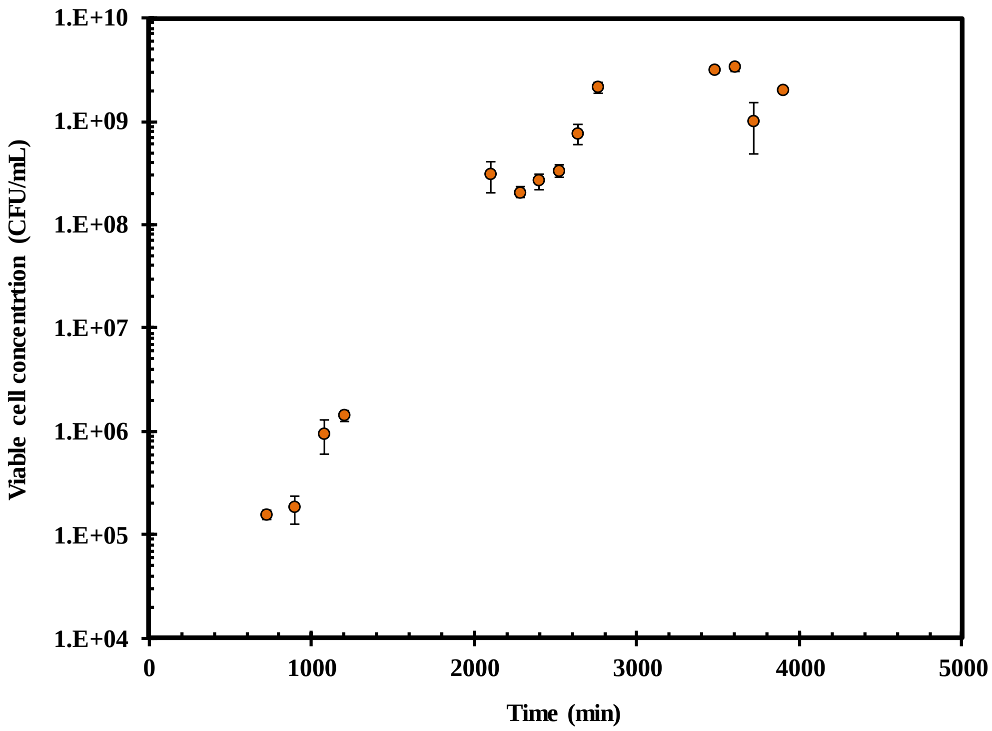

Figure 4-1 Representative growth profile for *S. pasteurii* (DSMZ 33) averaged from triplicates of viable plate counts (cell concentration vs. time) in UCSL media (pH 9) at 30°C; U: urea, CSL: corn steep liquor; Error bars represent the standard deviation based on triplicates of viable plate counts.

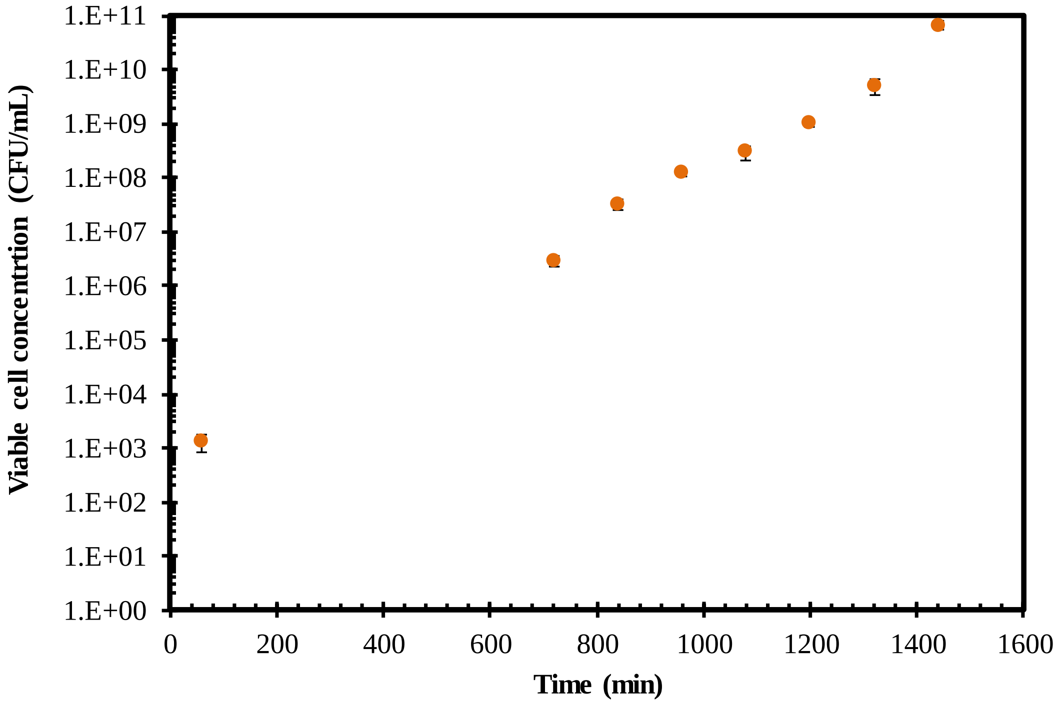

Figure 4-2 Representative growth profile for *P. polymyxa* averaged from triplicates of viable plate counts (cell concentration vs. time) in media (pH 7) at 30°C; Error bars represent the standard deviation based on triplicates of viable plate counts.

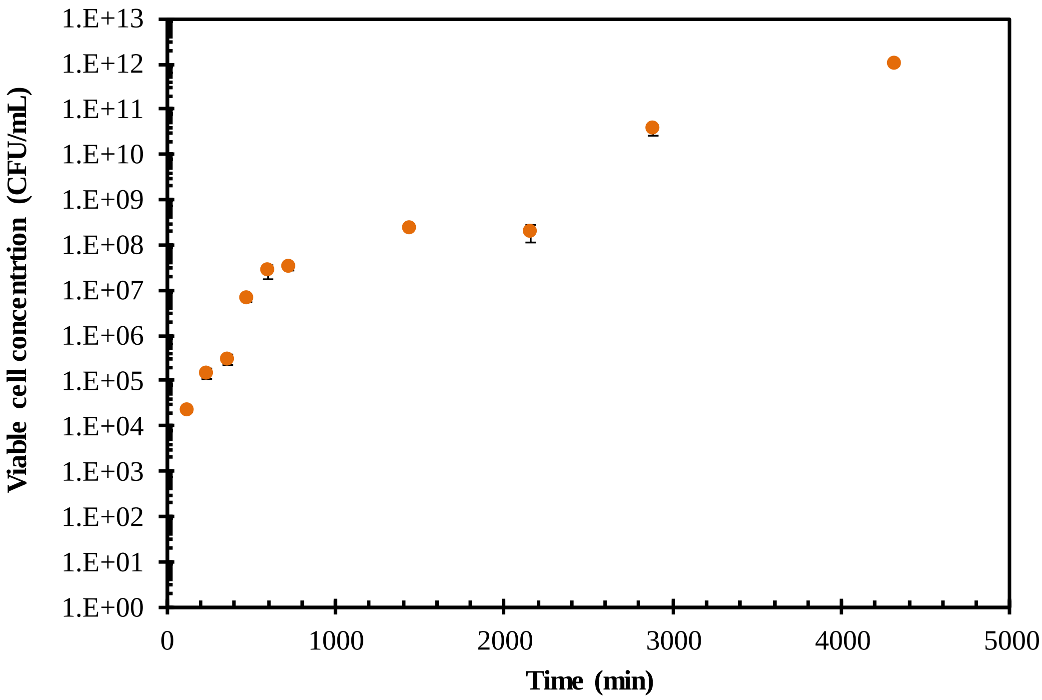

Figure 4-3 Representative growth profile for *B. megaterium* (ATCC 14581) averaged from triplicates of viable plate counts (cell concentration vs. time) in media (pH 8) at 30°C; Error bars represent the standard deviation based on triplicates of viable plate counts.

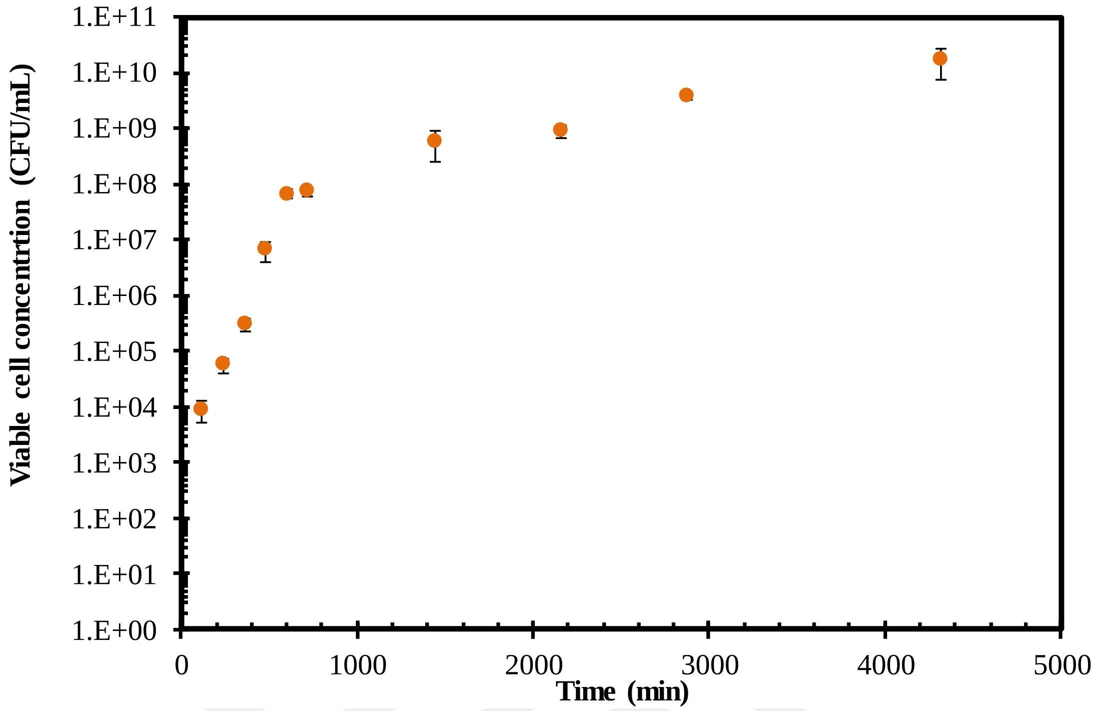

Figure 4-4 Representative growth profile for *B. subtilis* (ATCC 6051) averaged from triplicates of viable plate counts (cell concentration vs. time) in media (pH 8) at 30°C; Error bars represent the standard deviation based on triplicates of viable plate counts.

Metabolic activities, cell structures, and surface charge are important to understand the interaction of cells with cement particles. Thus, zeta potentials of cells kept at different environments upon cell growth were measured by DLS.

According to the zeta potential measurements, all of the four type strains had a negative surface charge, which was expected because all of them were known to be Gram-positive. While *P. polymyxa* and *S.pasteurii* have similar properties, *Bacillus* strains can be grouped within themselves. When *P. polymyxa* and *S.pasteurii* strains were added into DI water, there was a decrease in surface loads. The deterioration of the cell walls structures of these cells due to osmosis in the DI environment might be the reason[121]. The decrease in surface charge was more pronounced in *S.pasteurii* and *P.polymxa* compared to *B,subtilis,* and *B.megaterium*, when they were re-suspended in water. This might indicate that *B,subtilis*, and *B.megaterium* were more resistant to the restricted environment such as depletion of nutrients, low pH etc. The reason might be longer chains or the difference between the chemical structure of these strains. More investigation should be done regarding the long polysaccharide chains and their interaction with naphthalene-based SP in a medium.

In addition, it was observed that when the superplasticizer was added to the solution, the surface charge of the cells decreased slightly, regardless of the strain type. Although the exact reason for this is not known, it could be due to a similar effect to DI water resulting in the deterioration of the structure of the cell wall.

Table 4-1 Zeta Potential measurements for bacterial strains harvested in different media. 1- Added to the nutrient media and incubated for 8 hours.2. Cells were added into DI water and incubated for 8 hours.3. Cells were added to purified water containing superplasticizer (0.05% of solution volume) and incubated for 8 hours.4. Cells were added to Nutrient media and the superplasticizer (0.05% of the solution volume) and incubated for 8 hours; poly: *P.polymyxya*; sub: *B. subtilis*; mega: *B.megaterium*; past: *S.pasteurii*

sample name	Zeta Potential (mV)
1.poly-without waiting	-17.47 ± 0.97
2.poly-waited-water	-10.03 ± 0.92
3.poly-waited-water-sp	-8.68 ± 0.86
4.poly-waited-nut-sp	-16.12 ± 1.66
1.sub-without waiting	-21.23 ± 1.21
2.sub-waited-water	-18.08 ± 1.46
3.sub-waited-water-sp	-19.55 ± 0.54
4.sub-waited-nut-sp	-14.65 ± 1.21
1.mega-without waiting	-17.38 ± 10.78
2.mega-waited-water	-18.18 ± 1.16
3.mega-waited-water-sp	-17.8 ± 1.56
4.mega-waited-nut-sp	-12.95 ± 0.94
1.past-without waiting	-12.7 ± 0.71
2.past-waited-water	-11.95 ± 0.82
3.past-waited-water-sp	-8.84 ± 1.15
4.past-waited-nut-sp	-10.33 ± 0.55

4.2 Rheological experiments

Recent development in concrete technology mixes with improved flowability and stability, demand VMAs. Generally, VMAs use along with fly ash and superplasticizers. A combination of these three mixing ingredients and their influence on the mechanism of the bacterial cells also considered in this study. The first step to understanding the mechanisms of biological VMAs is to evaluate its rheological properties.

4.2.1 Influence of microorganism on rheological properties of cement paste

An extensive study was undertaken with the incorporation of *S. pasteurii, P.polymyxya, B. megaterium* and *B. subtilis* cells as VMAs and their influence on rheological properties of cement paste was evaluated. Tables 4-2 to 4-5 show the yield stress and viscosity curves under increasing shear stress in cement paste samples including different bacteria strains at different dosages to cement paste samples having w/c's of 0.36, 0.40, 0.45 and 0.50.

As shown in figures, the rheology of the mix significantly influenced by the incorporation of cells such that the shear stresses value and the apparent viscosity of the sample increased with increasing cell dosage. Based on our analysis, the apparent viscosity of the samples including cells was higher compared to control neat paste samples at low shear rates, particularly at low w/c's 0.36 and 0.40. However, with an increase in shear-rate the viscosity of the material decreases. As mentioned before, this is the nature of most cementitious mixes, also known as the shear-thinning response of the material. On the other hand, the shear-thinning response of the mix was more pronounced with increasing bacteria dosage. This might be directly related to increasing molecular weight of polysaccharides and peptidoglycans in the cells structure resulting in an increasing intertwining of chains and leading to a higher water retention at low shear rates [77]. However, with increasing shear rate this bonding break, release the entrapped water and leading to a decrease in viscosity. This might improve the fluctuation and agglomeration of the solids in the mix; reduce the flowability and a mechanism shows that the cells could work as VMAs.

Although the four of the chosen strains effectively affected the rheological properties, there was not a clear trend among the cell type, dosage and w/c.

a. Use of P.polymxa as a VMA:

The first cell type chosen was *P.polymyxa*, which was known to be a strain type having a high extracellular polysaccharide (EPS) production rate[122]. Moreover, the EPS extracted from these cells were previously used as a VMA in cement paste mortars[122]. As expected, incorporation of *P.polymxa* cells resulted with a significant increase in both yield stress and plastic viscosity, even at dosages as low as 0.05% cells per weight of cement even at high w/ cs like 0.50. At all w/c besides 0.45, there was a similar trend such that increasing cell dosage lead to a higher viscosity and the threshold bacterial dosage was 0.1%. Once the dosage was increased to 0.5%, the apparent viscosity of the material decreased compared to sample having a cell concentration of 0.1%. However, the value of the apparent viscosity and yield was still higher compared to the neat samples. Even though the exact mechanism is not known, the behavior of cells exhibited a great resemblance to commercially available bio-based VMAs. Such that the long polysaccharide chains adsorbed on to the water molecules, fill the surface after a specific dosage. With increasing dosage, polysaccharides and peptidoglycans in the cells structure might be crushed on the water molecules surfaces, thus resulting with a decrease in apparent viscosity. Such an effect was not observed when *P.polymxa* cells were added to the cement paste at a w/c of 0.45. This might be related to normal consistency of the mix, which enabled the required workability for the mix. Further experiments should be done to understand the relationship among the cells dosage, apparent viscosity, and consistency of the mix.

Comparing the average plastic viscosity of the mixes including *P.polymxa* cells (*see* Table 4.1), now there is actually a trend, where the threshold value for bacterial cells dosage was found to be 0.1% in terms of increasing the plastic viscosity and the cells found to be the most efficient at a w/c 0.45.

Another phenomenon evaluated in this study was the time-dependent behavior of cement paste such as thixotropy and hydration of the mix. Both of these processes would increase the viscosity of the material but the change due to hydration is irreversible while the change due to thixotropy is reversible. As for such a short waiting frame for 10 min., if there was a notable reduction in viscosity, this might be an indication for a segregating mix. Upon 10 min of waiting, the plastic viscosity and yield stress values were almost the same as the initial mix except for w/c of 0.36. Although with 0.1% and 0.5% dosages, and increase and decrease in viscosity were observed after 10 min waiting period. This might indicate the thixotropy behavior of the cement paste and also confirm the fact that, using cells as VMA would not affect the thixotropy behavior and hydration process of the cement paste negatively. This is while, segregation is known to happen in a higher w/c, like 0.45 or 0.50, and normally not happen in a w/c of 0.36. The long polysaccharide chains adsorbed on to the water molecules, fill the surface after a specific dosage. With increasing this dosage, polysaccharides and peptidoglycans in the cells structure could crush on the water molecules surfaces, thus resulting in a decreased viscosity.

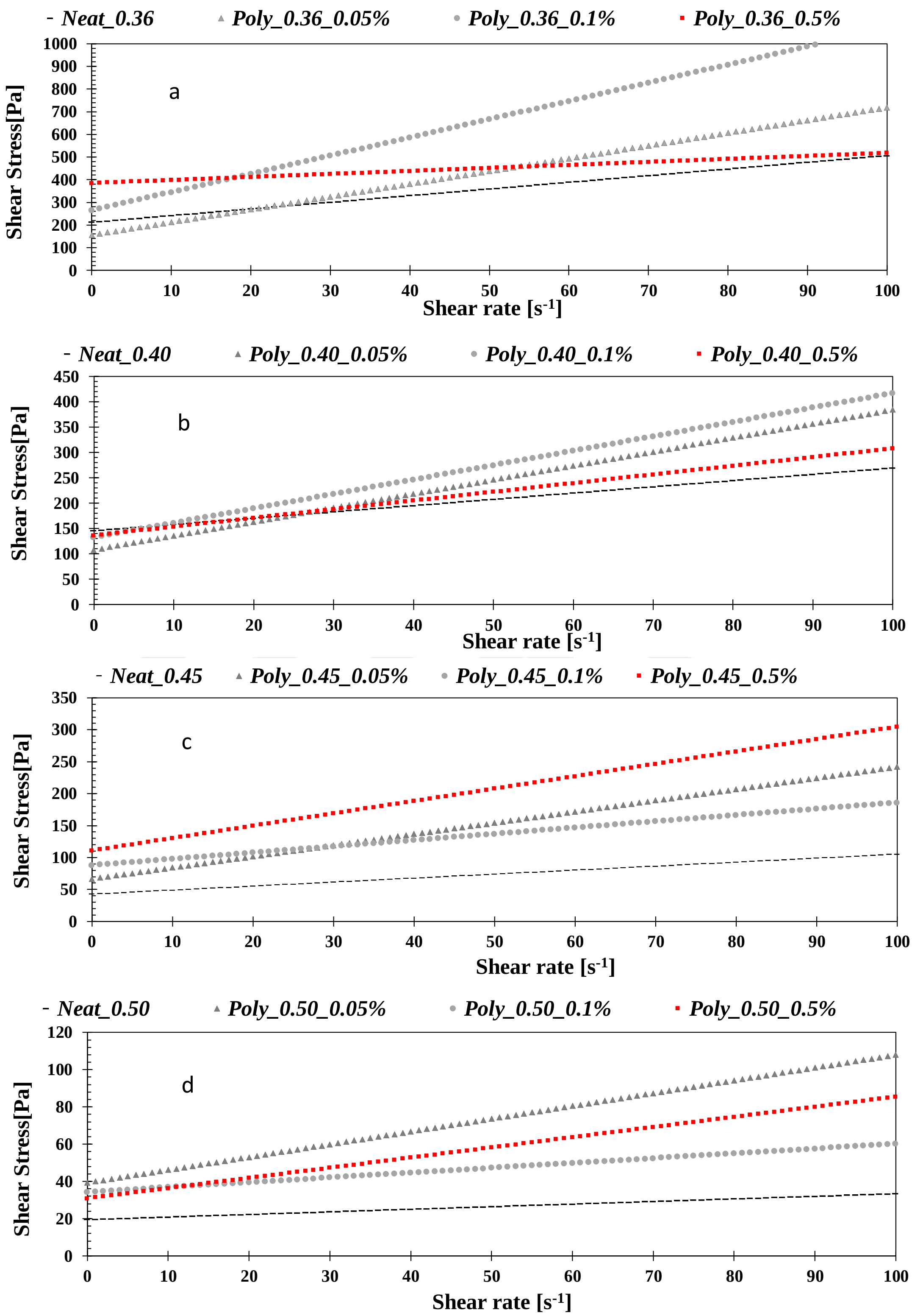

Figure 4-5 Rheological test results for cement paste samples with and without *P.polymyxa* at a w/c of (a)0.36, (b)0.40, (c)0.45 and (d) 0.50. Cell dosages were used as 0.05%, 0.1% and 0.5% per weight of cement.

Table 4-2 Rheological test results for samples with and without bacteria for *P. polymyxa* strain. The w/c were kept at 0.36, 0.40, 0.45 and 0.50. Cell dosages were used as 0, 0.05%, 0.0.1% and 0.5% g of 600g cement.

w/c	Bacteria	Cell dosage per cement weight	FA per cement weight	SP per cement weight	Mini Slump (cm)	Yield Stress [Pa]		Plastic Viscosity [mPa·s]	
						first	after 10 mins	first	after 10 mins
0.36	*P. polyxma*	0.00%	-	-	4.05	211.94	213.28	2943.10	2917.70
		0.05%			3.95	156.11	161.20	5628.60	5074.80
		0.10%			5.25	263.41	270.06	8034.90	7147.40
		0.50%			4.75	383.94	258.05	1327.90	3173.10
		0.00%	20%	-	4.65	359.90	364.45	5217.90	5437.20
		0.05%			5.45	137.73	141.73	3424.30	3373.30
		0.01%			5.65	242.63	210.99	4550.90	4654.70
		0.50%			6.30	335.48	247.47	2442.50	3486.30
		0.00%	-	1%	15.40	-2.15	-2.51	193.20	184.50
		0.05%			17.30	-4.46	-8.19	306.30	430.00
		0.10%			18.00	-11.56	-14.01	819.30	921.60
		0.50%			18.25	-5.03	-5.83	320.00	342.10
		0.00%	20%	1%	15.90	-1.36	-1.16	117.20	100.00
		0.05%			15.60	-8.76	-7.90	444.70	516.60
		0.10%			17.90	-11.56	-14.01	819.30	921.60
		0.50%			18.05	-4.38	-3.98	316.60	221.00
0.40	*P. polyxma*	0.00%	-	-	6.15	146.28	146.06	1237.3	1424.3
		0.05%			5.00	108.54	107.68	2764.8	2800.5
		0.10%			4.60	132.71	143.13	2843.2	2777.6
		0.50%			4.75	136.52	143.67	1714.9	1754.2
		0.00%	20%	-	7.15	92.18	96.47	1393.42	1375.92
		0.05%			7.45	83.59	88.98	2769.41	2662.8
		0.10%			8.00	136.97	139.47	2069.8	2031.5
		0.50%			6.00	156.47	152.62	1555.7	1656.8
		0.00%	-	1%	19.00	-1.60	-2.30	71.20	110.40
		0.05%			16.60	-2.84	-6.10	125.40	398.30
		0.10%			16.00	-1.80	-3.62	84.00	165.60
		0.50%			13.80	-1.85	-3.32	88.50	152.50
		0.00%	20%	1%	19.00	-1.50	-1.82	77.50	90.20
		0.05%			17.20	-7.00	-20.68	480.70	1462.30
		0.10%			18.30	-1.82	-3.62	83.80	165.60
		0.50%			16.35	3.43	-2.53	235.40	146.30
0.45	*P. polyxma*	0.00%	-	-	7.75	42.45	45.64	624.72	630.28
		0.05%			7.15	66.57	65.79	1753.8	1761.00
		0.10%			7.00	87.45	88.26	1897.02	1876.21
		0.50%			6.25	110.39	115.12	1939.10	1792.1
		0.00%	20%	-	8.20	46.61	48.82	534.68	511.05
		0.05%			8.75	71.72	71.33	1657.00	1811.4
		0.10%			8.50	62.66	64.71	855.29	880.31
		0.50%			8.15	54.90	56.15	728.83	720.66
		0.00%	-	1%	17.05	-0.67	-4.37	36.40	226.40
		0.05%			18.00	-0.96	-6.17	60.10	662.10
		0.10%			17.45	-0.71	-2.19	24.00	88.60
		0.50%			16.15	-1.56	-4.97	71.00	256.10
		0.00%	20%	1%	18.60	-0.84	-0.60	34.60	21.80
		0.05%			17.35	-0.62	-4.21	26.30	190.40
		0.10%			17.70	-1.92	-2.85	60.20	112.10
		0.50%			16.70	-1.53	-2.50	56.00	99.10
0.50	*P. polyxma*	0.00%	-	-	12.10	19.56	20.33	139.08	140.34
		0.05%			13.35	39.39	41.63	686.13	738.73
		0.10%			11.65	34.25	34.81	258.82	246.87
		0.50%			9.50	30.85	33.27	546.00	541.00
		0.00%	20%	-	11.20	21.67	21.94	114.57	113.25
		0.05%			13.00	26.81	28.72	374.10	398.68
		0.10%			11.05	27.93	29.17	355.08	344.54
		0.50%			10.20	26.16	27.50	454.50	461.80
		0.00%	-	1%	16.00	-0.90	-4.48	20.00	191.40
		0.05%			18.95	-0.77	-4.38	44.70	637.60
		0.10%			16.35	-0.40	-4.53	18.90	203.30
		0.50%			15.85	-1.55	-5.30	68.40	366.50
		0.00%	20%	1%	17.00	-0.32	-3.55	11.80	138.20
		0.05%			18.35	-0.45	-8.31	18.00	347.00
		0.10%			18.35	-0.31	-2.16	8.50	76.20
		0.50%			17.80	-4.40	-2.26	67.20	0.08

b. Use of S. pasteurii as a VMA:

The second strain evaluated as a VMA was *S. pasteurii*, which was commonly used as a self-healing agent in cement-based materials[123]. *S. pasteurii* is a rod-shaped Gram-negative, non-pathogenic bacteria, which could survive in mortar even after 1 year [124,125] and self-heal both internal [12] and external cracks [126]. However, the influence of *S. pasteurii* on rheological properties has not been studied in the literature.

Similar to *P. polymyxa* cells, incorporation of *S. pasteurii* cells also improved both the yield stress and the effective viscosity of the cement paste sample, particularly at lower w/c's (0. 36 and 0.40). However, compared *P. polymyxa* cells a higher dosage of *S. pasteurii* cells (0.5% by weight of cement) was required to observe the same amount of change in apparent viscosity. This indicates that in *P. polymyxa* cells both EPS and cell structure contributes to the viscosity however in the case of *S. pasteurii* addition, only the peptidoglycans in the cells structure influence the viscosity. Thus, to obtain the same efficiency, a higher concentration of cells was required.

The influence of microorganisms as a VMA was less pronounced at high w/c= 0.50. This was directly related to increasing the fluidity of the mix. Even though the flowability of the mix increased, the bacterial cells were able to increase the viscosity of the material by 7-20% even with the incorporation of 0.05% cells by weight of cement. It is simply the cells could not overcome the high fluidity of the mix. The increased amount of water content in the mix that might limit the attractive forces between the strains chain [5]. The performance of *S. pasteurii* cells as VMAs was rather similar to the behavior of welan and diutan gum [9]. Due to their relatively high molecular weight and polysaccharide structure both diutan gum and welan gum were also found to be effective additives as

VMAs for cement-based materials [127]. As so, the incorporation of welan gum by 0.04% of cement weight increased the plastic viscosity 1.6 to 2 times larger than that of a control sample without welan gum, which is similar to what was observed with use of cells. On a related note, there was not any simple explanation for the relationship between viscosity and welan gum dosage[9]. Comparing the constant dosage of diutan gum and welan gum (0.04%) showed that, although plastic viscosity increased with both of gums incorporation, the highest viscosity obtained by the diutan gum incorporation (2.5 to 2.7 times greater than control samples) [9]. Moreover, to obtain similar rheological properties, a lower dosage of diutan gum could be used. The reason explained as the higher molecular weight and greater water retention of diutan gum.

Table 4.3 summarizes the influence of *S. pasteurii* cells yield stress and viscosity of cement and the time-dependent change in these parameters. Comparing the plastic viscosity of the mixes containing *S. pasteurii*, a similar trend was observed at w/c of 0.36 and 0.40, where the threshold value for bacterial cells dosage was found to be 0.5% in terms of increasing the plastic viscosity. This is while by increasing the w/c up to 0.45, the threshold dosage decrease to 0.05% and by increasing the top 0.50, the most effective dosage was then found to be 0.1%. Although the exact reason is not known, the higher water content which reduces the attractive forces could result in effective dosages differences. Similar to the use of *P. polymyx*a, the time-dependent behavior of cement paste such as thixotropy and hydration of the mix was also studied for the mixes including *S. pasteurii*. Upon 10 min the plastic viscosity and yield stress values were almost the same as the initial mix. This might indicate that the incorporation of cells might not interfere with the thixotropy of the mix and possibly did not lead to any segregation neither.

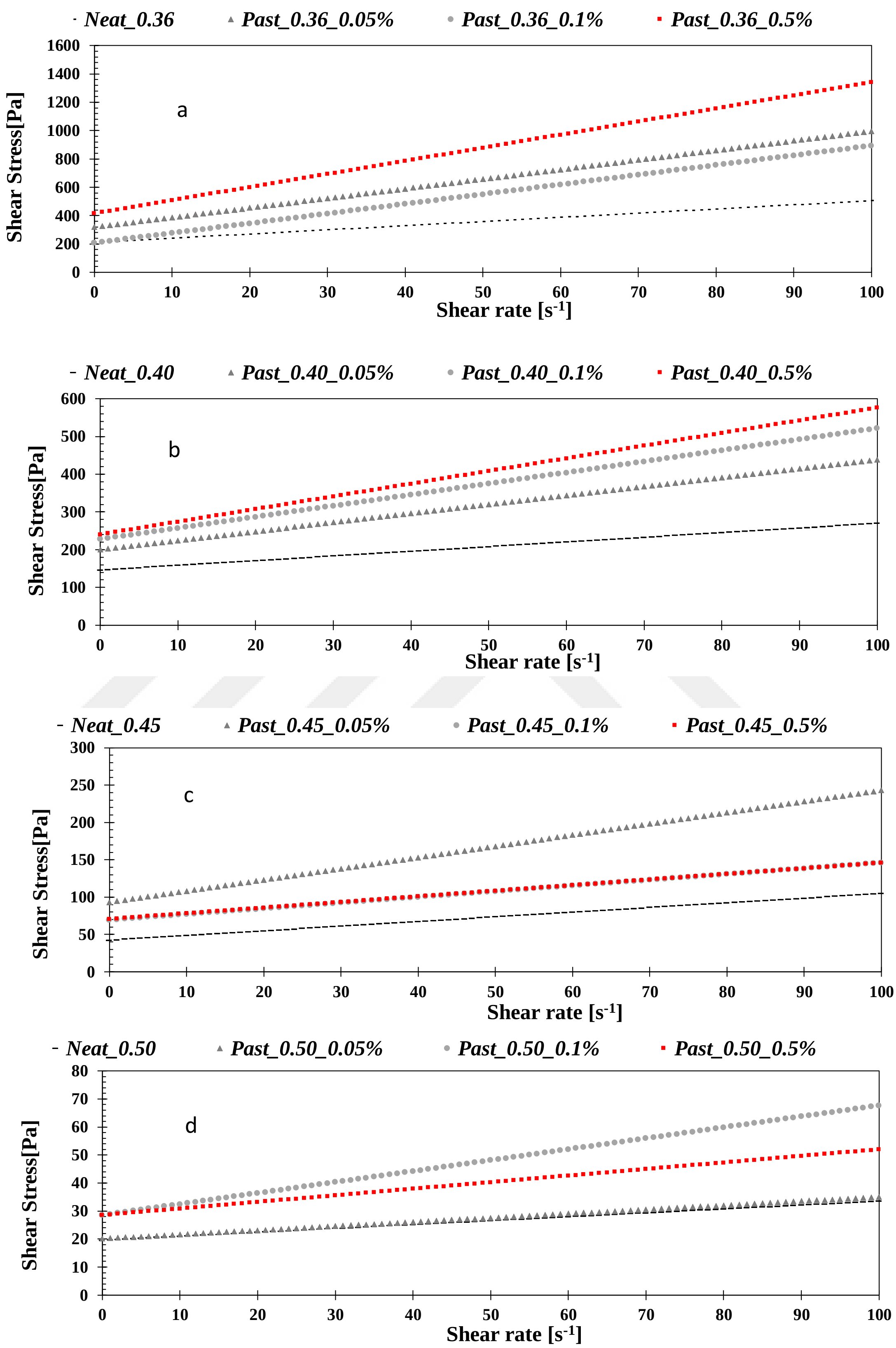

Figure 4-6 Rheological test results for cement paste samples with and without *S.pasteurii* at a w/c of (a)0.36, (b)0.40, (c)0.45 and (d) 0.50. Cell dosages were used as 0.05%, 0.1% and 0.5% per weight of cement.

Table 4-3 Summary of rheological test results for samples with and without bacteria for *S. pasteurii* strain. The w/c were kept at 0.36, 0.40, 0.45 and 0.50. Cell dosages were used as 0, 0.05%, 0.0.1% and 0.5% g of 600g cement.

w/c	Bacteria	Cell dosage per cement weight	FA per cement weight	SP per cement weight	Mini Slump (cm)	Yield Stress [Pa]		Plastic Viscosity [mPa·s]	
						first	after 10 mins	first	after 10 mins
0.36	*S. pasteurii*	0.00%	-	-	4.05	211.94	213.28	2943.10	2917.70
		0.05%			4.85	319.28	315.07	6768.10	6643.90
		0.10%			6.00	206.29	152.27	6854.20	7090.90
		0.50%			5.35	413.85	398.72	9253.50	8342.70
		0.00%	20%	-	4.65	359.90	364.45	5217.90	5437.20
		0.05%			5.40	280.75	313.49	4724.90	4630.10
		0.10%			6.15	187.96	177.90	4878.90	4683.70
		0.50%			5.25	239.61	247.13	4270.00	3782.50
		0.00%	-	1%	15.40	-2.15	-2.51	193.20	184.50
		0.05%			14.60	-7.95	-22.23	774.00	1580.60
		0.10%			16.35	-2.60	-2.64	134.50	133.80
		0.50%			14.95	-11.64	-10.62	772.40	697.30
		0.00%	20%	1%	15.90	-1.36	-1.16	117.20	100.00
		0.05%			16.40	-8.62	-5.85	603.20	382.80
		0.10%			16.20	-4.70	3.92	337.60	241.20
		0.50%			15.65	-6.75	-3.54	432.10	252.51
0.40	*S. pasteurii*	0.00%	-	-	6.15	146.28	146.06	1237.3	1424.3
		0.05%			5.25	200.67	209.9	2382.9	2409.7
		0.10%			4.80	227.13	238.26	2939.1	2906.20
		0.50%			4.80	239.61	238.98	3357.6	3272.9
		0.00%	20%	-	7.15	92.18	96.47	1393.42	1375.92
		0.05%			8.85	158.62	164.76	310.06	347.62
		0.10%			6.45	169.53	174.43	1468.2	1530.3
		0.50%			5.65	164.15	175.53	1995.2	1870.1
		0.00%	-	1%	19.00	-1.60	-2.30	71.20	110.40
		0.05%			17.10	-3.80	-7.41	353.30	646.50
		0.10%			16.00	-1.86	-1.95	141.00	148.50
		0.50%			16.90	-3.96	-1.54	308.40	225.20
		0.00%	20%	1%	19.00	-1.50	-1.82	77.50	90.20
		0.05%			18.25	-8.62	-1.16	603.20	58.50
		0.10%			17.65	-4.71	-1.68	337.60	80.00
		0.50%			17.85	-6.75	2.50	432.10	230.00
0.45	*S. pasteurii*	0.00%	-	-	7.75	42.45	45.64	624.72	630.28
		0.05%			7.60	93.18	96.10	1498.6	1547.4
		0.10%			8.25	69.21	70.26	766.03	748.94
		0.50%			6.95	70.46	72.13	752.52	749.7
		0.00%	20%	-	8.20	46.61	48.82	534.68	511.05
		0.05%			9.25	68.08	70.63	890.76	925.48
		0.10%			8.75	50.93	52.78	607.46	619.19
		0.50%			8.20	62.66	63.59	845.46	846.3
		0.00%	-	1%	17.05	-0.67	-4.37	36.40	226.40
		0.05%			18.40	-2.15	-3.62	147.00	325.60
		0.10%			18.30	-3.71	-0.54	189.20	28.20
		0.50%			16.75	-3.01	-1.23	158.60	58.40
		0.00%	20%	1%	18.60	-0.84	-0.60	34.60	21.80
		0.05%			18.55	-0.77	-0.41	36.90	18.10
		0.10%			18.45	-1.83	-3.80	83.30	353.30
		0.50%			18.50	-1.65	-1.11	89.10	45.60
0.50	*S. pasteurii*	0.00%	-	-	12.10	19.56	20.33	139.08	140.34
		0.05%			12.75	20.10	20.90	148.71	155.04
		0.10%			12.25	28.43	29.78	391.04	406.41
		0.50%			12.25	28.38	29.90	234.80	234.14
		0.00%	20%	-	11.20	21.67	21.94	114.57	113.25
		0.05%			12.15	24.70	26.16	276.25	293.35
		0.10%			11.25	26.43	28.23	341.77	366.55
		0.50%			11.25	31.22	32.87	346.29	350.81
		0.00%	-	1%	16.00	-0.90	-4.48	20.00	191.40
		0.05%			16.55	-0.92	-4.91	41.90	437.00
		0.10%			16.30	-0.78	-5.71	27.50	548.10
		0.50%			15.70	-1.15	-7.04	43.50	424.00
		0.00%	20%	1%	17.00	-0.32	-3.55	11.80	138.20
		0.05%			16.65	-0.88	-5.47	37.80	261.50
		0.10%			18.25	-0.68	-5.66	23.00	289.50
		0.50%			16.50	-1.00	-2.80	25.00	134.90

c. Use of B.megaterium as a VMA:

B.megaterium is a gram-positive bacteria having a thick cell wall and long rod-shaped with chain-like arrangement bacteria[128]. In particular, the "*megaterium*" name was given because there are one of the largest bacteria in the soil and it is classified in *Bacillus sp.*due to its ability for forming endospores and resistance to extreme conditions[129]. Based on the literature, different cell concentration of *B. megaterium* cells were used to increase the strength in concrete[3]. Incorporation of *B.megaterium* cells induced calcite precipitation in the pores and thus, improved the strength[128]. The results confirm the effect of *B.megaterium* incorporation on strength improvement.

Yet, this strain was selected due to its size and thick cell wall, which presumably have a relatively high volume of polysaccharides and peptidoglycans. Thus, *B. megaterium* cells could also be effective as a VMA. As so, *B. megaterium* cells were able to increase the viscosity regardless of the w/c and bacteria dosage used in the mix. Similar to *P.polymyxa* cells, increasing dosage of *B. megaterium* cells (from 0.1% to 0.5%) also led to a decrease in viscosity. Again, this was attributed to the particle to bacterium attraction such that above the threshold value, the negatively charged cells then could be able to induce a repulsive force against cement particles. Below that value, the cells could not induce a strong repulsive force. Another theory is that these rod shape motile cells could actually act as microswimmers and induce a push force in the suspension. It should be noted that even though, the cells selected for this study were motile, they might not be able to induce a drag force on thick cement paste suspension[102]. However, *B.megaterium* cells could actually be strong enough to induce a push force and decrease viscosity due to their size. In such a case, there could be a balance such that they could actually decrease or increase the viscosity based on dosage. Further microscale fluid dynamics have to be investigated to explain the mechanisms of microswimmer in complex fluids like cement paste.

Based on rheograms obtained from, 0.1% of cells per weight of cement was enough to improve the apparent viscosity at low w/c's, 0.36 and 0.40 (*see* Figure 4.7 (a) and Figure 4.7 (b)). Interestingly, at higher w/c. 0.45 and 0.50, the trend that normally observed in mixes including other strains, was totally different. The exact reason is not known, but it might be due to the longer chain and higher molecular weight of the strain. This can be attributed to the fact that the degree of water retention and the free water required to lubricate the cement paste increase with the adding dosages of cells that act on the aqueous phase. Moreover, at high w/c, the viscosity of the mix was almost doubled when bacteria dosage added by a dosage to 0.05% of cement weight which confirms the fact that high molecular weight caused a higher water retention. The cells were less effective in higher w/c in increasing viscosity compared to the lower w/c. Even though the resulted rheological properties were still higher than neat mixes, but the reduction of cells incorporation confirm that the increased water content, limits the cell motility in suspension. Further investigation should be done regarding the behavior of the cell in higher w/c and how fewer dosages were needed to decrease the fluidity.

Comparing the results obtained after 10 min waiting period (*see* Table 4-4 first 4 rows of each w/c), *B. megaterium* cells also exhibited a similar behavior with *S. pasteurii* and *P.polymxa* such that neither the yield stress not the viscosity has changed upon waiting even at high w/c at 0.50.

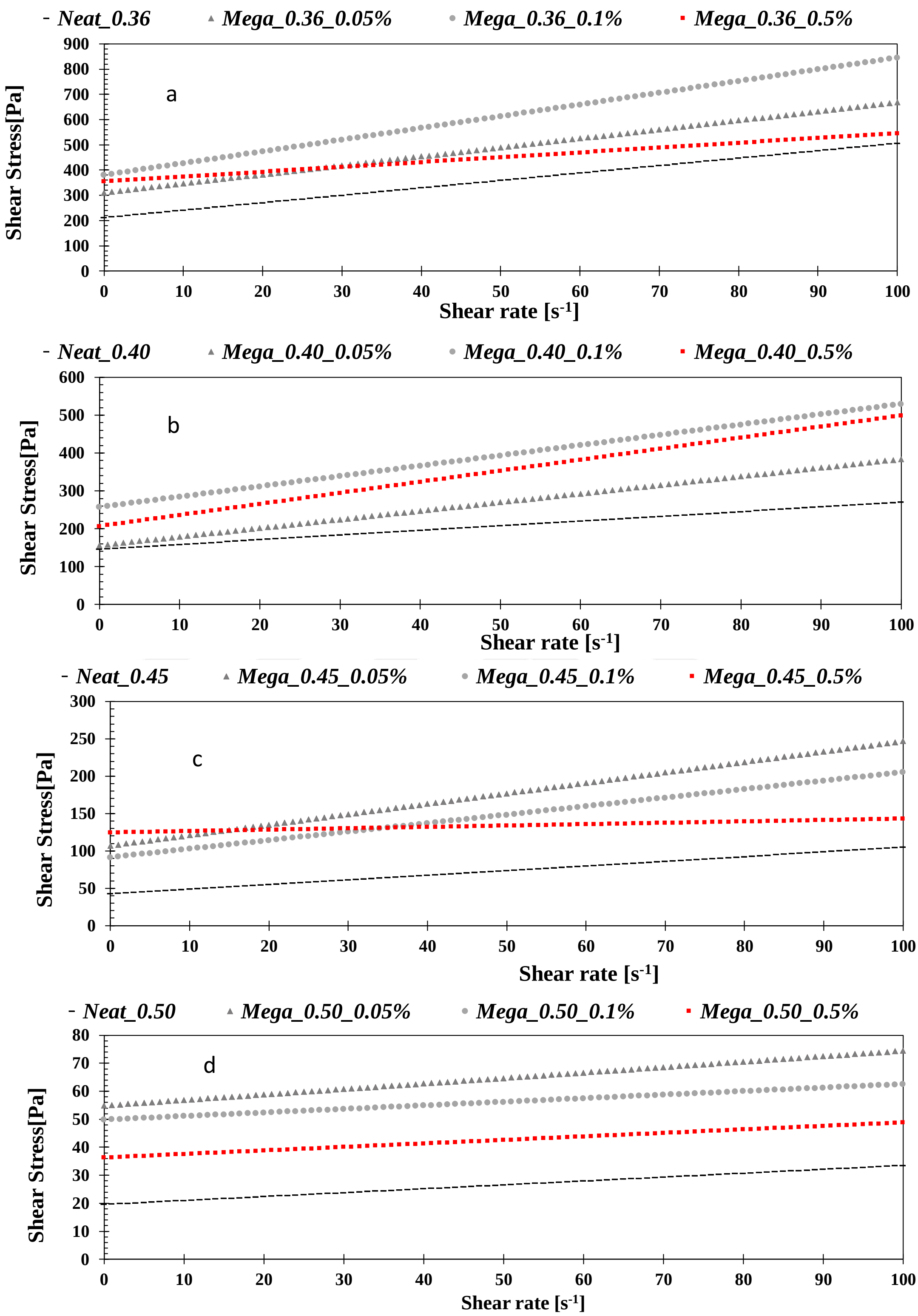

Figure 4-7 Rheological test results for cement paste samples with and without *B. megaterium* at a w/c of (a)0.36, (b)0.40, (c)0.45 and (d) 0.50. Cell dosages were used as 0.05%, 0.1% and 0.5% per weight of cement.

Table 4-4 Summary of rheological test results for samples with and without bacteria for *B. megaterium* strain. The w/c were kept at 0.36, 0.40, 0.45 and 0.50. Cell dosages were used as 0, 0.05%, 0.0.1% and 0.5% g of 600g cement.

w/c	Bacteria	Cell dosage per cement weight	FA per cement weight	SP per cement weight	Mini Slump (cm)	Yield Stress [Pa]		Plastic Viscosity [mPa·s]	
						first	after 10 mins	first	after 10 mins
0.36	*B. megaterium*	0.00%	-	-	4.05	211.94	213.28	2943.10	2917.70
		0.05%			4.30	311.06	317.46	3569.90	3582.20
		0.10%			4.50	379.45	383.54	4648.50	4592.10
		0.50%			4.00	353.36	358.38	1916.60	2151.60
		0.00%	20%	-	4.65	359.90	364.45	5217.90	5437.20
		0.05%			5.15	288.88	296.61	2042.90	2103.30
		0.10%			4.90	272.01	280.32	2161.20	2177.00
		0.50%			5.00	407.46	418.04	1560.50	1688.40
		0.00%	-	1%	15.40	-2.15	-2.51	193.20	184.50
		0.05%			18.45	-9.63	-11.93	847.00	1062.70
		0.10%			17.50	-1.12	-2.03	162.80	207.20
		0.50%			16.30	-1.23	-1.64	139.20	155.30
		0.00%	20%	1%	15.90	-1.36	-1.16	117.20	100.00
		0.05%			17.65	-1.17	-1.59	95.70	82.10
		0.10%			17.00	-1.12	-2.03	162.80	207.20
		0.50%			16.75	-1.34	-1.40	125.20	128.30
0.40	*B. megaterium*	0.00%	-	-	6.15	146.28	146.06	1237.3	1424.3
		0.05%			6.00	156.14	165.55	2277.70	2221.80
		0.10%			5.30	256.47	264.55	2723.30	2752.00
		0.50%			4.90	205.88	215.37	2923.40	2943.60
		0.00%	20%	-	7.15	92.18	96.47	1393.42	1375.92
		0.05%			5.50	146.21	154.42	2306.10	2205.60
		0.10%			5.80	171.02	174.88	2440.20	2467.10
		0.50%			7.00	140.82	146.09	547.75	590.31
		0.00%	-	1%	19.00	-1.60	-2.30	71.20	110.40
		0.05%			19.30	-1.14	-2.08	96.20	115.70
		0.10%			17.25	-1.10	-0.99	70.60	80.70
		0.50%			18.25	-2.82	-5.14	385.30	548.10
		0.00%	20%	1%	19.00	-1.50	-1.82	77.50	90.20
		0.05%			18.75	-3.40	-5.24	292.00	437.00
		0.10%			18.00	-1.08	-0.99	70.10	80.10
		0.50%			18.05	-0.84	-0.70	69.20	47.20
0.45	*B. megaterium*	0.00%	-	-	7.75	42.45	45.64	624.72	630.28
		0.05%			7.40	107.01	110.17	1398.90	1430.20
		0.10%			7.50	91.06	93.80	1139.20	1166.50
		0.50%			8.10	124.21	129.81	184.91	200.05
		0.00%	20%	-	8.20	46.61	48.82	534.68	511.05
		0.05%			8.50	76.47	79.56	895.55	926.48
		0.10%			8.15	69.98	72.60	919.82	964.92
		0.50%			10.00	78.27	82.23	202.13	213.03
		0.00%	-	1%	17.05	-0.67	-4.37	36.40	226.40
		0.05%			21.00	-1.31	-1.05	60.40	72.80
		0.10%			18.05	-1.44	-3.00	125.20	292.20
		0.50%			18.30	-1.54	-2.88	74.50	202.70
		0.00%	20%	1%	18.60	-0.84	-0.60	34.60	21.80
		0.05%			19.65	-0.99	-1.19	37.60	45.00
		0.10%			17.35	-0.77	-0.89	62.60	54.40
		0.50%			19.25	-0.75	-0.67	31.80	25.50
0.50	*B. megaterium*	0.00%	-	-	12.10	19.56	20.33	139.08	140.34
		0.05%			11.20	54.84	57.90	195.53	202.18
		0.10%			13.00	49.68	50.54	127.12	129.02
		0.50%			13.00	36.16	37.44	125.33	126.20
		0.00%	20%	-	11.20	21.67	21.94	114.57	113.25
		0.05%			13.20	44.51	46.72	128.45	131.42
		0.10%			11.60	38.47	39.01	225.36	230.17
		0.50%			12.00	33.42	35.25	230.10	244.77
		0.00%	-	1%	16.00	-0.90	-4.48	20.00	191.40
		0.05%			20.00	-0.80	-0.80	27.00	48.80
		0.10%			19.25	-0.44	-1.15	35.20	77.00
		0.50%			20.40	-0.43	-0.47	28.00	22.40
		0.00%	20%	1%	17.00	-0.32	-3.55	11.80	138.20
		0.05%			19.00	-0.78	-2.30	33.60	84.70
		0.10%			18.95	-0.43	-0.40	27.60	21.20
		0.50%			17.60	-0.53	-0.62	28.90	37.20

d. Use of B. subtilis as a VMA:

As mentioned above, previously the *B. subtilis* cells walls were used as VMAs in the literature [3]. Even though the results were promising in terms of improving the viscosity, the extraction of cells walls was rather a challenging and labor-intensive process. Herein, the *B. subtilis* cells were only collected from the culture and suspended directly in the mix water. With such a simple approach, only 0.05% of cells per weight of cement was enough to improve the apparent viscosity at low w/c’s, 0.36 and 0.40 (*see* Figure 4.8 (a) and Figure 4.8 (b)). However, a higher dosage of cells was required to improve the viscosity at high w/c mixes, which was again attributed to the high flowability of the mix. Even though a sound comparison could not be made since different rheometers were used in testing, Pei et al. showed that incorporation *B. subtilis* cell walls could only increase the viscosity at w/c’s of 0.36 and 0.40 and did not change the viscosity at w/c 0.45 and 0.50 on par to neat paste [3]. However, the direct addition of cells also resulted in an increase in viscosity at 0.45 and 0.50 but only required a higher dosage of cells. Moreover, at low w/c where both approaches worked, the viscosity of the mix was even doubled when bacteria dosage was increased to 0.1% of cement weight. Pei et al.[3] observed an increase in apparent viscosity within a range of 20-50% when only *B. subtilis* cell walls (0.34% by weight of cement) were incorporated in cement paste samples.

The difference of using active cells compared to the incorporation of only bacterial cells walls could be explained by the theory of pushing and pulling effect of bacteria cells. Rafai et al. [102] 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 [102]. Herein, the vegetative cells also showed a higher efficiency compared to what has been found in the literature, thus this might indicate that motility of these cells could actually impose a puller effect, also inducing additional resistance to flow. Further research has to be conducted to analyze how vegetative gram-positive bacterial cells actually influence the hydrodynamics of cement paste suspension.

Addition of almost all abovementioned strains increased the viscosity and yield stress in cement paste samples, at high and low shear rates, regardless of the w/c used. The mixes also waited for 10 min to examine the possible effects of thixotropy and segregation. The mixes containing cells exhibited a reduction in fluidity over even 10 min waiting period because of the cement paste thixotropic nature. While VMA can be used to enhance the resistance to segregation and bleeding[4,130]. No considerable difference observation between two viscosities before and after the waiting period might indicate the fact that cells increased the resistance to segregation. This might show that cells can act as VMA.

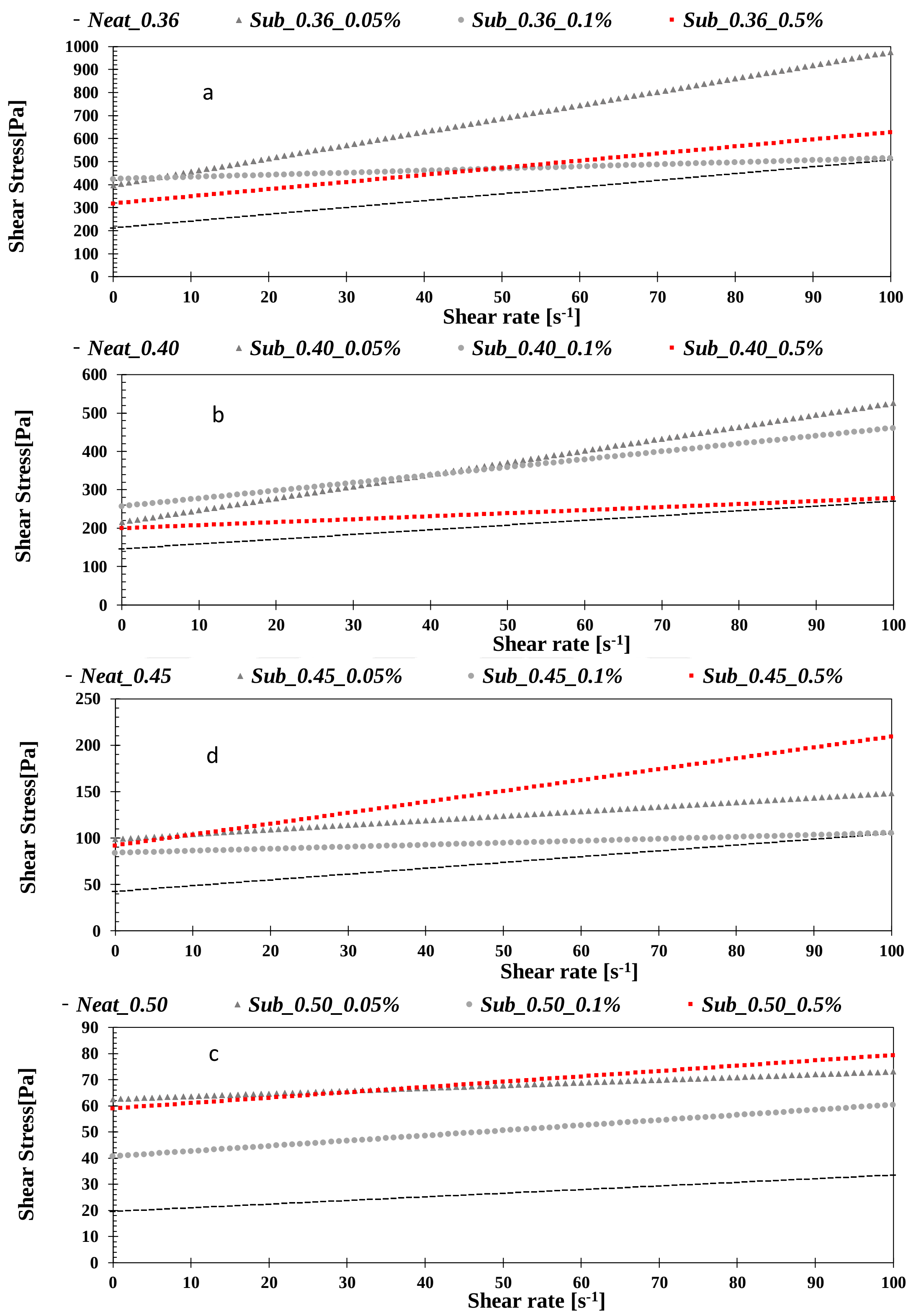

Figure 4-8 Rheological test results for cement paste samples with and without *B. subtilis* at a w/c of (a)0.36, (b)0.40, (c)0.45 and (d) 0.50. Cell dosages were used as 0.05%, 0.1% and 0.5% per weight of cement.

Table 4-5 Summary of rheological and mini-slump test results for samples with and without bacteria for *B. subtilis* strain. The w/c were kept at 0.36, 0.40, 0.45 and 0.50. Cell dosages were used as 0, 0.05%, 0.0.1% and 0.5% g of 600g cement.

w/c	Bacteria	Cell dosage per cement weight	FA per cement weight	SP per cement weight	Mini Slump (cm)	Yield Stress [Pa]		Plastic Viscosity [mPa·s]	
						first	after 10 mins	first	after 10 mins
0.36	B.subtilis	0.00%	-	-	4.05	211.94	213.28	2943.10	2917.70
		0.05%			5.00	397.36	407.55	5782.30	4925.30
		0.10%			4.70	422.51	420.59	907.80	924.50
		0.50%			4.75	316.23	322.74	3092.00	3016.70
		0.00%	20%	-	4.65	359.90	364.45	5217.90	5437.20
		0.05%			6.20	250.80	252.24	910.68	903.71
		0.10%			5.50	292.79	298.23	1678.60	1659.30
		0.50%			4.50	256.72	254.82	1817.70	1837.30
		0.00%	-	1%	15.40	-2.15	-2.51	193.20	184.50
		0.05%			18.50	-1.51	-3.01	255.70	318.90
		0.10%			18.50	-1.55	-1.57	112.70	113.60
		0.50%			17.00	3.31	0.13	160.70	246.90
		0.00%	20%	1%	15.90	-1.36	-1.16	117.20	100.00
		0.05%			18.00	-1.02	-1.62	79.90	85.30
		0.10%			17.75	-2.18	-2.98	166.60	183.70
		0.50%			18.00	-0.95	-1.05	87.10	82.00
0.40	B.subtilis	0.00%	-	-	6.15	146.28	146.06	1237.3	1424.3
		0.05%			6.00	215.71	218.55	3101.70	2838.50
		0.10%			5.30	256.19	259.01	2038.40	2051.90
		0.50%			4.90	198.77	198.14	785.20	778.70
		0.00%	20%	-	7.15	92.18	96.47	1393.42	1375.92
		0.05%			5.50	130.22	132.71	640.80	646.10
		0.10%			5.80	61.77	62.44	324.20	325.90
		0.50%			7.00	168.48	170.72	439.50	488.80
		0.00%	-	1%	19.00	-1.60	-2.30	71.20	110.40
		0.05%			19.30	-0.58	-0.60	50.00	51.50
		0.10%			17.25	-0.73	-0.99	98.00	118.00
		0.50%			18.25	-1.35	-1.41	69.80	70.80
		0.00%	20%	1%	19.00	-1.50	-1.82	77.50	90.20
		0.05%			18.75	-0.50	-0.87	50.10	47.20
		0.01%			18.00	-1.40	-1.05	72.80	61.70
		0.50%			18.05	-1.78	-1.83	127.40	133.50
0.45	B.subtilis	0.00%	-	-	7.75	42.45	45.64	624.72	630.28
		0.05%			7.40	99.02	99.77	493.68	512.20
		0.10%			7.50	83.77	85.61	213.70	223.00
		0.50%			8.10	91.53	91.44	1173.50	1064.90
		0.00%	20%	-	8.20	46.61	48.82	534.68	511.05
		0.05%			8.50	96.37	71.88	191.55	201.34
		0.10%			8.15	69.94	71.81	185.90	189.90
		0.50%			10.00	76.08	77.70	165.30	174.20
		0.00%	-	1%	17.05	-0.67	-4.37	36.40	226.40
		0.05%			21.00	-1.54	-1.30	104.10	99.10
		0.10%			18.05	-0.69	-0.93	41.80	48.00
		0.50%			18.30	-0.74	-0.90	37.70	38.30
		0.00%	20%	1%	18.60	-0.84	-0.60	34.60	21.80
		0.05%			19.65	-0.48	-0.67	31.50	25.70
		0.10%			17.35	-0.85	-0.62	39.50	32.50
		0.50%			19.25	-0.25	-0.38	27.70	26.90
0.50	B.subtilis	0.00%	-	-	12.10	19.56	20.33	139.08	140.34
		0.05%			11.90	62.53	64.67	104.77	108.72
		0.10%			11.50	40.52	41.59	197.40	198.31
		0.50%			9.85	58.83	59.71	204.00	197.90
		0.00%	20%	-	11.20	21.67	21.94	114.57	113.25
		0.05%			13.00	25.11	25.84	150.89	154.73
		0.10%			13.45	26.01	26.18	150.20	152.90
		0.50%			10.50	63.16	64.79	172.30	177.30
		0.00%	-	1%	16.00	-0.90	-4.48	20.00	191.40
		0.05%			19.75	-0.82	-0.17	29.00	12.10
		0.10%			18.60	-0.53	-0.91	30.70	33.60
		0.50%			23.50	-0.65	-0.67	43.40	42.70
		0.00%	20%	1%	17.00	-0.32	-3.55	11.80	138.20
		0.05%			19.75	-0.94	-0.77	35.60	51.80
		0.10%			18.60	-1.01	-0.79	53.00	46.30
		0.50%			23.50	-0.78	-0.43	34.30	23.50

e- Comparative evaluation of 4 cell types used as a VMA

The comparison between the influence of 4 type of cell strains showed that the influence of cells on plastic viscosity and yield stress was more pronounced when they were used in cement paste with a lower w/c,0.36 and 0.40, rather than higher ratios, 0.45 and 0.50. The viscosity of neat cement-paste samples decreased by 95% when w/c was increased to 0.50 from 0.36. Addition of *P.polymxa* and then *B.subtilis* and *B.megaterium* cells respectively, made this decreasing trend less pronounced, however, *S.pasteurii* cells were found to be less efficient in terms of overcoming the increase in w/c. This difference could be attributed to the different molecular length and weight each strain had. *P.polymxa* has the longest chain and the greatest molecular weight [131] between all these strains, this is while *S. pasteurii* has the shortest and lowest chain and weight [132].

Besides the fact that high molecular weight cause higher water retention which means higher viscosity, based on the results obtained from 256 test samples, it seems that every cell type used as a VMA had a different optimum dosage and this could also be attributed to the molecular weight and length. It was seen that after a specific dosage for each mix design and strain, viscosity decreased. It was hypothesized that these cells crushed on the surface of water molecules after adding more of them. The water molecules that could easily move regarded to their smooth surface, are not round anymore, which lead to higher flowability but lower viscosity. Further investigation on water retention of 4 of strains should be done to understand why there was a threshold value in cell concentration. Since in some samples although after optimum dosage (like 0.05% of cement weight), adding more cell (like 0.1% of cement weight) decreased viscosity, but also with increasing the dosage again (to 0.5% of cement weight), a significant increase recorded in plastic viscosity again. It was also assumed that although the chosen cells had a similar chemical structure, the action mechanism of each cell type might be different. The efficiency of *B. megaterium* and *B. subtilis* strains were not as significant as other two strains, however, they increased both yield stress and plastic viscosity up to 2 times greater that mixes without VMAs. In case of adding *B. megaterium*, 0.1% of the microorganism per cement weight generally showed the notable improvements, while 0.05% of the *B. subtilis* bacteria per cement weight was mostly efficient dosage. Among all, *P.polymxa* was found to be the most efficient cell type in terms of increasing the viscosity. It should be noted that the use of *S. pasteurii* might not only improve the fresh-state properties but also could provide self-healing ability to the cement-based material[125].

4.2.2 Influence of superplasticizers and fly ash on the efficiency of cells

The bacterial cells were efficient in terms of increasing viscosity when they were incorporated in mixing water of cement-paste. However, in a typical self-leveling cement-based mix with improved flowability and stability VMAs are generally used along with fly ash (FA) and superplasticizers (SP). Accordingly, as cells are anionic and have the ability to bind with cement particles, SP could counter the cement-binding tendency. A combination of these two mixing ingredients (SP and FA) might interfere with the mechanism of the bacterial cells. Figure 4-9 to 4-20 represent the yield stress and viscosity curves under increasing shear stress in cement-paste samples including different bacteria dosages, FA and SP at 4 different w/c's (0.36, 0.40, 0.45 and 0.50).

4.2.2.1 Interaction of 4 different cell types with FA

Since fly ash is finer than cement particles, adding fly ash to the mixture reduces the inter-particle distances and increases the pressure between particles. While this pressure leads to the removal of the water from inter-particle spaces, it also provides a flowing effect on cement paste [133]. Thus, incorporation of FA generally improves workability and provides stability with increasing viscosity, and in some cases the yield strength of the paste. Incorporation of FA by 20% for the weight of cement only influenced the viscosity and the yield stress of cement paste at a w/c of 0.36 such that both of these parameters were higher compared to its counter par near cement paste. In such a case, the fine FA particles acted as a filler material since there was not enough fluidity that would enable the spherical particles to flow and improve workability. As noted, FA particles could both improve the workability and act as a filler material due to their fine particle size. Incorporation of FA increases the specific surface area (SSA) of the powder content which improves the stability and segregation resistance of the mix. While incorporation of FA could improve workability at w/c higher than 0.40, it would also act as a stabilizer, which could be classified as an alternative VMA. Thus, it is crucial to explain the interaction of each cell type with FA.

a. Use of P.polymyxa in FA amended mixes:

Based on the results in section 4.1, all cell types were able to increase the viscosity regardless of the w/c. Interestingly, there was a completely opposite trend when the cells were used with FA even at low w/c where both cells and FA increased the viscosity separately (*see* Table 4-2). Incorporation of *P.polymyxa* cells with FA decreased the viscosity at a w/c of 0.36 compared to the counterpart sample without FA. The decreasing trend was still observed even if the cell concentration was increased from 0.05% to 0.5% by weight of cement. This was on the contrary of what has found previously when no FA was used. In contrast, this decreasing trend at w/c of 0.36 was not observed when the w/c was increased to 0.40. The viscosity was doubled when 0.05% of cells by weight of cement were added to the mix. Yet, there is not a simple explanation regarding the complex interaction of microorganisms with FA at low w/c, the cells might influence the particle to particle interaction and induce a repulsive force. This behavior only observed in highly packed mixes where even FA could not improve the workability. Further studies have to be conducted on the rheology of cement paste samples including microorganisms with different dosages and types of FA(or even different types of pozzolans). As the w/c increased both FA and cells exhibited an expected behavior such that viscosity of the FA amended mix has increased the viscosity with increasing cell concentration at w/c of 0.40, 0.45 and 0.50. This might be related to increasing water content enabled a flowable mix decreasing the impact of interparticle friction, which enabled both cells and FA functioned expectedly. Based on the results it can be concluded that *P. polymyxa*, incorporation as VMAs was compatible with the use of fly ash at w/c> 0.40. Below this w/c, the cells tend to decrease the viscosity rather than increasing it.

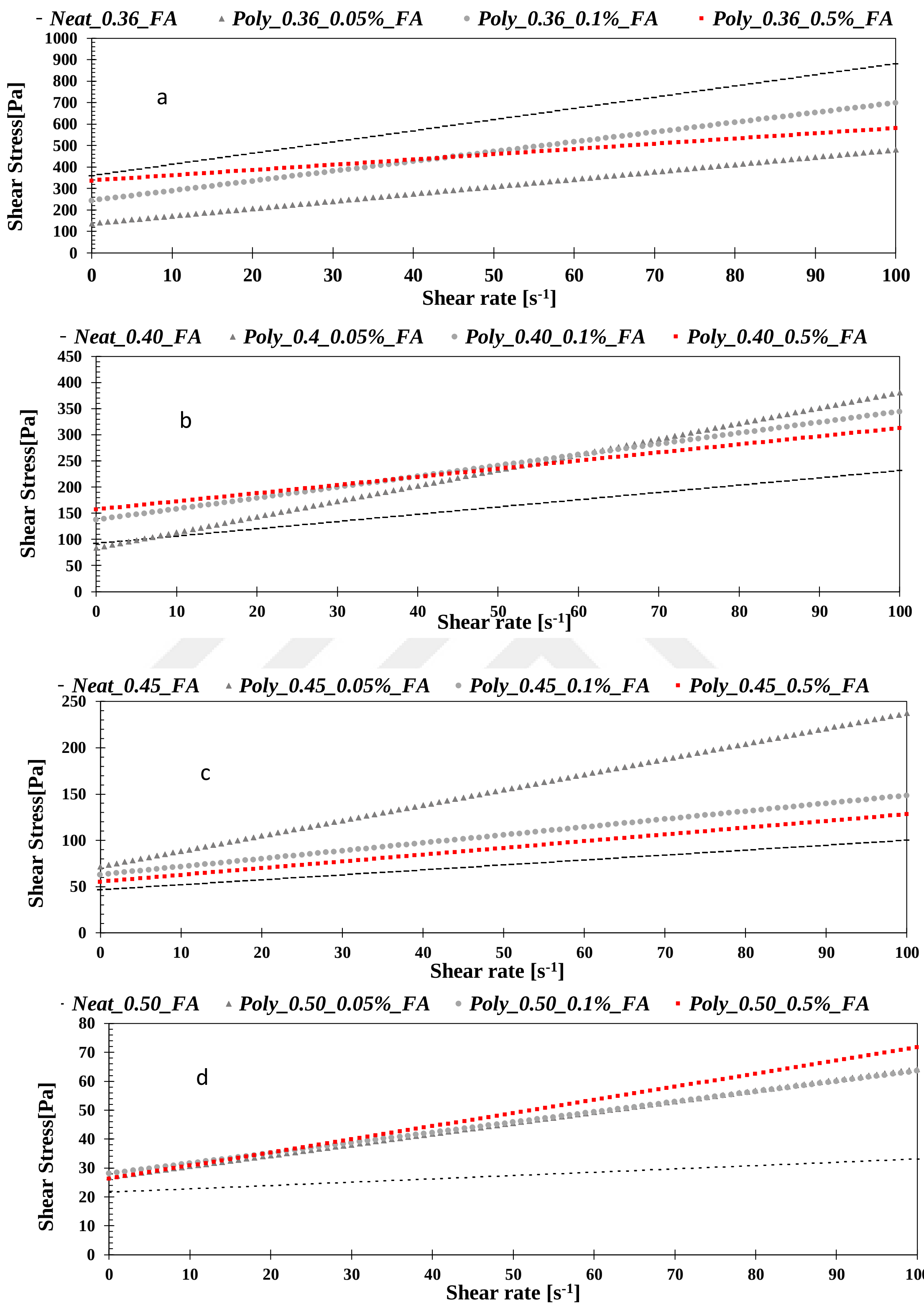

Figure 4-9 Rheological test results for cement paste samples including FA with and without *P.polymyxa* at a w/c of (a)0.36, (b)0.40, (c)0.45 and (d) 0.50. Cell dosages were used as 0.05%, 0.1% and 0.5% per weight of cement.

b. Use of S. pasteurii in FA amended mixes:

Similar to what was observed with *P.polymyxa*, *S.pasteurii* cells also had a decreasing effect on viscosity when they were incorporated to an FA amended cement paste. Addition of FA to neat paste without any microorganism lead to an increase in both yield stress and viscosity and incorporation of *S. pasteurii* cells again resulted with a decreased in both parameters. Interestingly, a similar trend was also observed at a w/c of 0.40. However, the plastic viscosity of an FA amended mortar was increased when cells were incorporated at a w/c of 0.45 and .50.

The neat samples showed higher viscosity and shear stress with adding FA and this increase was less observable by reaching from a w/c of 0.36 to 0.40. at a higher w/c of 0.45 and 0.50, the addition of FA even decreased the yield stress and viscosity. By adding *S.pasteurii* cells to the mix, the observed trend was different in every w/c. Diutan and welan gum also resulted in the same way[9], except with the addition of these cells, increasing w/c even slightly to 0.40, resulted in a higher plastic viscosity. The difference was more considerable by a cell dosage of 0.5%. however, in both 0.45 and 0.50 w/ c, cells dosages at 0.05% doubled the plastic viscosity and increased the yield value. But, based on the data obtained from these 2 strains, the decrease in plastic viscosity could be attributed to the interaction of FA with cells. Such that *P.polymxa* could overcome the high particle packing at a w/c of 0.40 but *S. pasteurii* can overcome at 0.45. In this kind of dense, stiff, low workability mixes, the cells rather act as superplasticizer. Thus, it would be more correct to say that these cells act as “rheology-modifying agents” instead of VMA. Further researches might be done regarding the different trends observed in each cell dosage and w/c.

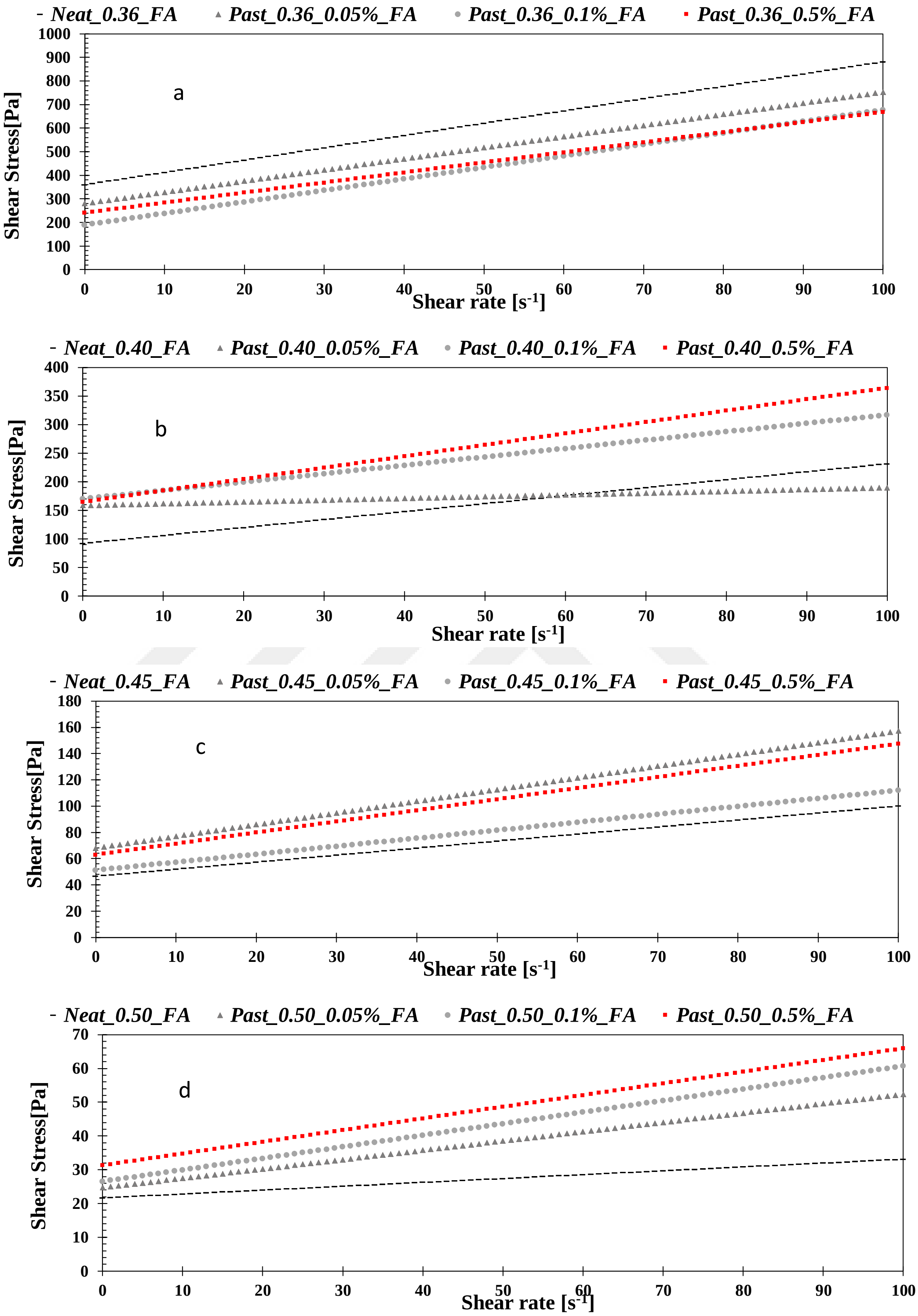

Figure 4-10 Rheological test results for cement paste samples including FA with and without *S.pasteurii* at a w/c of (a)0.36, (b)0.40, (c)0.45 and (d) 0.50. Cell dosages were used as 0.05%, 0.1% and 0.5% per weight of cement

c. Use of B.megaterium in FA amended mixes :

Since admixtures affecting the rheological properties of cementitious materials, select mineral admixtures to testing the cement paste seem reasonable. Adding mineral admixtures such FA, increase the water requirement in the mixture, since the FA addition increase the surface area in the mix. The most common and popular reason through the FA causing a decrease in viscosity is considered due to the spherical particle shape of FA that can roll on each other and reduce the interparticle friction[134]. However, in some works, it was reported that FA addition results in low water retention and further lower water demand for a specific workability[135,136]. *B. megaterium* showed a similar performance to *P.polymxa* such that the cells were able to improve the plastic viscosity of FA amended cement-paste starting with w/c of 0.40. While cells addition at a lower w/c of 0.36 decreased the viscosity, increasing water content made this drastic change inversely and at an optimum dosage of 0.1% of this cell in the w/c of 0.40, 0.45 and 0.50, cells addition made the viscosity 2 times greater than the neat samples. Yet, the exact reason is not known, the interaction of FA and cells might depend on (a) particle packing density (b) workability of the mix and (c) molecular weight of the cells. As the packing density increases and the workability of the mix is low, the cells act as a superplasticizer. The threshold w/c depends on the molecular weight.

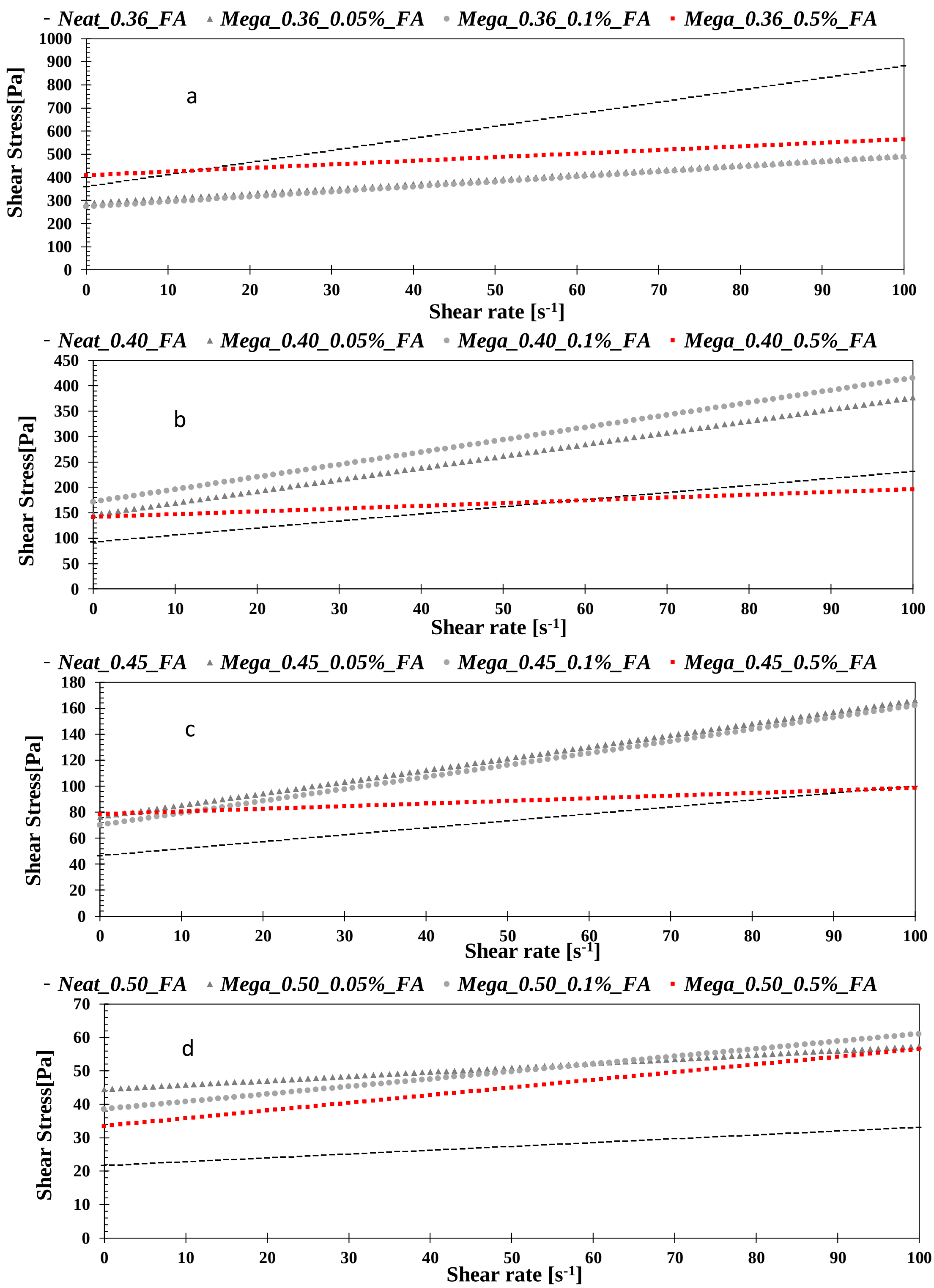

Figure 4-11 Rheological test results for cement paste samples including FA with and without *B.megaterium* at a w/c of (a)0.36, (b)0.40, (c)0.45 and (d) 0.50. Cell dosages were used as 0.05%, 0.1% and 0.5% per weight of cement.

d. Use of B. subtilis in FA amended mixes:

Similar to the experiments using cells alone, cells and FA were incorporated in the mixes. Previously *B. subtilis* cell walls were added to improve the rheological properties in cement paste[3,109]. In literature, the capability of *B. subtilis* cells with FA appearance was not reported. There was not a trend of incorporation of *B.subtilis* cells. In the w/c of 0.36, 0.40 and 0.45 and three dosages of this strain, viscosity decreased compare to the neat samples. At the highest w/c of 0.50, cells addition resulted in a slight increase in viscosity. By reaching to the 0.5% cell dosage, viscosity increased from 114 [mPa·s]to 172 [mPa·s]. Based on observations, B subtilis was not compatible with the use of FA.

The reason for the slightly different behavior of *B. subtilis* is not known. The molecular weight and length of this strain is almost the average one between the other three, and the reason might be that the rheology was not predominantly induced by adsorption mechanisms[7]. However, based on the results it can be concluded that the use of *P. polymyxa*, *S. pasteurii*, *B. megaterium* and *B.subtilis* cells as VMAs was compatible with the use of FA at w/c> 0.40 (or even 0.45). Below this w/c, the cells tend to decrease the viscosity rather than increasing it.

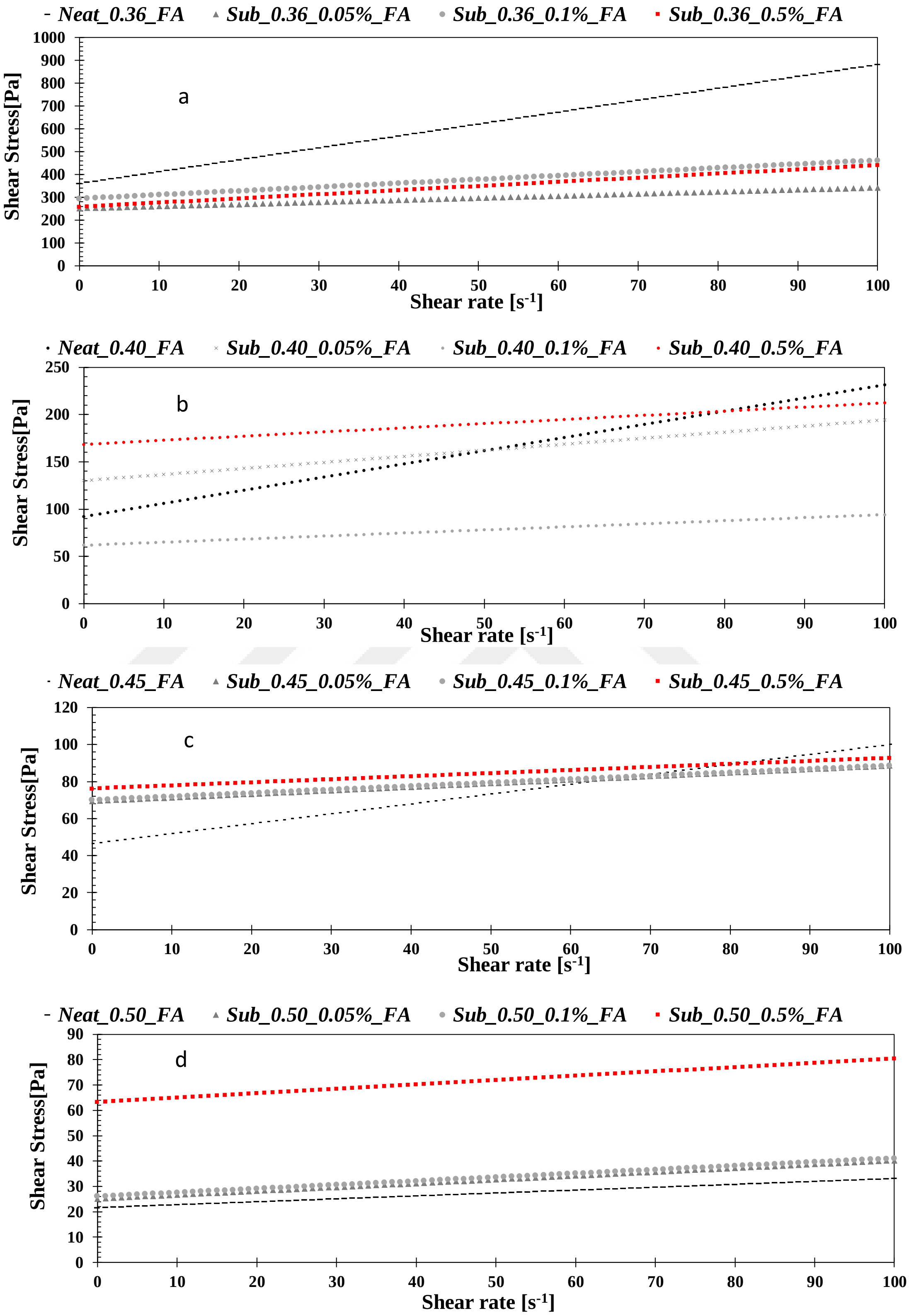

Figure 4-12 Rheological test results for cement paste samples including FA with and without *S.subtilis* at a w/c of (a)0.36, (b)0.40, (c)0.45 and (d) 0.50. Cell dosages were used as 0.05%, 0.1% and 0.5% per weight of cement

4.2.2.2 Interaction of cells with SP

.Although, unlike the linear polysaccharide VMAs, peptidoglycan has a tree-like 3-D structure, in which the long chains of the polysaccharide are cross-linked between the peptide side groups [99]. Viable cells generally include cable like intertwining peptidoglycan chains. Ata simple structural level, the rope is formed via cross-linking of a number of ordered chains, consisting of adjacent long polysaccharide backbones via peptide bonds. As a result, winding up these many ropes causes the formation of a long chain of the left-handed helix. These chains are considered to be side by side to form the cell structure [137]. These peptidoglycan chains intertwining at low shear rates cause an increase in apparent viscosity and then breaking at high shear rates cause a reduction in viscosity, thus exhibit the shear-thinning behavior. Like the other long-chain polysaccharide VMAs, peptidoglycan in cells might adsorb onto water molecules by forming a hydrogen bond between the water molecule and peptidoglycan. Continuously, the ropes of peptidoglycan can generate attractive forces that restrict the movement of water and cause the formation of viscous gels which cause an increase in viscosity. These functions of cells cause a reduction in fluidity which needs SP to restore the appropriate workability that is required for different concrete systems like SCC and HPC[138]. Thus, to test the compatibility of cells in high flowable mixes, a polycarboxylic ether- based SP was used (1% of cement weight)

Incorporation of superplasticizers completely changed the rheological behavior of cement paste samples regardless of the dosage of microorganisms and w/c used (see Figure 4-13 to 4-20). To compare the rheograms together with and without SP, while samples without SP showed a shear-thinning, the use of very strong Polycarboxylic ether based superplasticizer resulted with a shear-thickening behavior.

The shear-thickening cement paste behavior mix would not be simply explained by Bingham equation. Instead, Herschel-Bulkley model was used to analyze shear-thickening cement paste samples Equation 4-1, which could also explain the shear thinning behavior as well [116]:

$$\tau = \tau_0 + K\dot{\gamma}^n \qquad \text{Herschel and Bulkley} \qquad \text{Equation 2-5[36]}$$

where τ is the shear stress (Pa), τ_0 is the yield stress (Pa), K is constant and $\dot{\gamma}$ is the shear rate [116]. It is also mentioned in lithe terature that using this model would cause underestimation of the yield stress value and the consistency, and an overestimation of the shear thinning behavior. As expected, samples including superplasticizer resulted with a shear thickening behavior and this was directly related to type the of the superplasticizer used in the mix[116].

In general, the microorganism tends to increase the viscosity of the cement paste mix when they were incorporated to the mix with superplasticizers at lower (w/c < 0.50). Interestingly, the highest effective viscosity values were obtained when the bacterial cells were introduced even by 0.05% by cement weight, in cement paste mixes containing superplasticizers regardless of the w/c used. Yet, the interaction of the cells with Polycarboxylic ether cells are still unknown but they were compatible with their use as a VMA. It should be noted that both cells and superplasticizers are negatively charged. Thus, the increase in cell concentration might interfere with the dispersion of the particles and decrease the viscosity at higher dosages of cells. At last, the cells were added both with FA and SP. The cells were able to improve the viscosity at low w/c (0.36 and 0.40). But, at w/c of 0.50, there was a slight increase in viscosity when the cells were added by 0.5% of cement weight. This was mainly cells could not overcome the flowability of the mix. Thus, it might be possible to observe a higher increase in viscosity if the cell concentration was increased. Further studies have to conducted to evaluate the interaction of cells with different types of superplasticizers and puzzolans at various dosages.

a. *Compatibility of P.polymyxa cells with SP :*

Starting from the lowest w/c in neat paste samples, SP addition showed a dispersing effect that caused a significant reduction in both yield stress and plastic viscosity. *P.polymyxa* addition made this drastic change less pronounced comparing the samples including the same dosages of cells with SP. Similar to samples without any SP, there is a threshold value for optimum cell concentration. While at lower w/c's (0.36 and 0.40), the optimum dosage recorded was 0.1% of cement weight, this dosage was increased to 0.5% of cement weight. This was found to be related to the fluidity of the mix, such that as the w/c increase, incorporation of a strong SP results with a highly workable mix. To improve the viscosity, the dosage of VMA (in this case the cells) should be increased

The addition of VMA also increases the degree of shear thinning of cement paste regardless of the presence of SP. A sample including *P.polymyxa* cells exhibits high apparent viscosities at low shear rates and significantly lower viscosities at greater shear rates. The increased shear-thinning response in the presence of cells could be due to the entanglement or association of polysaccharide chains of the cells resulting in an increase in apparent viscosity, especially at the low shear rates. With the increase in shear rate, the entangled chains dislodge and align in the direction of flow, thus reducing the resistance of the cement paste to undergo deformation. The apparent viscosity is then decreased with an obvious improvement in flowability at the high shear rate. Thus, incorporation of cells was found to be more effective in increasing viscosity at a low shear rate than that at a high shear rate. To be concluded the result show that *P.polymyxa* was found to be compatible with SP appearance like welan gum [83].

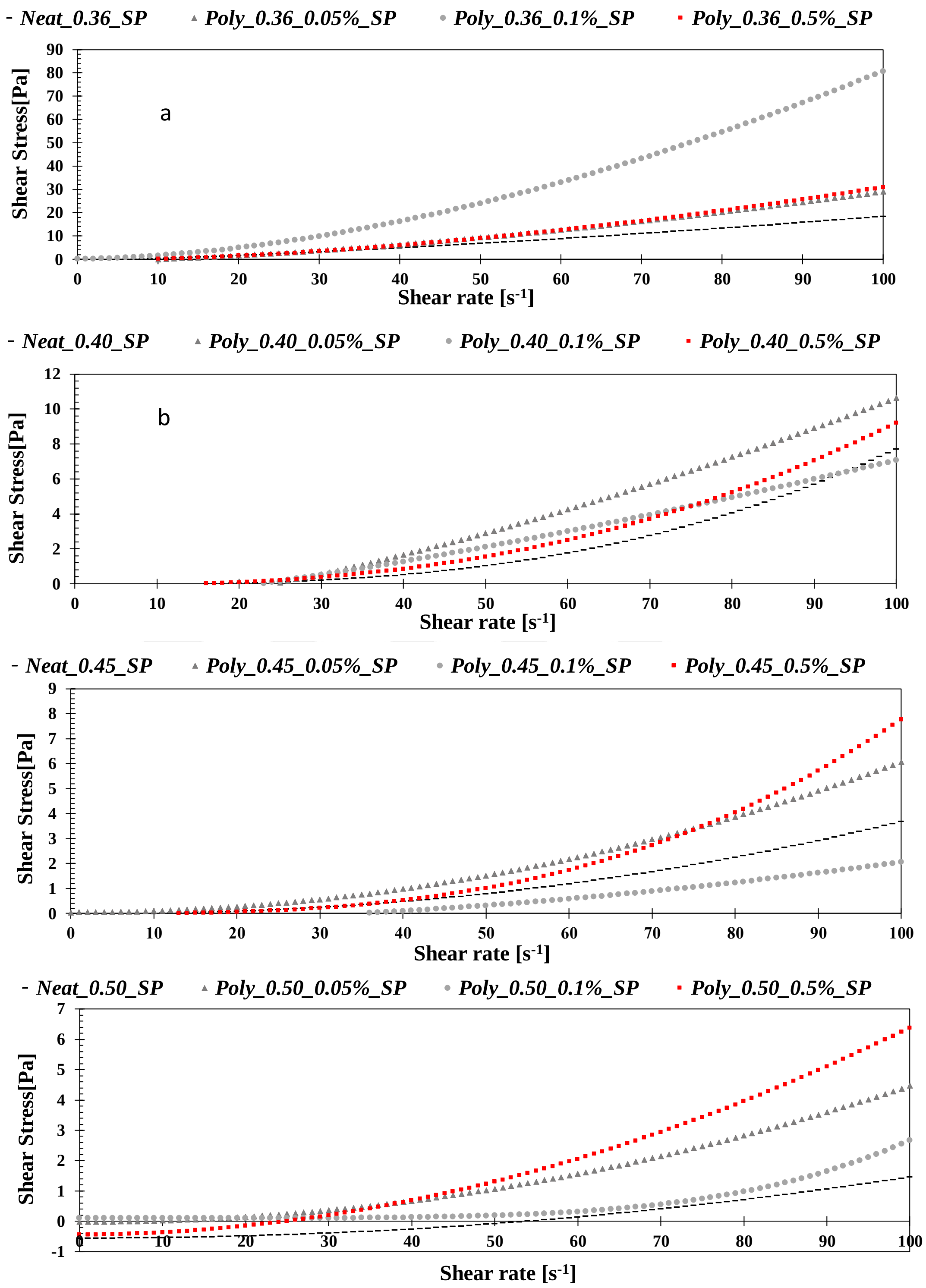

Figure 4-13 Rheological test results for cement paste samples including SP with and without *P.polymyxa* at a w/c of (a)0.36, (b)0.40, (c)0.45 and (d) 0.50. Cell dosages were used as 0.05%, 0.1% and 0.5% per weight of cement

b. Compatibility of S. pasteurii with SP :

From the rheological data, the plastic viscosity of cement paste amended with combinations of *S. pasteurii* cells and SP was obtained. Adding 0.05% of *S.pasteurii* cells for a dosage of 1% of SP resulted in an increase in the apparent viscosity at a low shear rate compared to similar neat cement paste without any cells. The increase in the dosage of cells increased the apparent viscosity at the low shear rate for a given dosage of SP. Comparing the neat samples with pastes including cells, the increasing shear-thinning behavior with cells addition is attributed to the polysaccharide-based chains of cells which intertwine, thus resulting in an increase in apparent viscosity, especially at the low shear rate. For the two w/c of 0.36 and 0.40, 0.05% of cell dosage incorporation resulted in an increase of the yield stress value and plastic viscosity, compared to the yield stress value of the neat paste samples, which confirms that cells have the ability to function as a VMA. Yet, the interaction of the cells with polycarboxylate cells are still unknown but they were compatible with their use as a VMA. It should be noted that both cells and superplasticizers are negatively charged. Thus, increase in cell concentration might interfere with the dispersion of the particles and decreasing the viscosity at higher dosages of cells. At last, the cells were added both with fly ash and superplasticizers. The cells were able to improve the viscosity at low w/c (0.36 and 0.40). But, at w/c of 0.50, there was a slight increase in viscosity when the cells were added by 0.5% of cement weight. This was mainly cells could not overcome the flowability of the mix. Thus, it might be possible to observe a higher increase in viscosity if the cell concentration was increased.

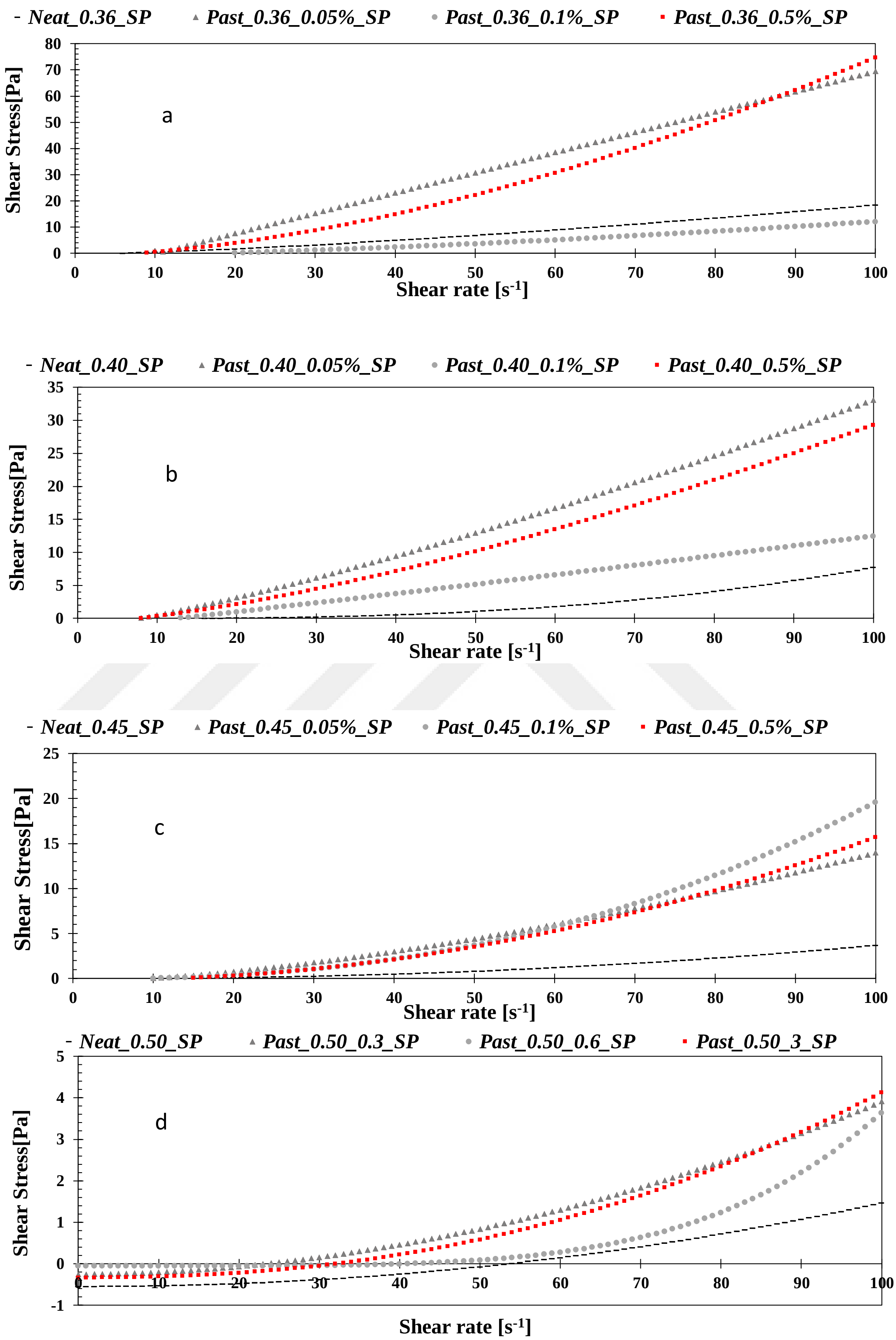

Figure 4-14 Rheological test results for cement paste samples including SP with and without *S.pasteurii* at a w/c of (a)0.36, (b)0.40, (c)0.45 and (d) 0.50. Cell dosages were used as 0.05%, 0.1% and 0.5% per weight of cement

c. Compatibility of B. megaterium with SP :

The viscosity and yield stress data presented in the Table 4-5 indicate that *B.megaterium* cells addition as VMAs exhibited an improvement in rheological properties. Figure 4-15 represents the rheological test results for cement paste samples including B. megaterium cells and 1% of SP by cement weight. For a given dosage of SP, the introduction of cells resulted in an increase in yield value and plastic viscosity. For instance, cement paste samples in a w/c=0.40 containing 1% of SP by cement weight, the increase in the dosages of *B.megaterium* cells from 0.05% to 0.5%, resulted in an increase of plastic viscosity from 96.20 MPa.s to 385 MPa.s. With increasing, w/c ratio, the effect of cells on both viscosity and yield stress was less notable but still, both parameters were higher than the neat paste samples. The performance of *B .megaterium* with the presence of SP was less effective compared to the samples without SP. Considering the thick cell wall of the *B.megaterium*, a notable decrease in shear-thickening behavior of the cement paste amendment with different dosages of the strain observed.

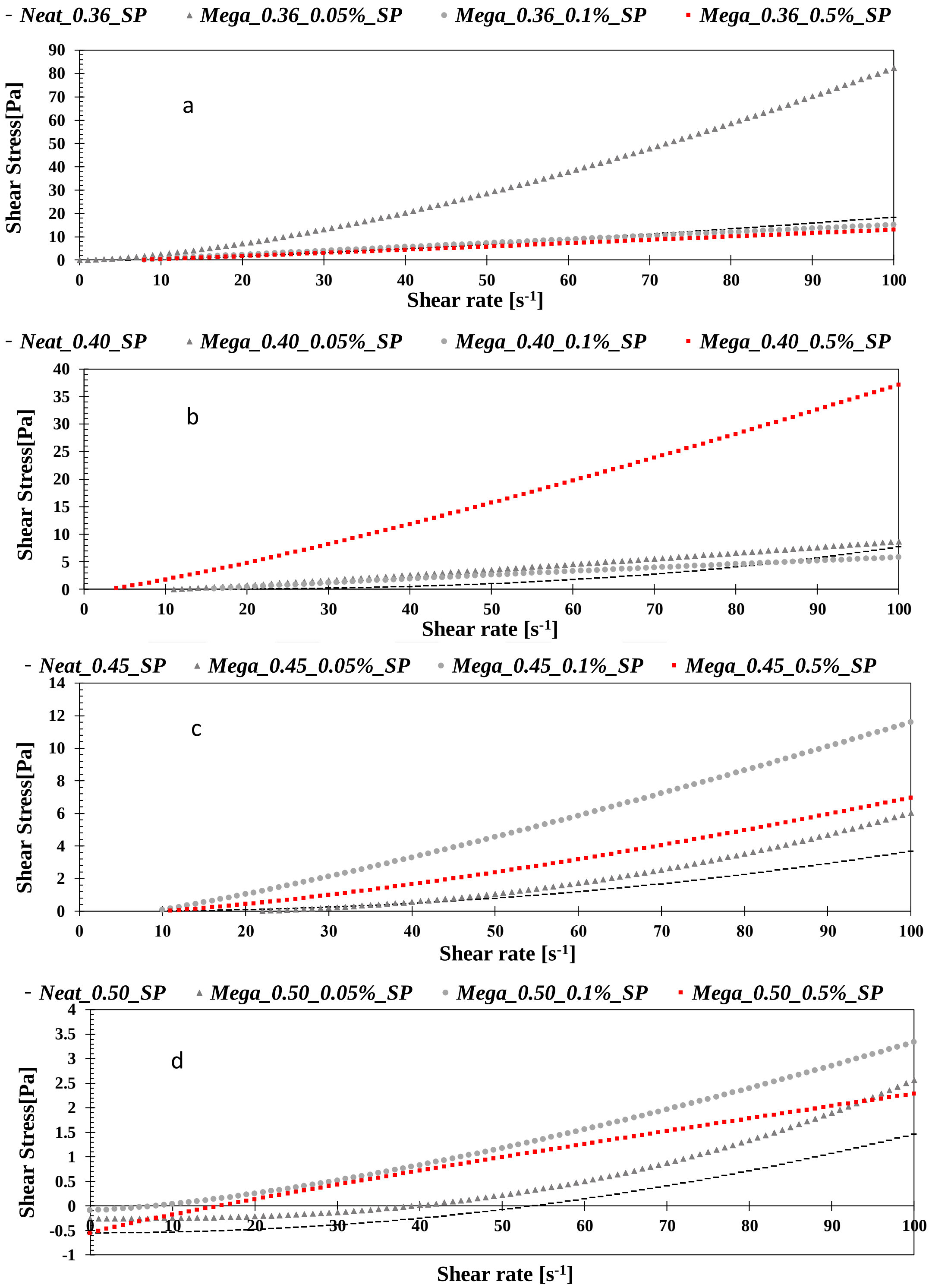

Figure 4-15 Rheological test results for cement paste samples including SP with and without *B.megaterium* at a w/c of (a)0.36, (b)0.40, (c)0.45 and (d) 0.50. Cell dosages were used as 0.05%, 0.1% and 0.5% per weight of cement.

d. Compatibility of B. subtilis with SP:

Incorporation of B. subtilis cells to mixes containing SP showed the most significant differences compared to the mixes without SP. Considering the 4 different strains used in the study, each type resulted with a different trend at each specified condition and there was not a general trend to summarize the general behavior. As so, the *B. subtilis* cells were the least efficient cells among all when they were incorporated to mixes without any SP or FA. However, incorporation of *B. subtilis* showed a significant viscosity and yield stress reduction. The reason might be the long polysaccharide base chains. Based on the results obtained from rheological measurements, at a 0.36 w/c cells addition in a dosage of 0.05% increased the viscosity significantly, with an increase in w/c to 0.40 , the optimum dosage increased to 0.1% but increasing the w/c to 0.45, once again the optimum dosage decreased to 0.05%. However, the trends observed were not the same at each w/c, but unlikely to the other studies, the strain was completely completable with the presence of SP and FA, which was the aim of the VMAs incorporation.

With adding dosages of *B.subtilis*, a shear thickening material changed to a shear thinning behavior and even Newtonian material. By adding more cells, a decrease in viscosity was recorded, which might confirm the fact of molecular weight and length effect on water retention of VMAs (see figure 4-16).

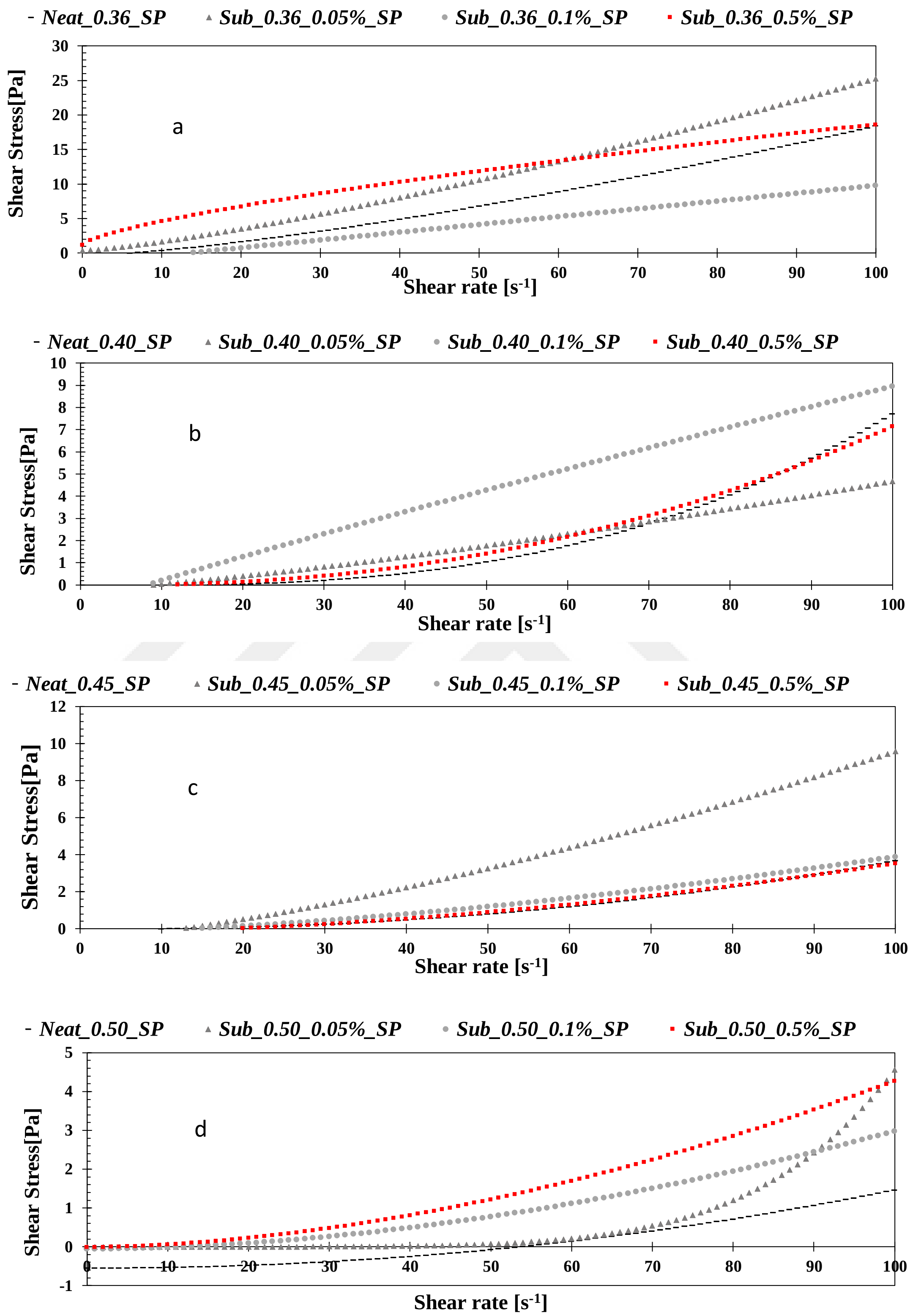

Figure 4-16 Rheological test results for cement paste samples including SP with and without *S.subtilis* at a w/c of (a)0.36, (b)0.40, (c)0.45 and (d) 0.50. Cell dosages were used as 0.05%, 0.1% and 0.5% per weight of cement

The rheological results showed that, at a constant dosage of SP, adding cells as VMA does not modify the plastic viscosity notably but controls the stability of cement pastes by increasing their yield stress. Moreover, the 4 types of VMAs behaved quite differently. To understand these differences, the action mechanism of cells should be further examined. First of all, SP works by adsorbing onto cement particles to induce a steric repulsion that decreases yield stress. Electrostatic forces, possibly mitigated by calcium ions, drove the adsorption of SP. They physically adsorb large amounts of water by hydrogen bonds, increasing their effective volume and consequently the viscosity of the interstitial fluid of cement paste. This increase is however much larger than that of the high shear viscosity of cement pastes. Apart from this specific effect on the continuous phase, VMAs can also adsorb onto cement particles [139]

In absence of SP, this adsorption has been shown to be responsible for an increase in yield stress which might be because of bridging flocculation. If this were also true in my study, it was expected that *B.subtilis or B.megaterium,* which might be the most adsorbed VMA, might increase yield stress most. However, the results show that the opposite was true. This suggests *P.polymyxa* cells may be responsible for the yield stress increase in presence of SP. Since the mechanism of adsorbing of each strain is not completely clear, further studies should be done regarding the adsorbing mechanism of cells. Depletion flocculation might be proportional to the molecular weight of the cells, which might explain the higher yield stress induced by *P.polymyxa* cells compared to the *S.pasteurii* cells. But the same hypothesize could not explain the reason of *P.polymyxa* strains being more effective *than the rest of the cells*. Further studies are needed to be done to explain the depletion flocculation mechanism. Specifically, measuring the adsorption of cells in cement pastes containing SP should provide more conclusive results.

4.2.3 Effect of time on the performance of cells

Another phenomenon tested was the rheological changes after waiting for a certain time period. To test this effect, the samples were kept at rest for 10 min. after the rheological test (see Section 3.3). Then, the viscosity and the yield stress were measured again without any pre-shearing. Tables 4-2 to 4-5 summarizes the results for the yield stress and effective viscosity values for cement paste samples kept at rest for 10 minutes.

As by its nature cement paste is complex structure due to on-going hydration reaction. It is known that when cement paste is left at rest, it generally builds up an internal structure which decreases the flowability of the material. Upon applied shear in a short time period (before irreversible structures are formed), the material can flow and viscosity can decrease back at a rate which increases with the applied shear rate. This is directly related to the thixotropic nature of the material [140]. Except for 6 samples (*Neat_0.50*, *Past_0.50_SP_FA*, *Poly_0.40_ SP_FA*, *Poly_0.45_ SP_FA*, *Poly_0.50_ SP_FA*, *Mega_0.40_ SP_FA*), there was not any significant change in neither yield strength and viscosity of the cement paste. This might indicate either the presence of cells delayed the structuring of the cement paste in such a short time period [113] or the cells did not interfere with the de-structuring of the cement paste upon shearing. A higher plastic viscosity, especially in mixes containing SP, might show the fact that hydration process happened even in a short period of time. This might be attributed to the hydration of cement with the time that made the paste stiffer as time progresses. This also indicates somehow that the new tested cells as VMAs are not inhibitors for the cement hydration.

The incorporated VMA's might take paste faster into the steady phase which caused higher fluctuation and as a result higher viscosity. However, the drastic change in the above-mentioned samples has to be further evaluated to find out the possible change in the steady phase and hydration.

4.3 Impact of microorganism on mini-slump diameter

Besides the rheological tests, workability and fresh state properties of mixes were also evaluated. Table 4-2 to 4-5 summarize (*see* Section 4.1) the average mini-slump diameters for all cement paste samples with and without the incorporation of cells.

As the addition of cells resulted in increasing the viscosity and yield strength of the cement paste in rheological measurements, they also resulted in a slight increase in the mini-slump value regardless of the w/c used in the mix. This was consistent with the rheological measurements such that the incorporation of cell improved not only the viscosity but also the yield strength.

Four type of the cells acted almost the same in cement pastes with the addition of FA. It should be noted that incorporation of FA would increase the workability due to its spherical shape and high specific gravity of FA, particularly at lower w/c. While *S.pasteurii. P.polymyxa and B.subtilis* caused an increase in mini-slump value with increasing dosages, their influence was less pronounced with increasing w/c.

With the addition of SP, comparing the neat samples with samples containing VMAs, it can be said that cell addition cause less significant increase in mini-slump test compared to the samples without SP. Incorporation of 3 different cell types, *P. polymxa*, *S.pasteurii*, and *B. megaterium*, with the SP at low w/c (0.36), cause slightly increased the mini-slump value which means higher yield stress. The behavior of the cement pastes including different dosages of *B. subtilis* cells was completely opposite. Increasing dosages of *B. subtilis* led to a decrease in mini-slump value. This indicated a decrease in yield value, which was not consistent with the rheological tests.

To be concluded, the workability tests showed almost the same result with rheological tests, except the addition of *B. subtilis* cells in the pastes had slightly different impact compare to the other three strains.

On a related note, cement pastes containing *P.polymxa,* and *S.pasteurii* had a slight thickening effect. The thickening effect was much more obvious in higher w/c and SP and FA addition. The spreading paste was much more steady and stable with cells addition (see Figure 4-17 and 4-18). In addition, to evaluate the flow behavior, the duration for cement paste samples to complete full spread was recorded. Cement paste samples containing four types of strains, even with 0.05% cells dosage per cement weight exhibited a slower spread time. This might indicate that the increase in viscosity and yield stress due to the incorporation of cells led to a slower spread time.

Comparing these results to the values obtained for the rheological test result indicates that the four additives reduced the mini-slump of the mortars. In addition, *P.polymyxa* had a much more significant effect in terms of improving viscosity and yield strength.

Figure 4-17 a neat sample with the addition of SP and w/c=50

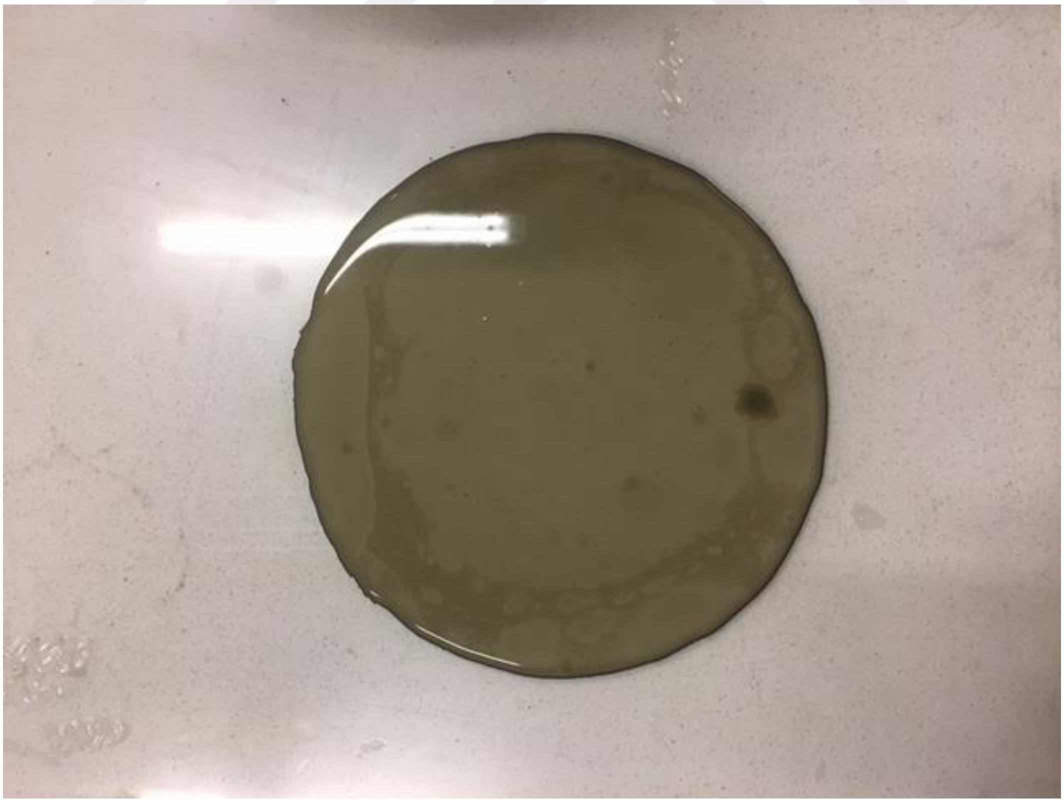

Figure 4-18 sample with the addition of 0.05% *P.polymyxa* and SP and w/c=50

4.4 Air Content Test

Tables 4-6 and 4-7 summarize the air-content test results in cement paste samples when 3 different dosages (0.05%, 0.1% and 0.5% g per cement weight) of *S. pasteurii P.polymyxya,* were added at a w/c of 0.36, 0.40. 0.45 and 0.50. Regarded to the results conducted in rheological and mini-cone slump values, it was the two mentioned strain had the most influence on workability and yield stress.

Comparing the different w/c in the samples it was observed that, increasing the w/c decreased the air-content in the samples without bacteria. A similar influence was also observed when FA and SP were added to the mix. This was mainly because of the high compatibility of mixes due to their high workability. Incorporation of cells to the mixture, cause a more drastic decrease in the air content of the mixture. This was consistent with the expected outcomes of using a VMA, providing a more stable and robust mix.

To be concluded the results obtained from this experiment, confirm the results of the rheological and mini-cone slump. Comparing the optimum dosage of these test, 0.5% of *S.pasteurii* cells were able to reduce the air-content the most and for *P.polymyxa* strain the most efficient dosage was observed to be 0.1% of cell per cement weight. Both results are overlapping the results of the experiments had done before.

Table 4-6 Air-content test results for different dosages of *P.polymyxa* compared with bacteria-free results

	Mix	w/c (%)	Air (%)		Mix	w/c (%)	Air (%)		Mix	w/c (%)	Air (%)		Mix	w/c (%)	Air (%)
Mixes without bacteria	Neat	36	30.9	**Bacteria content 0.05% of cement content**	Neat	36	29.4	**Bacteria content 0.1% of cement content**	Neat	36	26.6	**Bacteria content 0.5% of cement content**	**Neat**	36	32.52
		40	26.3			40	26.9			40	19.9			40	28.09
		45	27.6			45	23.6			45	16.9			45	16.18
		50	18.0			50	11.2			50	11.7			50	9.04
	SP	36	31.6		SP	36	23.7		SP	36	23.3		**SP**	36	20.89
		40	19.2			40	18.3			40	12.5			40	16.90
		45	20.6			45	7.56			45	10.2			45	6.47
		50	5.5			50	0.04			50	7.82			50	0.00
	FA	36	37.0		FA	36	38.4		FA	36	34.9		**FA**	36	21.25
		40	29.4			40	30.9			40	25.9			40	17.00
		45	30.0			45	19.3			45	17.9			45	14.09
		50	17.0			50	10.4			50	11.2			50	13.27
	SP +FA	36	27.1		SP +FA	36	13.6		SP +FA	36	17.5		**SP +FA**	36	21.40
		40	17.3			40	11.2			40	11.3			40	18.39
		45	12.2			45	9.65			45	12.1			45	8.82
		50	4.17			50	6.60			50	9.4			50	6.02

Table 4-7 Air content test results for different dosages of *S. pasteurii* compared with bacteria-free results

	Mix	w/c (%)	Air (%)		Mix	w/c (%)	Air (%)		Mix	w/c (%)	Air (%)		Mix	w/c (%)	Air (%)
Mixes without bacteria	Neat	36	30.9	**Bacteria content 0.05% of cement content**	Neat	36	30.9	**Bacteria content 0.1% of cement content**	Neat	36	31.0	**Bacteria content 0.5% of cement content**	**Neat**	36	24.97
		40	26.3			40	27.9			40	26.8			40	21.49
		45	27.6			45	20.9			45	21.4			45	17.95
		50	18.0			50	9.2			50	10.7			50	13.43
	SP	36	31.6		SP	36	28.6		SP	36	24.8		**SP**	36	24.72
		40	19.2			40	18.2			40	16.2			40	12.83
		45	20.6			45	11.79			45	10.2			45	7.56
		50	5.5			50	7.66			50	5.81			50	4.33
	FA	36	37.0		FA	36	27.0		FA	36	33.4		**FA**	36	22.78
		40	29.4			40	25.4			40	32.1			40	21.80
		45	30.0			45	21.2			45	22.8			45	14.61
		50	17.0			50	14.6			50	14.9			50	10.62
	SP +FA	36	27.1		SP +FA	36	21.6		SP +FA	36	22.5		**SP +FA**	36	21.51
		40	17.3			40	18.4			40	20.6			40	10.92
		45	12.2			45	14.82			45	11.1			45	5.79
		50	4.17			50	10.89			50	0.0			50	4.80

CHAPTER V

CONCLUSION AND SUGGESTED WORK

5.1 Conclusion

In the last decade, reducing the negative impacts of concrete production such as CO_2 footprint, use fuel sources and production of wastes has gained attention from researchers and civil engineers. Among many approaches, development of bio-based admixtures could be a remedy to the potential problems arisen due to the production of concrete. This study represented the results of an extensive study undertaken to evaluate the possible use of microorganism VMA, or more precisely as a rheology modifying agent.

The flow behavior of cement paste samples including *P.polymyxa*, *S.Pasteurii*, *B. megaterium and B. subtilis* cells were evaluated through rheological tests and mini-slump tests. Moreover, the compatibility of cells with the use of superplasticizer and fly ash, as well as the influence of w/c was investigated. Finally, the fresh state performance was tested air content and wash-out tests with the most effective strains founded in rheological tests.

Based on the results presented in this study, the following conclusions can be drawn:

I. Incorporation of cells modified the rheological parameters (plastic viscosity and yield stress) regardless of the w/c used for the mixes. However, there was not any specified trend in the action mechanism of the microorganisms.

II. This influence is attributed to the interwinding of peptidoglycan chains leading to an increase in viscosity of mix water. However, further studies have to be conducted to understand the other possible parameters of cells influencing the rheology such as the effect of cells on drag forces and particle-to-particle interaction.

III. The most efficient dosages for the specific cells 0.1%, 0.5%, 0.1% and 0.05% for the *P.polymyxa, S.Pasteurii, B.megaterium and B.subtilis* cells respectively in w/c of 0.45.

IV. Increasing w/c, decrease the effect of cells, the difference between the viscosity of the cement pastes. The influence of cells was much more pronounced at a w/c of 0.36 than it was at 0.50.

V. The cells were compatible with both FA and SP. However, since the flow behavior of cement paste was significantly modified with the incorporation of these additives, the influence of cells on rheology was also different. As so, the cells lead to higher shear thinning behavior when they were added to the mixes including FA and SP at low w/c's.

VI. Regarding the rheological properties described, with the addition of cells, the comparison between viscosity after 10 minutes waiting with a viscosity of the paste right after mixing, present the effectiveness of the chosen strains in decreasing segregation.

VII. The mini-slump and air content tests have strongly supported the rheology-based observations. In other words, samples with VMAs addition can also be detected based on the air content and the workability, however, these test methods are time-consuming and labor-intensive compared with rheology testing.

VIII. There exists a good correlation between the result of the mini-slump cone tests and the yield stress by the rheology tests.

P.polymyxa strain with an optimum dosage of 0.1% by weight of cement showed the most significant results in rheological properties improvements.

5.2 Suggested work

The approach summarized herein is a novel idea which was firstly studied in the literature. Relatedly, there is a patent pending (PCT/TR2016/050472)[124]for the idea presented in this study. Thus, there can be many prospective studies conducted to understand the mechanism of bacterial rheology modifying agents and take this nutshell idea out in the field. Some of the possible further research ideas could be:

- The interaction and behavior of cells should be evaluated in cement paste suspension, possibly through a cryo-scanning electron microscopy (SEM) at liquid state. This will reveal both the active suspension behavior of microswimmers and their absorbance on particles.

- Also, the hardened state properties such as compressive strength, flexural strength, chemical composition and durability of the bacteria amended concrete have to be evaluated.

- Influence of different types and dosages of pozzolans and superplasticizers on the performance of bacterial rheology modifiers should be evaluated.

The product of these studies may in the future compete with the known additives in the market to modify the rheology and reduce the segregation during conducting new types of concretes such as SCC. The obtained concrete with microorganism might be more stable during the high pressure of pumping and hard casting situations compared to the concretes containing additives are known in the market. Although it should be mentioned that the incorporation of the microorganism to cement paste showed a promising result in the laboratory scale and optimization of nutrient media and large-scale demonstration with different type and dosages of SP and FA might be needed before introducing it to market. Since the production process and the demanded time to produce the required VMA by using bacteria cells reduced noticeably during this study, the use of microorganism for practical applications may not be the way forward. Furthermore, the efficiency of the incorporated cells, specially *P.polymyxa* strain, need to be further proven in larger concrete productions, in different environments.

Vita

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Mahzad Azima was born in Urmia/ Iran on May 20.1992. Mahzad enrolled in the University of Tabriz, in August 2010. She received her Bachelor of Science in civil engineering degree in December 2014. In January 2016, Mahzad started her MSc program in Ozyegin University. She is an M.Sc. student in civil engineering at Ozyegin University, Istanbul, Turkey under the supervision of Dr. Zeynep Basaran Bundur. Her research area focuses on cementitious material, construction material, concrete workability, and sustainability. She will continue with her graduate studies

to pursue a Doctor of Philosophy degree in civil engineering.

REFERENCES

[1] H. Okamura, M. Ouchi, Self-Compacting Concrete, J. Adv. Concr. Technol. 1 (2003) 5–15. doi:10.3151/jact.1.5.

[2] W.-J. Long, K. Khayat, G. Lemieux, F. Xing, W.-L. Wang, Factorial Design Approach in Proportioning Prestressed Self-Compacting Concrete, Materials (Basel). 8 (2015) 1089–1107. doi:10.3390/ma8031089.

[3] R. Pei, J. Liu, S. Wang, Use of bacterial cell walls as a viscosity-modifying admixture of concrete, Cem. Concr. Compos. 55 (2015) 186–195. doi:10.1016/j.cemconcomp.2014.08.007.

[4] K.H. Khayat, Viscosity-enhancing admixtures for cement-based materials — An overview, Cem. Concr. Compos. 20 (1998) 171–188. doi:10.1016/S0958-9465(98)80006-1.

[5] J. Plank, Applications of biopolymers and other biotechnological products in building materials, Appl. Microbiol. Biotechnol. 66 (2004) 1–9. doi:10.1007/s00253-004-1714-3.

[6] F.M. León-Martínez, P.F.D.J. Cano-Barrita, L. Lagunez-Rivera, L. Medina-Torres, Study of nopal mucilage and marine brown algae extract as viscosity-enhancing admixtures for cement based materials, Constr. Build. Mater. 53 (2014) 109–202. doi:10.1016/j.conbuildmat.2013.11.068.

[7] W. Schmidt, H.J.H. Brouwers, H.C. Kühne, B. Meng, The working mechanism of starch and diutan gum in cementitious and limestone dispersions in presence of polycarboxylate ether superplasticizers, Appl. Rheol. 23 (2013). doi:10.3933/ApplRheol-23-52903.

[8] D. Saintillan, Kinetic models for biologically active suspensions, Urbana, n.d.

[9] M. Sonebi, Rheological properties of grouts with viscosity modifying agents as diutan gum and welan gum incorporating pulverised fly ash, Cem. Concr. Res. 36 (2006) 1609–1618. doi:10.1016/j.cemconres.2006.05.016.

[10] H. Guan, S. Liu, Y. Duan, Expanded polystyrene as an admixture in cement-based

composites for electromagnetic absorbing, J. Mater. Eng. Perform. (2007). doi:10.1007/s11665-006-9010-2.

[11] P. Ghosh, S. Mandal, B.D. Chattopadhyay, S. Pal, Use of microorganism to improve the strength of cement mortar, Cem. Concr. Res. 35 (2005) 1980–1983. doi:10.1016/j.cemconres.2005.03.005.

[12] S. Liu, Z.B. Bundur, J. Zhu, R.D. Ferron, Evaluation of self-healing of internal cracks in biomimetic mortar using coda wave interferometry, Cem. Concr. Res. 83 (2016) 70–78. doi:10.1016/j.cemconres.2016.01.006.

[13] P.F.G. Banfill, Rheology of fresh cement and concrete. Rheology Reviews vol. . Edinburgh, The British Society of Rheology, Rheol. Rev. 2006 (2006) 61–130.

[14] P.F.G. Banfill, Poster - Theme VI - Rheology of setting Portland cement pastes, in: 7th Int. Congr. Chem. Cem. Vol. 4, 1980: pp. 324–328.

[15] G.H. Tattersall, CONTROL OF HIGH WORKABILITY CONCRETE., Concr. 16 (1982) 12–24.

[16] P.S. Roller, Plastic flow of dispersions and a new approach to the study of plasticity, J. Phys. Chem. (n.d.).

[17] R. L’Hermite, The rheology of fresh concrete and vibration, Publ. Tech. No 2. (n.d.).

[18] P.S. Roller, Theory and measurement of plasticity and workability of mortars, Proc. Amer. Soc. Test. Mater. (n.d.).

[19] K. Hattori, K. Izumi, A RHEOLOGICAL EXPRESSION OP COAGULATION RATE THEORY, J. Dispers. Sci. Technol. 3 (1982) 169–193. doi:10.1080/01932698208943632.

[20] R.J. Flatt, P. Bowen, Yodel: A yield stress model for suspensions, J. Am. Ceram. Soc. 89 (2006) 1244–1256. doi:10.1111/j.1551-2916.2005.00888.x.

[21] K. Sujata, H.M. Jennings, Formation of a Protective Layer During the Hydration of Cement, J. Am. Ceram. Soc. 75 (1992) 1669–1673. doi:10.1111/j.1151-2916.1992.tb04243.x.

[22] P. Banfill, The rheology of fresh cement and concrete-a review, Proc. 11th Int. Cem. Chem. Congr. 1 (2003) 50–62. doi:10.1016/0008-8846(90)90053-Z.

[23] V.A. Ghio, P.J.M. Monteiro, L.A. Demsetz, The rheology of fresh cement paste containing polysaccharide gums, Cem. Concr. Res. 24 (1994) 243–249. doi:10.1016/0008-8846(94)90049-3.

[24] N.Q. Dzuy, D. V. Boger, Direct Yield Stress Measurement with the Vane Method, J. Rheol. (N. Y. N. Y). 29 (1985) 335–347. doi:10.1122/1.549794.

[25] O.J. Uzomaka, A concrete rheometer and its application to a rheological study of concrete mixes, Rheol. Acta. 13 (1974) 12–21. doi:10.1007/BF01526878.

[26] S.A. Khedr, M.N. Abou-Zeid, Characteristics of Silica-Fume Concrete, J. Mater. Civ. Eng. 6 (1994) 357–375. doi:10.1061/(ASCE)0899-1561(1994)6:3(357).

[27] P.L.J. Domone, X. Yongmo, P.F.G. Banfill, Developments of the two-point workability test for high-performance concrete, Mag. Concr. Res. 51 (1999) 171–179. doi:10.1680/macr.1999.51.3.171.

[28] M. Nehdi, S. Mindess, P.C. Aïtcin, Rheology of high-performance concrete: Effect of ultrafine particles, Cem. Concr. Res. 28 (1998) 687–697. doi:10.1016/S0008-8846(98)00022-2.

[29] P.F.G. Banfill, Rheological methods for assessing the flow properties of mortar and related materials, Constr. Build. Mater. 8 (1994) 43–50. doi:10.1016/0950-0618(94)90007-8.

[30] G.H. TATTERSALL, COMPONENTS OF WORKABILITY AND RHEOLOGICAL MEASUREMENTS ON MORTARS AND FRESH CONCRETE, in: 8th Int. Congr. Chem. Cem. Vol. 6, 1986: pp. 228–238.

[31] C. Hu, F. De Larrard, The rheology of fresh high-performance concrete, Cem. Concr. Res. 26 (1996) 283–294. doi:10.1016/0008-8846(95)00213-8.

[32] P.F.G. Banfill, A discussion of the papers “rheological properties of cement mixes” by M. Daimon and D. M. Roy, Cem. Concr. Res. 9 (1979) 795–796. doi:10.1016/0008-8846(79)90075-9.

[33] P.F.G. Banfill, Feasibility study of a coaxial cylinders viscometer for mortar, Cem. Concr. Res. 17 (1987) 329–339. doi:10.1016/0008-8846(87)90115-3.

[34] O.E. Petel, J.D. Hogan, An investigation of shear thickening fluids using ejecta analysis techniques, Int. J. Impact Eng. 93 (2016) 39–48. doi:10.1016/j.ijimpeng.2016.02.001.

[35] P.F.G. Banfill, R.E. Carter, P.J. Weaver, Simultaneous rheological and kinetic measurements on cement pastes, Cem. Concr. Res. 21 (1991) 1148–1154. doi:10.1016/0008-8846(91)90075-S.

[36] C. Atzeni, L. Massidda, U. Sanna, Comparison between rheological models for portland cement pastes, Cem. Concr. Res. 15 (1985) 511–519. doi:10.1016/0008-8846(85)90125-5.

[37] J. Assaad, K.H. Khayat, H. Mesbah, Assessment of thixotropy of flowable and self-consolidating concrete, ACI Mater. J. (2003). doi:10.14359/12548.

[38] M. Nehdi, M.A. Rahman, Estimating rheological properties of cement pastes using various rheological models for different test geometry, gap and surface friction, Cem. Concr. Res. 34 (2004) 1993–2007. doi:10.1016/j.cemconres.2004.02.020.

[39] A. Papo, Rheological models for cement pastes, Mater. Struct. (1988). doi:10.1007/BF02472527.

[40] T.E.R. Jones, S. Taylor, A mathematical model relating the flow curve of a cement paste to its Water/Cement ratio, Mag. Concr. Res. (1977). doi:10.1680/macr.1977.29.101.207.

[41] P.F.G. Banfill, A Review of Fresh Cement and Concrete Rheology, Proc 11th Int. Cem. Chem. Congr. (2013).

[42] C.F. Ferraris, Measurement of the rheological properties of high performance concrete: State of the art report, J. Res. Natl. Inst. Stand. Technol. 104 (1999) 461. doi:10.6028/jres.104.028.

[43] G.H. Tattersall, S.J. Bloomer, Further development of the two-point test for workability and extension of its range, Mag. Concr. Res. 31 (1979) 202–210.

doi:10.1680/macr.1979.31.109.202.

[44] D.A. Williams, A.W. Saak, H.M. Jennings, Influence of mixing on the rheology of fresh cement paste, Cem. Concr. Res. 29 (1999) 1491–1496. doi:10.1016/S0008-8846(99)00124-6.

[45] S. Hanehara, K. Yamada, Rheology and early age properties of cement systems, Cem. Concr. Res. (2008). doi:10.1016/j.cemconres.2007.09.006.

[46] O.H. Wallevik, J.E. Wallevik, Rheology as a tool in concrete science: The use of rheographs and workability boxes, Cem. Concr. Res. (2011). doi:10.1016/j.cemconres.2011.01.009.

[47] R.B. Polder, Test methods for on site measurement of resistivity of concrete - a RILEM TC-154 technical recommendation, Constr. Build. Mater. (2001). doi:10.1016/S0950-0618(00)00061-1.

[48] M. Daimon, D.M. Roy, A reply to the discussion by P.F.G. Banfill of "rheological properties of cement mixes, Cem. Concr. Res. 9 (1979) 797. doi:10.1016/0008-8846(79)90076-0.

[49] J.E. Wallevick, Rheology of particle suspension: fresh concrete, mortar and cement paste with various types of lignosulfates, (2003) 1–397.

[50] J.E. Wallevik, Minimizing end-effects in the coaxial cylinders viscometer: Viscoplastic flow inside the ConTec BML Viscometer 3, J. Nonnewton. Fluid Mech. (2008). doi:10.1016/j.jnnfm.2008.05.006.

[51] L.E. Brower, C.F. Ferraris, Comparison of concrete rheometers, Concr. Int. (2003).

[52] P.C. Hewlett, Lea's Chemistry of Cement and Concrete, 2003. doi:10.1016/B978-0-7506-6256-7.X5007-3.

[53] D.P. Bentz, E.J. Garboczi, C.J. Haecker, O.M. Jensen, Effects of cement particle size distribution on performance properties of Portland cement-based materials, Cem. Concr. Res. (1999). doi:10.1016/S0008-8846(99)00163-5.

[54] S. Tsivilis, E. Chaniotakis, E. Badogiannis, G. Pahoulas, A. Ilias, A study on the parameters affecting the properties of Portland limestone cements, Cem. Concr.

Compos. (1999). doi:10.1016/S0958-9465(98)00031-6.

[55] K. Andersson, B. Allard, M. Bengtsson, B. Magnusson, Chemical composition of cement pore solutions, Cem. Concr. Res. (1989). doi:10.1016/0008-8846(89)90022-7.

[56] M.H. Blees, Foundations of Colloid Science, Colloids Surfaces A Physicochem. Eng. Asp. (2002). doi:10.1016/S0927-7757(02)00170-X.

[57] C.F. Ferraris, N.S. Martys, Concrete rheometers, in: Underst. Rheol. Concr., 2011. doi:10.1016/B978-0-85709-028-7.50003-0.

[58] J. Mewis, Thixotropy - a general review, J. Nonnewton. Fluid Mech. (1979). doi:10.1016/0377-0257(79)87001-9.

[59] R.G. Larson, The Structure and Rheology of Complex Fluids, 1999. doi:10.1029/2006EO230009.

[60] P.E. Clark, L. Sundaram, M. Balakrishnan, Yield Points in Oilfield Cement Slurries, SPE East. Reg. Meet. (1990).

[61] B.H. Min, L. Erwin, H.M. Jennings, Rheological behaviour of fresh cement paste as measured by squeeze flow, J. Mater. Sci. (1994). doi:10.1007/BF00975091.

[62] R.A. Helmuth, SUB-THEME VI-0 Structure and rheology of fresh cement paste, in: 7th Int. Congr. Chem. Cem. Vol. 3, 1980.

[63] H.A. Barnes, J.O. Carnali, The vane-in-cup as a novel rheometer geometry for shear thinning and thixotropic materials, J. Rheol. (N. Y. N. Y). (1990). doi:10.1122/1.550103.

[64] J.F. Raffle, A. King, The effect of cellulose ethers and lignosulphonates on the settling and flow properties of cement suspensions, J. Phys. D. Appl. Phys. (1977). doi:10.1088/0022-3727/10/1/004.

[65] R.J. Mannheimer, Laminar and turbulent flow of cement slurries in large diameter pipe: A comparison with laboratory viscometers, J. Rheol. (1991). doi:10.1122/1.550223.

[66] T.T. Roshavelov, New viscometer for measuring flow properties of fluid concrete,

ACI Mater. J. (2005). doi:10.14359/14802.

[67] M.H. Mohammed, R. Pusch, S. Knutsson, G. Hellström, Rheological Properties of Cement-Based Grouts Determined by Different Techniques, Engineering. (2014). doi:10.4236/eng.2014.65026.

[68] M.A. Schultz, L.J. Struble, Use of oscillatory shear to study flow behavior of fresh cement paste, Cem. Concr. Res. (1993). doi:10.1016/0008-8846(93)90092-N.

[69] F. Winnefeld, L. Holzer, MONITORING EARLY CEMENT HYDRATION BY RHEOLOGICAL MEASUREMENTS, in: 11th Int. Congr. Chem. Cem., 2003.

[70] P.V. Vlachou, J.M. Piau, The influence of the shear field on the microstructural and chemical evolution of an oil well cement slurry and its rheometric impact, Cem. Concr. Res. (1997). doi:10.1016/S0008-8846(97)00089-6.

[71] R.J. Flatt, P. Bowen, Electrostatic repulsion between particles in cement suspensions: Domain of validity of linearized Poisson-Boltzmann equation for nonideal electrolytes, Cem. Concr. Res. (2003). doi:10.1016/S0008-8846(02)01059-1.

[72] S. Maschio, E. Lucchuni, V. Sergo, Piezospectroscopic analysis of the residual stresses in the strontium hexaluminate/zirconia. Soc. (1999). doi:10.1111/j.1151-2916.1999.tb02215.x.

[73] M. Daimon, D.M. Roy, Rheological properties of cement mixes: II. Zeta potential and preliminary viscosity studies, Cem. Concr. Res. (1979). doi:10.1016/0008-8846(79)90100-5.

[74] K.H. Khayat, G. De Schutter, S. Concrete, Mechanical Properties of Self-Compacting Concrete, RILEM State-of-the-Art Reports. 14 (2014). doi:10.1007/978-3-319-03245-0.

[75] L. Rudzinski, The effect of fly ashes on the rheological behaviour of cement pastes, Matériaux Constr. (1984). doi:10.1007/BF02478709.

[76] L. Yijin, Z. Shiqiong, Y. Jian, G. Yingli, The effect of fly ash on the fluidity of cement paste, mortar and concrete, Proc. Int. Work. Sustain. Dev. Concr. Technol.

(2004).

[77] M. Lachemi, K.M.A. Hossain, V. Lambros, P.C. Nkinamubanzi, N. Bouzoubaâ, Self-consolidating concrete incorporating new viscosity modifying admixtures, Cem. Concr. Res. 34 (2004) 917–926. doi:10.1016/j.cemconres.2003.10.024.

[78] M. Lachemi, K.M.A. Hossain, V. Lambros, P.C. Nkinamubanzi, N. Bouzoubaâ, Performance of new viscosity modifying admixtures in enhancing the rheological properties of cement paste, Cem. Concr. Res. (2004). doi:10.1016/S0008-8846(03)00233-3.

[79] N.P. Mailvaganam, Miscellaneous Admixtures, in: Concr. Admixtures Handb., 1996. doi:10.1016/B978-081551373-5.50019-2.

[80] T. Matsumoto, M. Kawai, T. Masuda, Rheological properties and fractal structure of concentrated polyion complexes of chitosan and alginate, Biorheology. (1993). doi:10.3233/BIR-1993-305-614.

[81] D.F. Zhang, B.Z. Ju, S.F. Zhang, L. He, J.Z. Yang, The study on the dispersing mechanism of starch sulfonate as a water-reducing agent for cement, Carbohydr. Polym. (2007). doi:10.1016/j.carbpol.2007.04.024.

[82] W. Schmidt, H.J.H. Brouwers, H.C. Kühne, B. Meng, Interactions of polysaccharide stabilising agents with early cement hydration without and in the presence of superplasticizers, Constr. Build. Mater. (2017). doi:10.1016/j.conbuildmat.2016.11.022.

[83] K.H. Khayat, A. Yahia, Effect of welan gum-high-range water reducer combinations on rheology of cement grout, ACI Mater. J. 94 (1997) 365–372.

[84] L. Xu, G. Xu, T. Liu, Y. Chen, H. Gong, The comparison of rheological properties of aqueous welan gum and xanthan gum solutions, Carbohydr. Polym. (2013). doi:10.1016/j.carbpol.2012.09.082.

[85] M.H. Ozkul, Revolution in concrete technology: Self-compacting concrete, 2002.

[86] B.W. Staynes, I.C. on P. Concretes, The production, performance & potential of polymers in concrete : 5th International Congress on Polymers in Concrete,

Brighton, England : 22-24 September 1987, Brighton Polytechnic, Brighton, 1987.

[87] S. Rols, J. Ambroise, J. Péra, Effects of different viscosity agents on the properties of self-leveling concrete, Cem. Concr. Res. (1999). doi:10.1016/S0008-8846(98)00095-7.

[88] A. Leemann, F. Winnefeld, The effect of viscosity modifying agents on mortar and concrete, Cem. Concr. Compos. (2007). doi:10.1016/j.cemconcomp.2007.01.004.

[89] S. Nagataki and H. Fujiwara, Self-Compacting Property of Highly Flowable Concrete, On-Line J. (1995). doi:10.14359/960.

[90] R. Hooton, K. Khayat, A. Yahia, Simple Field Tests to Characterize Fluidity and Washout Resistance of Structural Cement Grout, Cem. Concr. Aggregates. (1998). doi:10.1520/CCA10448J.

[91] H. a. Barnes, Rheology of industrial polysaccharides — Theory and applications, 1995. doi:10.1016/0377-0257(95)90025-X.

[92] P.A. Williams, G.O. Phillips, Introduction to food hydrocolloids, 2009. doi:doi:10.1094/1891127381.toc.

[93] Z.H. Pan, H.P. Cai, P.P. Jiang, Q.Y. Fan, Properties of a calcium phosphate cement synergistically reinforced by chitosan fiber and gelatin, J. Polym. Res. (2006). doi:10.1007/s10965-006-9041-2.

[94] M.H. Alkhraisat, C. Rueda, L.B. Jerez, F. Tamimi Mariño, J. Torres, U. Gbureck, E. Lopez Cabarcos, Effect of silica gel on the cohesion, properties and biological performance of brushite cement, Acta Biomater. (2010). doi:10.1016/j.actbio.2009.06.010.

[95] M. Lasheras-Zubiate, I. Navarro-Blasco, J.M. Fernández, J.I. Álvarez, Effect of the addition of chitosan ethers on the fresh state properties of cement mortars, Cem. Concr. Compos. (2012). doi:10.1016/j.cemconcomp.2012.04.010.

[96] G. Kahng, S. Lim, H. Yun, W. Seo, Production of Extracellular Polysaccharide , EPS WN9 , from Paenibacillus sp . WN9 KCTC 8951P and Its Usefulness as a Cement Mortar Admixture, (2001) 112–116.

[97] M. Lasheras-Zubiate, I. Navarro-Blasco, J.M. Fernández, 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). doi:10.1002/app.33048.

[98] I.E. Isik, M.H. Ozkul, Utilization of polysaccharides as viscosity modifying agent in self-compacting concrete, Constr. Build. Mater. (2014). doi:10.1016/j.conbuildmat.2014.09.017.

[99] W. Vollmer, D. Blanot, M.A. De Pedro, Peptidoglycan structure and architecture, FEMS Microbiol. Rev. 32 (2008) 149–167. http://dx.doi.org/10.1111/j.1574-6976.2007.00094.x.

[100] J. Plank, N.R. Lummer, F. Dugonjić-Bilić, Competitive adsorption between an AMPSV®-based fluid loss polymer and Welan gum biopolymer in oil well cement, J. Appl. Polym. Sci. (2010). doi:10.1002/app.31865.

[101] P.J. Wyatt, Cell Wall Thickness, Size Distribution, Refractive Index Ratio and Dry Weight Content of Living Bacteria (Stapylococcus aureus), Nature. 226 (1970) 277–279. doi:10.1038/226277a0.

[102] S. Rafaï, L. Jibuti, P. Peyla, Effective Viscosity of Microswimmer Suspensions, Phys. Rev. Lett. 104 (2010) 1–4. doi:10.1103/PhysRevLett.104.098102.

[103] T. Pedley, Hydrodynamic Phenomena In Suspensions Of Swimming Microorganisms, Annu. Rev. Fluid Mech. (1992). doi:10.1146/annurev.fluid.24.1.313.

[104] C. Dombrowski, L. Cisneros, S. Chatkaew, R.E. Goldstein, J.O. Kessler, Self-concentration and large-scale coherence in bacterial dynamics, Phys. Rev. Lett. (2004). doi:10.1103/PhysRevLett.93.098103.

[105] T. Ishikawa, T.J. Pedley, The rheology of a semi-dilute suspension of swimming model micro-organisms, J. Fluid Mech. (2007). doi:10.1017/S0022112007007835.

[106] B.M. Haines, I.S. Aranson, L. Berlyand, D.A. Karpeev, Effective viscosity of dilute bacterial suspensions: A two-dimensional model, Phys. Biol. (2008). doi:10.1088/1478-3975/5/4/046003.

[107] D. Saintillan, M.J. Shelley, Active suspensions and their nonlinear models, Comptes Rendus Phys. (2013). doi:10.1016/j.crhy.2013.04.001.

[108] Y. Hatwalne, S. Ramaswamy, M. Rao, R.A. Simha, Rheology of Active-Particle Suspensions, Phys. Rev. Lett. (2004). doi:10.1103/PhysRevLett.92.118101.

[109] A. Sokolov, I.S. Aranson, Reduction of viscosity in suspension of swimming bacteria, Phys. Rev. Lett. (2009). doi:10.1103/PhysRevLett.103.148101.

[110] P. Johann, Applications of Biopolymers in Construction Engineering, Biopolym. Online. (2005). doi:doi:10.1002/3527600035.bpola002.

[111] Z. Başaran Bundur, S. Bae, M.J. Kirisits, R.D. Ferron, Biomineralization in Self-Healing Cement-Based Materials: Investigating the Temporal Evolution of Microbial Metabolic State and Material Porosity, J. Mater. Civ. Eng. (2017). doi:10.1061/(ASCE)MT.1943-5533.0001838.

[112] V. Achal, X. Pan, N. Özyurt, Improved strength and durability of fly ash-amended concrete by microbial calcite precipitation, Ecol. Eng. 37 (2011) 554–559. doi:10.1016/j.ecoleng.2010.11.009.

[113] Z.B.B. Ali Amiri, Use of corn-steep liquor as an alternative carbon source for biomineralization in cement-based materials and its impact on performance, Constr. Build. Mater. (2017).

[114] ASTM C305, Standard Practice for Mechanical Mixing of Hydraulic Cement Pastes and Mortars of Plastic Consistency, ASTM Lnternational. (2011) 1–3. doi:10.1520/C0305-13.2.

[115] M. Benaicha, X. Roguiez, O. Jalbaud, Y. Burtschell, A.H. Alaoui, Influence of silica fume and viscosity modifying agent on the mechanical and rheological behavior of self compacting concrete, Constr. Build. Mater. (2015). doi:10.1016/j.conbuildmat.2015.03.061.

[116] D. Feys, R. Verhoeven, G. De Schutter, Fresh self compacting concrete, a shear thickening material, Cem. Concr. Res. 38 (2008) 920–929. doi:10.1016/j.cemconres.2008.02.008.

[117] P. Domone, The Slump Flow Test for High-Workability Concrete, Cem. Concr. Res. 28 (1998) 177–182. doi:10.1016/S0008-8846(97)00224-X.

[118] H. Uchikawa, D. Sawaki, S. Hanehara, Influence of kind and added timing of organic admixture on the composition, structure and property of fresh cement paste, Cem. Concr. Res. (1995). doi:10.1016/0008-8846(95)00021-6.

[119] A. Maksoud, A. Ashour, Calculating air content in cement mortars, Batim. Int. Build. Res. Pract. 4 (1976) 44. doi:10.1080/09613217608550448.

[120] ASTM C1437-15, Standard Test Method for Flow of Hydraulic Cement Mortar, in: Annu. B. ASTM Stand., 2015. doi:10.1520/C1437-15.

[121] Z.B. Bundur, A. Amiri, Y.C. Ersan, N. Boon, N. De Belie, Impact of air entraining admixtures on biogenic calcium carbonate precipitation and bacterial viability, Cem. Concr. Res. 98 (2017) 44–49. doi:10.1016/j.cemconres.2017.04.005.

[122] G.G. Kahng, S.H. Lim, H.D. Yun, 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). doi:10.1007/BF02931956.

[123] D. Sarda, H.S. Choonia, D.D. Sarode, S.S. Lele, Biocalcification by Bacillus pasteurii urease: A novel application, J. Ind. Microbiol. Biotechnol. (2009). doi:10.1007/s10295-009-0581-4.

[124] B.Z. BASARAN, A. Amiri, Cement-based compositions with improved rheological properties and methods for production thereof, PCT/TR2016/050472, 2016.

[125] A. Amiri, Use of biomineralization in self-healing cement-based materials, Özyeğin University, 2017.

[126] B.Z. BASARAN, Biomineralization in cement based materials : inoculation of vegetative cells, University of Texas – Austin, 2013. doi:2152/21141

[127] J. Zhang, E.A. Weissinger, S. Peethamparan, G.W. Scherer, Early hydration and setting of oil well cement, Cem. Concr. Res. (2010).

doi:10.1016/j.cemconres.2010.03.014.

[128] R. Andalib, M.Z. Abd Majid, M.W. Hussin, M. Ponraj, A. Keyvanfar, J. Mirza, H.S. Lee, Optimum concentration of Bacillus megaterium for strengthening structural concrete, Constr. Build. Mater. (2016). doi:10.1016/j.conbuildmat.2016.04.142.

[129] P.S. Vary, R. Biedendieck, T. Fuerch, F. Meinhardt, M. Rohde, W.D. Deckwer, D. Jahn, Bacillus megaterium-from simple soil bacterium to industrial protein production host, Appl. Microbiol. Biotechnol. (2007). doi:10.1007/s00253-007-1089-3.

[130] K.H. Khayat, Z. Guizani, Use of viscosity-modifying admixture to enhance stability of fluid concrete, ACI Mater. J. (1997). doi:10.14359/317.

[131] S.J. Park, S.H. Park, S.Y. Ghim, The effects of paenibacillus polymyxa E681 on antifungal and crack remediation of cement paste, Curr. Microbiol. 69 (2014) 412–416. doi:10.1007/s00284-014-0604-x.

[132] B. Mahanty, S. Kim, C.G. Kim, Biokinetic modeling of ureolysis in Sporosarcina pasteurii and its integration into a numerical chemodynamic biocalcification model, Chem. Geol. 383 (2014) 13–25. doi:10.1016/j.chemgeo.2014.05.034.

[133] B. Esmaeilkhanian, D. Feys, K.H. Khayat, A. Yahia, New test method to evaluate dynamic stability of self-consolidating concrete, ACI Mater. J. 111 (2014) 299–307. doi:10.14359/51686573.

[134] V.S. Ramachandran, Concrete Admixtures Handbook – Properties, Science, and Technology, 1995. doi:10.1016/B978-081551373-5.50011-8.

[135] F. Collins, J.G. Sanjayan, Effects of ultra-fine materials on workability and strength of concrete containing alkali-activated slag as the binder, Cem. Concr. Res. (1999). doi:10.1016/S0008-8846(98)00237-3.

[136] A. Hallal, E.H. Kadri, K. Ezziane, A. Kadri, H. Khelafi, Combined effect of mineral admixtures with superplasticizers on the fluidity of the blended cement paste, Constr. Build. Mater. (2010). doi:10.1016/j.conbuildmat.2010.01.015.

[137] E.J. Hayhurst, L. Kailas, J.K. Hobbs, S.J. Foster, Cell wall peptidoglycan architecture in Bacillus subtilis, Proc. Natl. Acad. Sci. (2008). doi:10.1073/pnas.0804138105.

[138] M. Saric-Coric, K.H. Khayat, 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). doi:10.1016/S0008-8846(03)00214-X.

[139] F. Oosawa, S. Asakura, Surface tension of high-polymer solutions, J. Chem. Phys. (1954). doi:10.1063/1.1740346.

[140] K. Kovler, N. Roussel, Properties of fresh and hardened concrete, Cem. Concr. Res. 41 (2011) 775–792. doi:10.1016/j.cemconres.2011.03.009.