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BIO-BASED RHEOLOGY MODIFYING AGENTS

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Abstract
Advances in construction technology increased the importance of sustainability and reinforce the use of biological admixtures. Using bio-based admixtures brings new horizons to the construction material science by improving concrete properties through a more environmentally friendly and cost-efficient approach. Generally, bio-based additives are used as viscosity modifyin agents (VMAs) in concrete mixes. Traditional bio-based VMAs are water-soluble bipolymers that can be produced from polysaccharide-based biopolymers obtained from cellulose, starch or bacterial fermentation. Through the literature, nopal mucilage, brown algae and bacterial cell walls were proposed as alternatives to these bio-based admixtures. However, these alternatives also require extra processing which results again with a higher unit cost compared to admixtures that could be directly obtained from nature, like the direct addition of cells.

The aim of this study is to improve the rheology of cement paste by incorporating *Peanibacillus polymxa* (*P.polymxa*) cells and biofilm collected from *Bacillus amyloliquefaciens (EPI 60)* as viscosity modifying agents (VMAs). To achieve this goal, the bacterial cells and biofilms were directly incorporated into the mix water and influence of cells on viscosity and yield strength was evaluated by rheological tests. The impact of fly ash and superplasticizer on performance of cells were also investigated. Our results showed that the apparent viscosity and yield stress of the cement-paste mix were increased with the addition of the bio-based additives. Biofilm obtained from *EPI 60* cells were found to be more efficient in terms of improving rheological parameters. Moreover, both biological VMAs was found to be compatible with the use of fly ash and superplasticizers.

Keywords:
Concrete; Self-leveling concrete; Water reducers; Viscosity modifying agents

1 INTRODUCTION

Recent developments in the field of materials, lead researchers focus on 3D printing applications in construction. The innovative additive manufacturing processes mostly rely on high-performance cement-based materials. Herein, the parameters define the cement-based materials are extrudability, flowability, buildability, flowability, open time and hardened performance [Soltan and Li 2018].

Generally, HPC requires the use of admixtures such as superplasticizers and viscosity modifying agents (VMAs) to provide high strength without sacrificing from workability. While additives have a significant impact on most of the concrete's properties, the majority of them are chemicals and their production processes have a consequential influence on the environment. The most commonly used VMA in construction is generally produced from polymers and bio-based polysaccharides (cellulose, chitosan etc.). These polymers are highly effective to reduce the bleeding since the long chain molecules stick to the water molecules, decrease their relative motion and forms a gel; increasing the yield stress a plastic viscosity [Feys, Verhoeven, and De Schutter 2008]. In presence of most VMAs, shear-thinning behavior is preserved, which means the viscosity of the material still decreases with increasing shear rate [K.H Khayat and Yahia 1998].

Throughout the literature, polysaccharide and cellulose-containing biological additives and bacterial fermentation products frequently used are welan gum [Jiménez et al. 1991; Sonebi 2006; Plank 2004], diutan gum [Schmidt et al. 2013] xanthan gum [Plank 2004]. These natural VMAs have long polysaccharides backbones which could trigger 3 different action mechanisms [K. H. Khayat and Yahia 1997]: (1)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; (2)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; (3) 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 a shear-thinning behavior. Water adsorption on polysaccharides molecules can result with a higher entanglement after critical concentration and cause a significant increase in viscosity. The shear-thinning behavior that can be observed at a high shear rate can be attributed to the rearrangement of the molecules lead to a release of water back to the mix [Barnes 1995].

According to Plank [Plank 2004], most of the bio-derived polysaccharide VMAs are not economically feasible due to the labor-intensive process such as extraction of polysaccharides. 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, which resembles the long molecular chains of VMAs [Schmidt et al. 2013] .

Sonobi [Sonebi 2006] 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 [Sonebi 2006].

Besides welan and diutan gum, Kahng et al. [Kahng et al. 2001] extracted EPS from *Paenibacillus sp.* and incorporated in cement paste as a VMA. With the addition of cells by 0.02% of cement weight, the segregation resistance of mortar was improved significantly. At this dosage, there was not any adverse effect neither on initial set nor on compressive strength. However, with increasing EPS content there was also an increase in entrapped air, which led to a decrease in compressive strength.

Leon Martinez et al. [León-Martínez et al. 2014] conducted a comparative study on the use of nopal mucilage and marine brown algae extract as VMA in mortar. Both of these additives improved the rheology of cement-paste and resulted with a shear-thinning response with increasing shear rate. However, use of brown algae extract increased the viscosity more than mucilage at lower shear rates. In contrast, mucilage performed better compared to algae extract in terms of increasing the viscosity at higher shear rates. The authors preliminary SCC mixes revealed that both of these bio-based additives induced similar workability indicating that they could both serve as VMAs. However, incorporation of algae extract increased the volume of entrapped air due to its protein content, thus mucilage was found to be a better option to improve the overall quality of the material [León-Martínez et al. 2014].

However, these bio-based additives listed above, EPS, welan gum and diutan gum, required labor-intensive fermentation process that can increase the production rate and cost, which limits the use of these materials in the field [Ivanov, Chu, and Stabnikov 2015; Plank 2004]. Another possible bio-based VMA was bacterial cell walls. Pei et al. [Pei, Liu, and Wang 2015] 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. Addition of cell walls by 0.34% of cement weight increased the apparent viscosity approximately by 20% compared to control samples without any cell walls [Pei, Liu, and Wang 2015]. However, extraction of cell walls again requires a labor-intensive and special processing.

To-date most of the approaches using bio-derived VMA were found to be successful in enhancing the viscosity of cement-based materials. However, it might not be necessary to generate an extraction of the fermentation products to obtain similar improvement in rheology. A possible way to provide this is to directly use the vegetative bacterial cells in the mix. These bacterial cells could be used as VMAs not only due to their complicated cell wall structure but also they can influence rheology of suspensions as being microswimmers [Saintillan 2012]. Based on the mathematical models developed in the literature [Rafaï, Jibuti, and Peyla 2010; Saintillan 2010], in microbial suspensions, the flagella located at the back of the microorganism exerts a force at their tail and were called pushers. Pushers, such as bacteria, are also known as ‘tensile swimmers’ and they decrease the viscosity of the solution. In contrast, the pullers exert a thrust force at their head and called ‘contractile swimmers’ by increasing the viscosity of the solution [Rafaï, Jibuti, and Peyla 2010]. Yet, it is still not clear if the microorganisms could actually influence the rheology of a complex fluid like cement paste. These cells can act as a VMA not only the peptidoglycans and polysaccharides in cell structure, but they can also influence the rheology due to their motility.

This paper summarizes the results of a novel study where gram-positive *Peanibacillus* polymxa cells and biofilm collected from *Bacillus amyloliquefaciens (EPI60)* could be used as VMAs in cement-based materials. To achieve this goal the bacterial cells and biofilm were suspended in mixing water without any extra intervention, thus the system is much more simplified compared to the use of similar bio-based products. The rheology of cement paste samples was evaluated at different dosages of cells. The influence superplasticizers and fly ash on the performance of biological VMA were also investigated.

2 MATERIALS AND METHODS

2.1 Microorganism growth

P.polymxa is Gram-positive bacteria, which have thick cell walls containing peptidoglycans and polysaccharides [Yegorenkova et al. 2011]. In addition, *P. polymxa* cells can also produce extracellular polysaccharides (EPS) during growth, which might also contribute to the VMA mechanism.

P. polymyxa cells were cultured in a nutrient medium including yeast extract (5 g), NaCl (5 g), and peptone (10 g) to 1-liter distilled water (DI) water and the pH was adjusted to 7. First, the nutrient medium was sterilized at 121 ° C for 45 minutes. Then, cells were added in 300 mL of sterilized liquid nutrient medium and incubated aerobically with shaking conditions (210 rpm) at 30°C. Then, the cells were collected from the culture by centrifuging at 6300g for 15 min. Then the cells were washed twice by PBS (Phosphate buffered solution) and stored at 4°C until testing.

2.2 Biofilm formation by *EPI 60*

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

2.3 Preparation of cement paste samples

Cement paste samples were prepared by Ordinary Portland Cement (OPC) CEM 42.5 R accordingly to the norm EN 197-1 and had Blaine fineness value of 3954 cm^2/g. The mixtures were prepared at a w/c of 0.40. To determine the effects of superplasticizers and fly ash on the performance of the biological VMA, a polycarboxylate superplasticizer (by 0.1 kg/kg cement) and F-type fly ash (by 20% of the cement weight) was added to samples. To investigate the effect of microorganisms on the rheology of the cement paste, the abovementioned collected cells (*see* Section 2.1) were directly added to the mixing water. The number of cells was adjusted in terms of percent weight of cement, such as 0.05%, 0.10%, and 0.50% of the cement weight. The cement paste samples were prepared according to ASTM C305-14 Standard Practice for Mechanical Mixing of Hydraulic Cement Pastes and Mortars. The cells were added to mixing water prior to mixing, homogenized with hand-mixing for 30s, and then mixed with cement. In case of superplasticizer addition, the cells were added to initial $2/3^{rd}$ of the mix water and the superplasticizer was added to the $1/3^{rd}$ of the mix water. The last $1/3^{rd}$ portion of the mix water including superplasticizer was added during the last 60s of the mixing. The mixing procedure for cement paste samples was completed within the 4 minutes period specified by the ASTM C305-14 standard.

2.4 Rheological measurements

Rheological measurements were made at room temperature (23°C) with ANTON PAAR RheolabQC rotational rheometer (Anton Paar, Graz, Austria). To ensure the homogeneity of the mixtures, the cement paste samples were first mixed for 60 seconds at a shear rate of at $100s^{-1}$. Then, a pre-shear stage where the shear rate was kept constant at $100s^{-1}$ for another 60s. Following the pre-shear stage, the analysis was conducted by decreasing 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 (1). Where τ_0 is the yield stress (Pa), µ is the plastic viscosity (Pa.s), and $\dot{\gamma}$ is the shear rate (s^{-1}).

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

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 was used to analyze shear-thickening cement paste samples (2), which could also explain the shear thinning behavior as well [Feys, Verhoeven, and De Schutter 2008].

$$\tau = \tau + \mu\gamma \dot{+} c\dot{\gamma}^{n} \qquad (2)$$

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 a 2^{nd}-degree parameter ($Pa.s^2$) [Feys, Verhoeven, and De Schutter 2008]. When c/ µ > 0 the materials were classified as shear-thickening and when c/ µ < 0, the materials exhibited shear thinning behavior.

2.5 Mini-slump test

The mini-slump test was conducted based on the measurement of the spread of cement paste placed into a cone-shaped mold on smooth plexiglass. For this approach, a specially manufactured stainless-steel cone with a height of 57.2 mm, an upper diameter of 19 mm and a lower diameter of 38.1 mm was used [Kamal H. Khayat 1998] (Figure 1). The cone was placed on the center of the plexiglass, and, immediately after mixing, the sample was filled into the cone. 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.

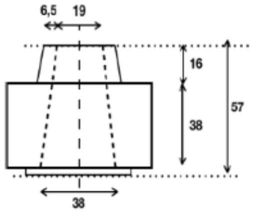


Fig. 1: Schematic of the mini-slump cone test.

3 RESULTS AND DISCUSSION

3.1 Effects of microorganisms on rheology of cement paste

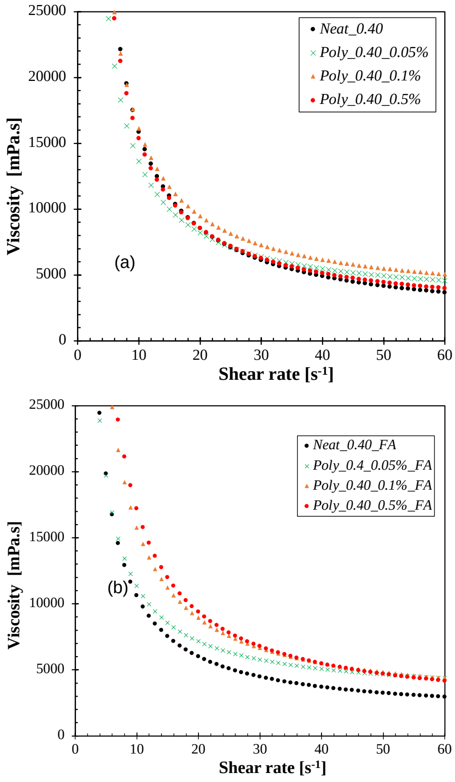
An extensive study was undertaken to use *P. polymxa* cells and biofilms as a VMA and evaluate its influence on the rheological properties of cement paste. The rheological behavior was evaluated based on Bingham model or modified Bingham model as it was stated in Section 2.4. Yield stress values were calculated from the intersection of the regression line and the viscosity was defined by the slope of the line. Figure 2 and 3 show the viscosity curves for cement paste samples (w/c:0.40) including different dosages of bacterial cells and biofilm content, respectively.

As seen in Figure 2 (a) and Figure 3 (a) all cement paste samples exhibited a shear-thinning response regardless of the type of bio-based VMA used. Incorporation of *P. polymxa* cells to the mortar mix slightly increased the viscosity of samples compared to the control neat paste with a w/c of 0.40. Previously, addition *P. polymxa* cells induced a higher impact on viscosity in cement paste samples with a w/c of 0.36 [Azima et al. 2018]. This influence of cells was attributed to the incorporation of polysaccharides and peptidoglycans in the cells structure resulting in an increasing intertwining of chains and leading to higher water retention particularly at low shear-rates [Lachemi et al. 2004]. However, with increasing shear rate the chains (or bonds) would break and releasing the water to the mix, leading to a more pronounced decrease in viscosity compared to low shear rates. Thus, resulting in a higher shear-thinning response. However, with increasing w/c ratio the efficiency of *P. polymxa* cells on viscosity.

In contrast, incorporation of EPI 60 biofilm to cement paste at a w/c of 0.40 significantly increased the viscosity compared to the control neat paste. Biofilms are different than vegetative cells. Under stress conditions, some species can form colonies on surfaces and form a thin layer. This layer is embedded in a slimy extracellular matrix composed of extracellular polymeric substances (EPS). This might be directly related to increasing molecular weight of polysaccharides and peptidoglycans in the mix resulting in an increasing intertwining of chains and leading to higher water retention at lower shear-rates [Lachemi et al. 2004].

But, interestingly the increase in viscosity compared to neat paste was slightly less pronounced with increasing biofilm content from 0.1% to 0.5%. Even though the actual mechanism is not known yet, it could be due to the slimy matrix of the biofilms. Biofilms used in the mix were more fluid like additives while cells were rather thick gel-like structures. Thus, by significantly increasing the amount of biofilm (from 0.1% to 0.5%) might also increase the liquid content of the mix. Further studies have to be done to evaluate the characterization of biofilms.

Based on abovementioned data, particularly incorporation biofilm obtained from *EPI 60* in mixing water were efficient in terms of increasing viscosity compared to neat cement paste. However, VMAs are generally used along with fly ash and superplasticizers in typical self-leveling mixes. Thus, it is important to define the compatibility of bacterial cells with the use of fly ash and superplasticizers. Figure 1(b) and Figure 2(b) represent viscosity curves for fly ash amended cement paste samples including different bacteria and biofilm dosages.



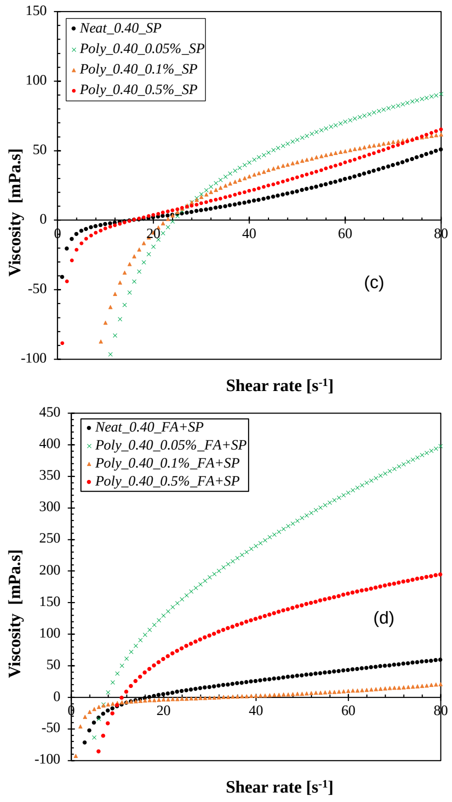
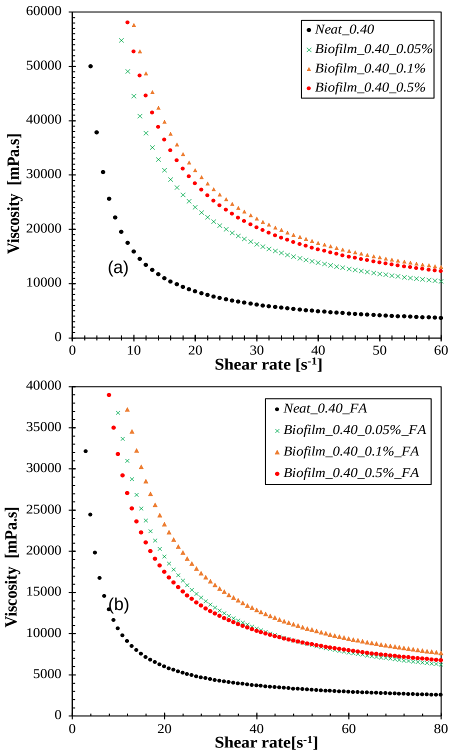


Fig. 2: Viscosity curves for cement paste samples with and without P. polymxa cells at a w/c of 0.40. (a) Samples without any additives (b) with 20% fly ash (FA) replacement (c) with 1% superplasticizer (SP) addition (d) use of both FA and SP. Cell dosages were used as 0.05%, 0.1% and 0.5% per weight of cement.



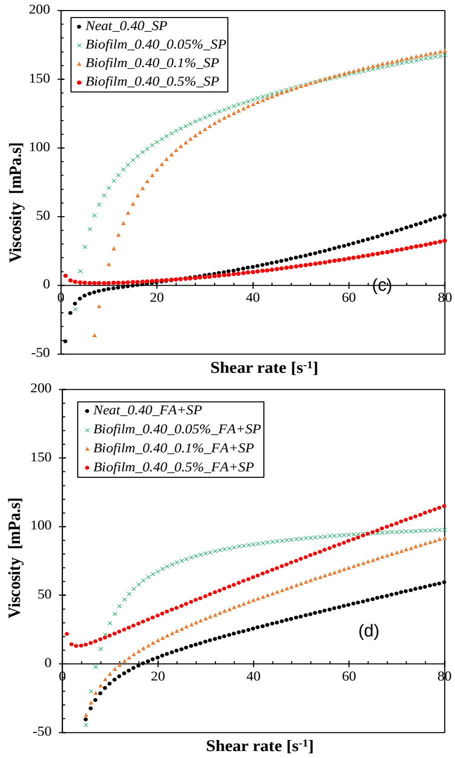


Fig. 3: Viscosity curves for cement paste samples with and without EPI 60 biofilm cells at a w/c of 0.40. (a) Samples without any additives (b) with 20% fly ash (FA) replacement (c) with 1% superplasticizer (SP) addition (d) use of both FA and SP. Cell dosages were used as 0.05%, 0.1% and 0.5% per weight of cement.

Interestingly, the influence of *P. polymxa* cells on increasing viscosity was more pronounced in fly ash amended samples compared their counterpart samples without any fly ash. This was in line with the fact that *P. polymxa* was more efficient in increasing viscosity in low w/c mixes with high solid particle concentration. This might bring up the discussion of whether *P. polymxa* cells influencing the water retention or actually interfering by the particles. Biofilms were also found to be compatible with fly ash. Their interaction as a VMA was similar to those without any fly ash. This might indicate that biofilms most likely act on water phase and increase the water retention rather than acting on particles. Further analysis should be done to understand the action mechanism of these two different additives.

Incorporation of superplasticizers completely changed the rheological behavior of cement paste samples regardless of the dosage of microorganisms and w/c used (*see* Figure 2(c) and 3(c)). The use of a very strong polycarboxylate superplasticizer resulted with a shear-thickening behavior rather than shear-thinning behavior previously observed in rest of the samples. In this case, the shear-thickening cement paste behavior mix would not be simply explained by Bingham equation (see Section 2.4). Instead, a modified Bingham equation was used to analyze shear-thickening cement paste samples [Feys, Verhoeven, and De Schutter 2008]. Moreover, incorporation of a very strong superplasticizer yielded negative viscosity values at very slow shear rates. High flowability of the mix resulted with inconsistencies in rheological parameters such that negative viscosity values were recorded at low shear rates. This might indicate possible segregation in the mix and invalid test results. Use of EPI 60 biofilm (at dosages of 0.05% and 0.1%) significantly increased the apparent viscosity at low shear rates, which might indicate that they could also increase the segregation resistance of the mix. To validate the influence of superplasticizer dosage on rheological behavior, a new set of samples were prepared by 0.3% superplasticizers by weight of cement (see Figure 4). As expected, decreasing superplasticizer content reduced the possible segregation at low shear rates, thus the negative viscosity values were also eliminated. Moreover, the material showed a shear-thinning response to the increasing shear rates. This might suggest that the bio-based VMAs were compatible with superplasticizers up to a certain dosage.

Figure 5 summarizes the yield stress values for cement paste samples including different bacteria and biofilm dosages at w/c of 0.40. Even though, yield stress and viscosity are independent variables, the change in yield stress was found to be related to the change in viscosity Such that, addition of cells did not significantly affect neither the yield stress nor the viscosity of mixes without any additives (*comparing* Neat_0.40 sample with Poly_0.40_0.05% in Figure 5 (a)). However, incorporation of biofilms resulted with an increase in yield stress of mixes without any additives regardless of the dosage (Figure 5 (b)). This behavior was in line with the change in viscosity and the highest change was observed at a dosage of 0.1%. Thus, the behavior of biofilms functions as a VMA; they could increase both viscosity and yield stress. As expected, the cells were more efficient in terms of increasing yield stress in fly ash amended cement paste compared to their counterpart samples without any fly ash.

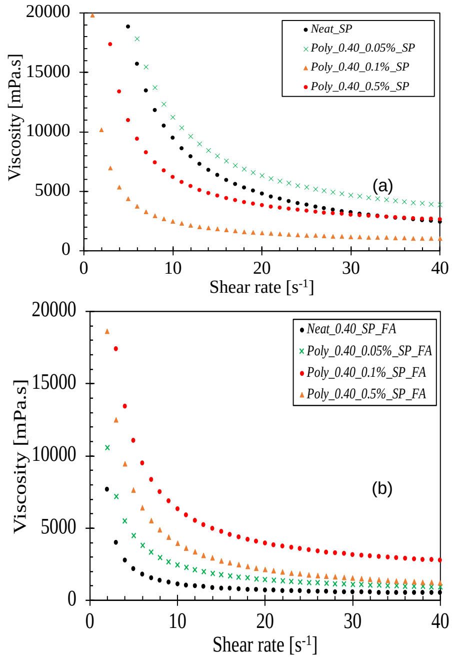


Fig. 4: Viscosity curves for cement paste samples with and without P. polymxa cells at a w/c of 0.40. (a) samples with 0.3 % superplasticizer (SP) (b) with 20% fly ash (FA) replacement and 0.3 % superplasticizer. Cell dosages were used as 0.05%, 0.1% and 0.5% per weight of cement.

However, use of superplasticizers to the mix significantly reduced the yield stress of the mix, where the biofilms were also found to be less effective in increasing the yield stress in highly flowable mixes including superplasticizers. With the addition of fly ash and superplasticizers, *P. polymxa* cells slightly increased the yield stress in line with their influence on viscosity.

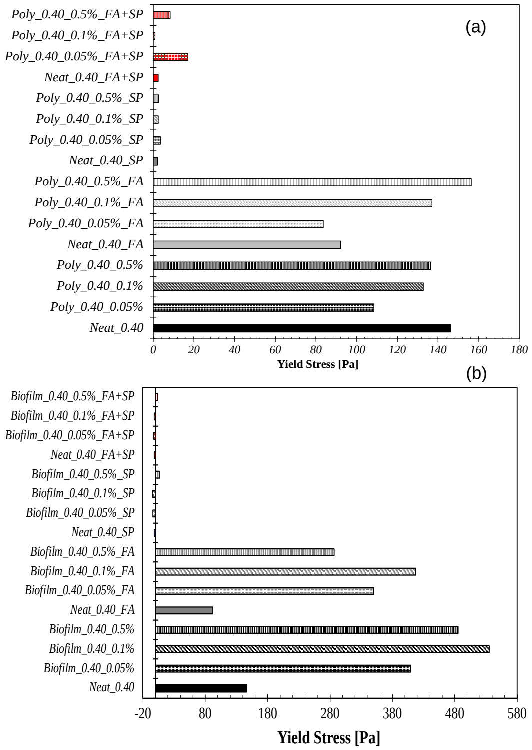


Fig. 5: Yield stress results for cement paste samples with and without (a) P. polymxa cells (b) EPI 60 biofilm at a w/c of 0.40. Cell dosages were used as 0.05%, 0.1% and 0.5% per weight of cement.).

Besides the rheological tests, workability and robustness of the mixes were evaluated by mini-slump test. Figure 6 summarizes the average mini-slump diameters for all cement paste samples. Throughout the literature mini-slump test was correlated with the yield stress of the material. With the incorporation of a VMA, it was expected to observe a decrease in mini-slump diameter along with an increase in yield stress. Incorporation of *P. polymxa* cells decreased the mini-slump diameters particularly in use with superplasticizers. The difference of mini-slump test and rheological analysis could be attributed to a difference in applied shear rates. The mini-slump cone test was employed with a significantly lower shear rate than that was imposed during the rheological analysis [K.H Khayat and Yahia 1998]. Considering the pseudo-plastic behavior of cement paste, the material response to these two different shear modes and rates was not the same. As expected, the addition of biofilms decreased the mini-slump value, which was in line with their influence on viscosity and yield stress.

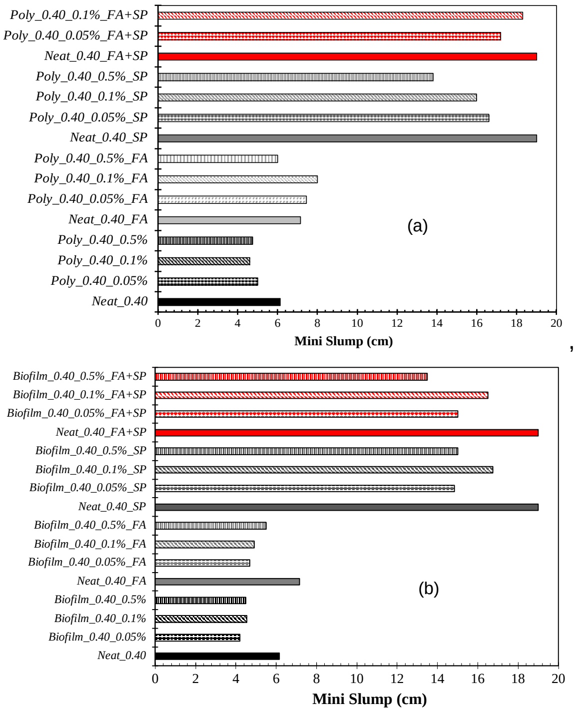


Fig. 6: Mini-slump results for cement paste samples with and without (a) cells (b) EPI 60 biofilm at a w/c of 0.40. Cell dosages were used as 0.05%, 0.1% and 0.5% per weight of cement.).

4 CONCLUSIONS

This study represented the results of an extensive study undertaken to evaluate the possible use of vegetative S. pasteurii cells as a VMA to improve the rheology of cement paste. The flow behavior of cement paste samples including *P. polymxa* cells and biofilm obtained from EPI 60 *B. amyloliquefaciens* 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 were investigated. In general EPI 60 biofilm was found to be much more effective in terms of increasing the viscosity and yield stress compared to *P. polymxa* cells. This was attributed to increasing EPS content leading to an increase in polysaccharide molecular weight in the mix resulting which results with a higher intertwining of chains higher water retention at lower shear-rates. Moreover, the use of biofilms reduced the segregation of mixes including superplasticizers at low shear rates. The optimum dosage for EPI 60 biofilm was 0.1% and above this value, the additive lost its efficiency in terms of increasing rheological parameters. Both cells and biofilm were found to be compatible with both fly ash and superplasticizers.

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