Materiales de Construcción
Vol. 72, Issue 348, October-December 2022, e296
ISSN-L: 0465-2746
https://doi.org/10.3989/mc.2022.02422

Suitable yeast extract concentration for the production of self-healing mortar with expanded clay as bacterial carrier

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Received 16 March 2022
Accepted 27 June 2022
Available on line 14 October 2022

ABSTRACT: In microbial induced calcium carbonate precipitation (MICP) system, yeast extract (YE) is needed for spores germination. The aim of this research is to evaluate the minimum amount of YE in mortar that allows spores of *Bacillus sphaericus* to germinate with limited negative effect on mortar properties. Two YE concentrations of 2 and 5 g/l were tested and compared to a reference without YE. To protect the bacteria in the mortar matrix, spores or cells were encapsulated into porous expanded clay. The ureolytic activity of bacteria with YE variation, the mechanical properties and the healing ability of mortar were assessed. The results show that a YE concentration of 2 g/l provided acceptable mortar properties, while it was sufficient for spores to germinate and provide a satisfactory healing ability to resulting mortar. When vegetative cells are used as a healing agent, it is best to omit yeast extract from the mortar mixture.

KEY WORDS: MICP; Yeast extract concentration; Self-healing mortar; Mechanical properties; *Bacillus sphaericus;* Expanded clay.

Citation/Citar como: Risdanareni, P.; Ma, L.; Wang, J.; De Belie, N. (2022) Suitable yeast extract concentration for the production of self-healing mortar with expanded clay as bacterial carrier. Mater. Construcc. 72 [348], e296. https://doi.org/10.3989/mc.2022.02422.

RESUMEN: *Concentración adecuada de extracto de levadura para la producción de mortero autorreparable con arcilla expandida como portador de bacterias.* En el sistema de precipitación de carbonato de calcio inducido por microbios (MICP), el extracto de levadura (YE) es necesario para la germinación de las esporas. El objetivo de esta investigación es evaluar la cantidad mínima de YE en el mortero que permite que las esporas de *Bacillus sphaericus* germinen con un efecto negativo limitado en las propiedades del mortero. Se ensayaron dos concentraciones de YE de 2 y 5 g/l y se compararon con una referencia sin YE. Para proteger las bacterias en la matriz del mortero, las esporas o las células se encapsularon en arcilla expandida porosa. Se evaluó la actividad ureolítica de las bacterias con la variación de YE, las propiedades mecánicas y la capacidad de reparación del mortero. Los resultados muestran que una concentración de YE de 2 g/l proporcionó propiedades aceptables del mortero, mientras que fue suficiente para que las esporas germinaran y proporcionaran una capacidad de reparación satisfactoria al mortero resultante. Cuando se utilizan células vegetativas como agente de reparación, es mejor omitir el extracto de levadura en la mezcla de mortero.

PALABRAS CLAVE: MICP; Concentración de extracto de levadura; Mortero autorreparable; Propiedades mecánicas; Bacillus sphaericus; Arcilla expandida.

1. INTRODUCTION

Microbially induced calcium carbonate precipitation (MICP) has been widely studied in geological and structural engineering to remediate cracks and degradation of natural stone or concrete (1–4). In this approach, various bacterial strains such as *Bacillus sphaericus* (2, 5–7), *Bacillus alkalinitrilicus* (8, 9), *Bacillus cereus* (10)*, Sporosarcina pasteurii* (11, 12), *Bacillus subtillis* (13) *Diaphorobacter nitroreducens* and *Pseudomonas aeruginosa* (14) have been used as bacterial agent.

In MICP processes that use the urea hydrolysis metabolic pathway, agents such as nutrients, urea, and calcium source are needed to complete the reaction. A nutrient obtained from yeast extract is needed to induce germination of spores and keep the viability of bacteria. At the same time, urea is needed to complete the metabolic pathway (15). With the help of the bacterial urease enzyme, the urea can be decomposed to ammonium and carbonate as described in Equation [1] and [2]:

$$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-} \qquad [1]$$

With sufficient amount of calcium ions in the solution, the carbonate can react and form calcium carbonate according to Equation [2]:

$$CO_3^{2-} + Ca^{2+} \leftrightarrow CaCO_3 \qquad [2]$$

Several studies on screening calcium sources reported that different calcium sources mainly affect the morphology of the healing products, and different bacteria strains could induce different reactions (2, 15, 16). Thus in this research, calcium nitrate was chosen as a calcium source as it has been reported to deliver a sufficient amount of calcium carbonate precipitation when *Bacillus sphaericus* is employed as a healing agent (4). Furthermore, it would not provoke a risk of rebar corrosion in reinforced concrete structures, as would be the case if calcium chloride were used.

However, bioagents, especially yeast, could negatively affect the properties of the resulting mortar (17). The presence of yeast extract tends to delay the hydration process, and due to its autolysis, hydrogen gas is released in contact with water that later on could induce the formation of pores in concrete (18). With the presence of extra pores in mortar, mortar's mechanical properties could decrease to an unacceptable level. Vandervoort et al. also found a 49% strength decrease relative to the reference sample when yeast extract with the concentration of 5 g/l mortar was added into a mortar mixture (19).

Regarding the function of yeast as a germination agent for spores, in a previous study by Wang et al. that monitored the outgrowth of *B. sphaericus* spores, the minimum yeast extract concentration that allowed spores to significantly grow was 5 g/l (5). However, through the Nessler test, it was found that the urea decomposition rates of spores cultivated in a medium containing 2 g/l or 5 g/l yeast extract were similar (5). From the same literature, by omitting the yeast from the culture medium, spores still can slowly decompose the urea. For comparison, when spores were cultivated in yeast extract solution 2 g/l and 5 g/l, the amount of urea decomposed was 20 g/l, while it was less than 5 g/l urea that could be decomposed in no-yeast extract samples in a time span of 72 hours.

Considering previous findings in utilizing yeast extract, when the presence of yeast is compulsory in the mortar (this is when spores are used as healing agent), yeast with a concentration of 2 g/l or 5 g/l would be enough for spores to germinate (5). However, the properties of bio-mortar containing those two yeast concentrations still need to be further investigated. At the same time, the feasibility of omitting yeast from the matrix should also be investigated for self-healing mortar that employs vegetative cells as a healing agent.

To summarize, this part of the study aims to find suitable yeast concentrations that have a less negative effect on the properties of lightweight mortar prepared for bacteria-based self-healing mortar but sufficient for spores to germinate into cells and heal the cracks in mortar. The possibility of omitting yeast from the mortar matrix was also studied. The setting time, workability, and heat evolution of fresh mortar were studied. In the hardened state, the compressive strength, bulk density, and porosity determined by mercury intrusion porosimetry (MIP) were investigated. The viability of spores and vegetative cells of *Bacillus sphaericus* at a specific concentration of yeast extract medium was monitored. In the end, the effect of minimizing the usage of yeast extract in the mixture on the healing performance of mortar containing encapsulated vegetative cells or spores was observed.

2. MATERIALS AND METHODS

2.1 Materials

Cement type I 52.5 from Holcim was used as a binder. An Expanded Clay Lightweight Aggregate (EC LWA) with a size of 2-4 mm was used as fine aggregate replacement. This fraction was obtained by sieving the 0/4 fraction of commercial EC LWA from Argex Nv. River sand with a size of 0-2 mm was used as fine aggregate. This fraction was obtained by sieving the 0/4 river sand. Based on the particle size distribution of river sand 0/4, the volume of sand with particle size larger than 2 mm was 16% (Figure 1). Thus, in this research, the whole volume of riv-

er sand with the size of 2-4 mm was replaced with EC LWA. Extra water equal to its water absorption value was added into EC LWA, in order to bring the aggregates in saturated surface dry (SSD) condition. Pre-conditioning of EC LWA before mixing was performed to avoid that the EC LWA would absorb water during mixing. The properties of EC LWA and river sand are presented in Table 1, while the mix design compositions to investigate the properties of fresh and hardened mortar is displayed in Table 2.

Yeast extract, urea, and calcium nitrate tetrahydrate were utilized as bio-agents in this research. A powder form of yeast extract with a purity of 99% (Carl Roth Belgium) was used as a bacterial nutrient, while calcium nitrate tetrahydrate with a purity of 99% (Carl Roth Belgium) was used as a calcium source needed for bacteria-based self-healing. Urea with a purity of 98 % was added as a substrate for bacteria's metabolism into the mixture as well. The amount of urea and calcium nitrate was kept constant while the amount of yeast was varied and set at 0, 2 g/l, and 5 g/l (5, 19) (Table 2). To keep the water/cement ratio of 0.5, the mixing water of samples with calcium nitrate was reduced to accommodate the excess water released from calcium nitrate tetrahydrate (4 H_2O molecules). The chosen concentration of urea and calcium nitrate was reported to deliver good healing capacity in self-healing mortar (20).

The vegetative cell pellets were obtained by cultivating *B. sphaericus strain* LMG 22257 (Belgian Co-ordinated Collections of Micro-organisms, Ghent) in a sterile yeast extract and urea medium with a concentration of 20 g/l for 24 hours on a shaking table incubator (120 rpm, 28°C). The cells were harvested by centrifuging the cells culture (15050 g for 7 minutes). The vegetative cells pellets were then re-suspended in a sterile yeast extract solution with a concentration of 5 g/l for further use. The final concentration of vegetative cells suspension was $2x10^9$ cells/ml.

Spores of *Bacillus sphaericus* were obtained by transferring vegetative cell pellets into a sterile sporulation medium. The sporulation medium consisted of yeast extract (2 g/L), peptone (3 g/L), glucose (4 g/L), malt extract (3 g/L), K_2HPO_4 (1 g/L), $(NH_4)_2SO_4$ (4 g/L), $CaCl_2$ (0.1 g/L), $MgSO_4$ (0.8 g/L), $MnSO_4.H_2O$, (0.1 g/L), $FeSO_4.7H_2O$ (0.001 g/L), $ZnSO_4$ (0.01 g/L) and $CuSO_4.5H_2O$ (0.01 g/L). The culture was then incubated on the same shaking table as mentioned earlier (120 rpm, 28°C) for 7-14 days until at least 80% of the vegetative cells turned into spores. The spores were harvested by centrifuging (15050 g for 7 minutes). The spore pellets were resuspended into a sterile saline solution (NaCl 8.5 g/l). A pasteurization process (80°C for 20 minutes followed by 5 minutes in ice water) was then applied to kill the remaining vegetative cells in the spore suspension. Finally, spore suspension with a final concentration of $2x10^9$ cells/ml was obtained and was kept in the fridge with a temperature of 4°C until further use.

TABLE 1. Physical properties of EC LWA and river sand with the size of 2-4 mm.

Test	EC LWA	Sand 2-4 mm
Apparent particle density (AD) (g/cm^3)	1.25 ± 0.01	2.63 ± 0.04
Oven dried density (OD) (g/cm^3)	0.99 ± 0.01	2.48 ± 0.03
SSD particle density (SSD) (g/cm^3)	1.19 ± 0.01	2.54 ± 0.03
Water absorption 24 hours (WA_{24h}) (%)	20.85 ± 0.09	2.19 ± 0.14
Open Porosity (OP) (%)	21.14 ± 0.3	2.15±0.14

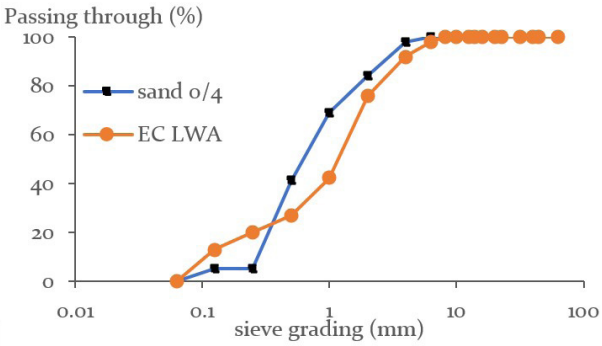

FIGURE 1. Particle size distribution of sand 0/4 and EC LWA 0/4.

TABLE 2. Mix design of mortars.

Type	Sand 0-2 (g)	LWA 2-4 (g)	Cement (g)	Entrained water (g)	Water (g)	Yeast extract (g)	Urea (g)	Calcium nitrate (g)
Ref	1251	82	450	17	225	0	0	0
NY0	1251	82	450	17	214	0	18	36
NY2	1251	82	450	17	214	1.53	18	36
NY5	1251	82	450	17	214	3.83	18	36

2.2 Experiment set up

The research was divided into 2 stages, as shown in Figure 2. In the first stage, a suitable yeast extract concentration that suitable for cells and spores were investigated. The bacteria activity, the fresh and hard properties of resulting mortar with designed YE concentration were investigated. After the suitable yeast extract concentrations that has limited negative effect to the resulting mortar was found, new samples were manufactured to investigate the healing ability of resulting mortar containing suitable amount of yeast extract. A list of tests in all stages including the number of samples and their dimensions is presented in Table 3.

2.3 Fresh properties

The setting time of paste was tested according to NBN EN 196-3. The paste containing cement, water, and bio-agents (yeast extract, urea, and calcium nitrate) was mixed and cast into a conical mould with a height of 400 mm, an upper diameter of 65 mm, and a bottom diameter of 75 mm. The paste was then tested with a Vicatronic E044N by Matest. The initial setting time was determined when the penetration depth of the needle from the top of the mold was recorded as 34 ± 3 mm, while the final setting time was determined when the needle could not penetrate into a paste, indicated by the penetration depth of 5 mm.

The heat evolution of the paste was monitored using an isothermal calorimeter (TA instrument, TAM Air) at the temperature of 20ºC. To keep all the materials at the same temperature, all the raw materials were kept in the curing chamber at 20 ± 2 °C one day before the test. The yeast extract, urea, and calcium nitrate were dissolved into the water required to obtain a water/cement ratio of 0.5.

The workability of fresh mortar was determined by a flow table test. This test was performed following the guidance from NBN EN 1015-3 (1999). A fresh mortar was poured gradually (2 layers) into a

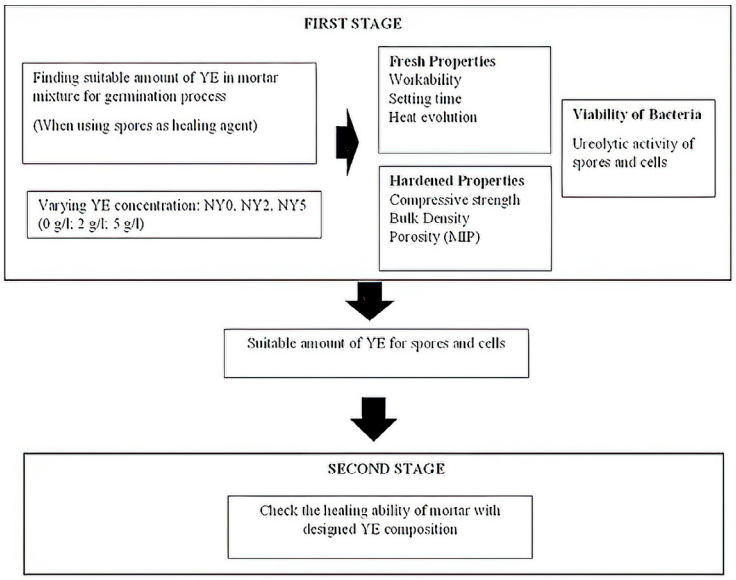

FIGURE 2. Experiment set up.

TABLE 3. List of tests and samples.

No	Test	Types of specimens	Dimension	Number of samples
1	Heat evolution	cement paste	-	1
2	Workability	fresh mortar	-	3
3	Setting time	fresh cement paste	cylinder	1
4	Compressive strength	hardened mortar	prism 40x40x160 mm³	3
5	Bulk density	hardened mortar	prism 40x40x160 mm³	3
6	Porosity	hardened mortar	pieces from mortar prism	1
7	Ureolytic activity	liquid medium + cells/spores	-	3
8	Crack closure observation	hardened mortar	prism 30x30x360 mm³	2

conical mold on the shaking table. The mixture was compacted by stroking each layer 10 times with a small stick. Finally, the mold was removed, and the table was jolted 10 times. The flow value was then determined by measuring the diameter of the spread-out mortar.

2.4 Hardened state properties

The mortar mixtures were cast into prismatic molds with the dimension of 40x40x160 mm³. Immediately after being cast, the mortar samples were covered with plastic film to avoid evaporation and cured in a curing chamber with a temperature of 20±2ºC and relative humidity of 95±5% for 24 hours. After curing for 24 hours, samples were demolded and covered with plastic film and cured in the same chamber until reaching the age of 28 days.

The bulk density and the compressive strength of 28 days old mortar were tested according to EN 196-1 standard. After the dimensions and the weight of samples were recorded, the mortar samples were tested in a machine for three-point bending (Walter and Bai) which has a loading speed of 2400±200 N/s. Half of the broken prisms from the bending test were then subjected to uniform load to determine their compressive strength.

The pore structure of mortar was studied by Mercury Intrusion Porosimetry (MIP) test. The 28 days old mortar was broken into pieces with a diameter of more or less 20 mm, not containing EC LWA. The hydration was stopped by immersing the samples into iso-propanol and shaking it on a shaking table with a speed of 70 rpm for 15 minutes. The procedure was repeated twice followed by drying the samples in a 40ºC oven for 24 hours. The samples were further dried in a vacuum chamber with a pressure of -0.8 bar for approximately 2 weeks until reaching constant mass.

2.5 Ureolytic activity of spores

The ureolytic activity of spores was monitored for 72 hours. Spores need to germinate into cells before they can decompose the urea in the medium. Thus, monitoring the ureolytic activity of free spores in the medium is a tool to see whether the spores germinate successfully. The medium used for spores germination monitoring was urea (20 g/l) mixed with yeast extract medium with concentrations of 2 and 5 g/l. A Nessler test was performed to determine the ureolytic activity of bacteria. One ml of spores was added into 100 ml medium for conducting the Nessler test. In each group, three sample series were made. The urease enzyme in the bacterial cells can decompose the urea into two ammonium molecules and one carbonate molecule. Thus, by measuring the total ammonium formed, the amount of urea decomposed could be calculated (Equation [3]):

$$U = TAN \times dilution \times M \frac{Urea\ (\frac{60g}{mol})}{2Nitrogen\ (2x14\frac{g}{mol})} \quad [3]$$

Where DU is decomposed urea (g/l), TAN (g/l) is total ammonium nitrogen recorded with a spectrometer at wavelength 425 nm. The dilution used in this research was 2.5. The relative molecular mass (Mr) of urea and two nitrogen molecules were 60g and 28g, respectively.

2.6 Crack closure observation

Samples for crack closure observation were prisms with dimensions of 30x30x360 mm³ with a 6 mm diameter reinforcing bar. The length of the reinforcement bar was 600 mm. Cracks were fabricated at the age of 28 days. The central rebar was clamped at the ends and the prisms were subjected to tensile load using an Instron universal testing machine with a load rate of 0.01 mm/s. The displacement of the crack was monitored after the specimen was in the plastic zone. The loading was stopped when the average displacement in each crack reached between 0.4-0.5 mm. Per sample, the average number of cracks obtained was between 5-9 cracks. Four series were made to observe the effect of utilizing the minimum amount of yeast extract in the mixture on the healing performance of the resulting mortar. The control sample contain no LWA and nutrients, reference sample contained EC LWA as fine aggregate replacement. When vegetative cells were used as a healing agent, yeast extract was omitted from the mixture (16 VY0). When spores were employed as a healing agent, yeast extract with the concentration of 2 g/l mortar was added to the mixture (16 SY2). The yeast extract concentration of 2 g/l mortar equal to 1.53 gram yeast extract / mortar batch. The mix design to produce two mortar prisms of 30x30x360 mm³ is displayed in Table 4. The control samples (without EC LWA) were produced in this series to see the effect of autogenous healing provided by EC LWA itself (without nutrient and bacteria).

The spores/cells or water was loaded into EC LWA by vacuum and pressure treatment. Under a vacuum condition (-0.85 bar) the healing agent was loaded into EC LWA. This vacuum pressure was maintained for 20 minutes following by introducing pressure +1 bar for 24 hours to push the healing agent deeper into the pores of EC LWA.

After cracks were fabricated, the initial crack width was measured under an optical microscope (Leica DMC 2900). The prims were then subjected to wet-dry curing in tap water with the cycle of 4 hours wet and 4 hours dry. The wet and dry cycles were activated automatically by connecting the

Table 4. Mix design of two mortar prims with the dimensions of 30x30x360 mm³.

Type	LWA OD 2/4 (g)	cells/spores / water* (ml)	Sand (g)	Cement (g)	Water (g)	Yeast (g)	Urea (g)	calcium nitrate (g)
Control	0	0	1350	450	225	0	0	0
16 R	82	17	1251	450	225	0	0	0
16 VY0	82	17	1251	450	214	0	18	36
16 SY2	82	17	1251	450	214	1.53	18	36

*cells/spores/water: cells for 16 VYO, spores for 16SY2 and water for 16R

pumps for emptying and filling the tank with water to a timer. The crack closure was monitored under the microscope after the samples had been cured for 30 days. For the crack closure monitoring, the top side surface cracks were observed. The crack was divided into three observation zones; 4 to 5 images were taken in each zone, and the width of the crack reported in each zone was the average value of those measurements. Finally, the healing ratio (HR) was then calculated for each zone based on Equation [4]:

$$HR = (C_{wi} - C_{wf})/C_{wi} \times 100\% \quad [4]$$

where HR is the healing ratio, C_{wi} was the initial crack width, and C_{wf} was the final crack width.

3. RESULTS AND DISCUSSION

3.1 Setting time

In general, samples containing bio-agents showed a shorter setting time compared to the reference sample (Table 5); although the initial setting was delayed, the final setting was reached earlier. Indeed, three elements were interacting in the nutrient sample. The urea and yeast extract were expected to act as a retarder that delays the setting time, while calcium has been reported to accelerate the hydration process (18, 21, 22). For the compositions applied in this research, it seems that calcium successfully repressed the retarding effect provoked by yeast extract and urea. In previous research by Vandervoort, the final setting time of samples only containing yeast extract 5 g/l mortar reached almost 650 minutes (19). In comparison, with the same amount of yeast extract but with the addition of calcium nitrate and urea, the final setting time of the NY5 sample was 80 minutes faster than Vandervoort's result.

Without the addition of yeast in the mixture, the final setting time of NY0 decreased, while the initial setting time increased. Slight retardation due to the presence of urea in the NY0 sample might occur. But this was less dominant compared to the acceleration effect contributed by calcium coming from the addition of calcium nitrate into the mixture. As the calcium element (in alite) tends to dominate the early hydration process, the extra addition of calcium could accelerate the main hydration process and even lead to the flash setting of the paste (23). A flash setting of paste was also reported in the literature when calcium nitrate was used as a plasticizer (22). Still from the same literature, by adding calcium nitrate at a dosage of 10% of cement weight, the final setting time occurred 25 minutes faster than the reference sample.

A clear delay in setting time was observed in the sample containing yeast extract, compared to a no-yeast sample containing only the calcium source and urea (NY0). Increasing the yeast extract concentration up to 5 g/l mortar delayed the final setting time for 75 minutes compared to the no-yeast sample (NY0). A slight delay in setting due to the addition of nutrient medium containing yeast was also reported in the literature (17, 24). The presence of carbohydrates and proteins in yeast could contribute to this behavior. It is reported that sugars could stimulate the dissolution of calcium in cement clinker and form a sucrose half-salt. The absorption of this product at the surface of C-S-H gel could inhibit the formation of stable C-S-H precipitation which mainly occurs in the early hydration process (25).

Table 5. The effect of nutrients on the setting time of paste.

Sample	Initial setting time (minutes)	Final setting time (minutes)
Ref	135	660
NY0	225	495
NY2	330	555
NY5	390	570

3.2 Heat evolution

Significant differences in the heat released were observed among samples (Figure 3). The refer-

ence sample showed less heat release followed by the samples NY2, NY5, and NY0. It was also observed that the second peak, which corresponds to the formation of ettringite by the dissolution of tri-calcium aluminate disappeared in samples with nutrients (23). It seems that the addition of nutrients oppressed the hydration of aluminates. Bundur et al. also obtained a single hydration peak by adding yeast extract at 0.67 % of the paste weight (17).

When increasing the concentration of yeast (in combination with calcium source and urea), a significant retarder effect was not observed in this research. The first hydration peak of the reference sample and samples containing yeast (NY2 and NY5) were similar. It seems that in this mix composition, the role of calcium as an accelerator was more dominant than the retarder effect of yeast. The effect of urea in the mixture, which was expected to delay the hydration process, might occur but not enough to shift the hydration peak location to later times.

No significant difference was observed in the cumulative heat release obtained by all samples monitored for 140 hours (Figure 3). A sharper hydration peak in the nutrient samples due to a fast heat release followed by a quick decrease makes that the final cumulative heat release obtained in nutrient samples are not as high as expected. The total heat release after 140 h obtained in the Ref, NY0, NY2, and NY5 samples was 335, 345, 329, and 334 J/g. Compared to the heat release obtained with only calcium nitrate, the heat release obtained in the NY0 sample was 13% lower (26). The presence of urea seems to slightly repress the heat release in the NY0 sample.

In addition, more heat release was observed in the nutrient sample, and this was higher when yeast was not present (Figures 3 and 4). This behaviour was also attributed to the heat released by the dissolution of C_3S, which is accelerated by the addition of extra calcium in the matrix (23). An increase in heat release was also reported for the heat evolution of paste with a higher C_3S content (25).

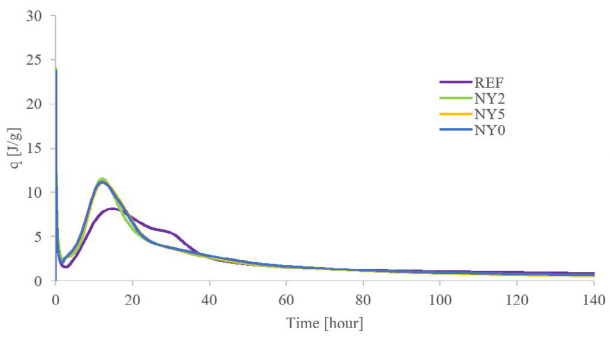

FIGURE 3. Heat evolution of paste with various yeast extract concentrations.

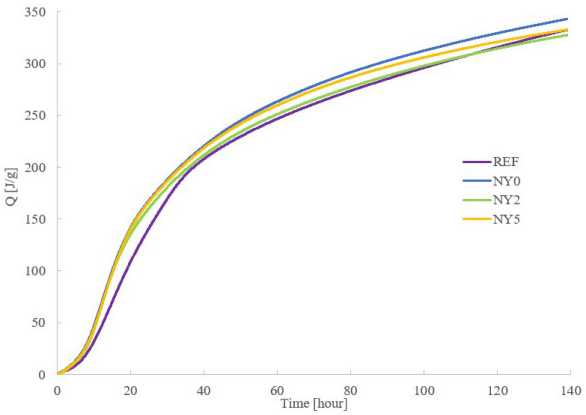

FIGURE 4. Cumulative heat release of paste with various yeast extract concentrations.

3.3 Workability of fresh mortar

It can be seen that the workability of fresh mortar was increased as the concentration of yeast extract increased (Figure 5). The flow value was increased by 3% and 19% by adding yeast extract up to 2 g/l and 5 g/L, respectively, compared to that of the reference sample. The formation of hydrogen as a result of yeast autolysis reaction could explain the increase of workability observed in nutrient samples containing yeast (26). The presence of gas has a lubricating effect, and the slurry texture of the mixture leads to the increase of flowability of fresh mortar.

The role of calcium that is widely used as an accelerator could be seen in the no-yeast sample. The mixture became stiffer in a short period. Without the presence of yeast extract in the mixture, the flowability of fresh mortar dropped up to 25% compared to the reference sample. The stiff fresh mortar of NY0 is well correlated with the fast setting of its paste (Table 3).

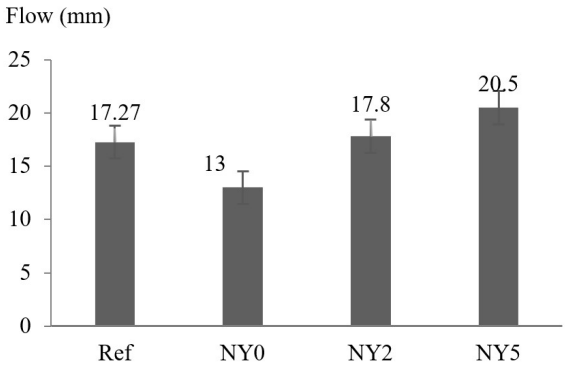

FIGURE 5. The effect of yeast extract concentration on the flowability of fresh lightweight mortar. The dashed line corresponds to the average value of the reference sample, while the error bar represents the standard deviation on the mean (n=3).

3.4 Porosity

It can be seen that increasing the concentration of yeast extract increased the cumulative volume of mercury that intruded into the pores of the mortar (Figures 6a and b). A higher cumulative volume intruded into mortar pores is an indication that the mortar sample has high porosity. The results were then confirmed with the characterization of the pores presented in Figure 6c. Sample NY5 which has a high level of yeast extract possessed a high amount of macropores. The number of macropores decreased with the decreasing yeast extract concentration in the mixture. The gas which was released due to the hydrolysis of proteins in yeast created bubbles that remained as pores when the mortar hardens (26).

A noticeable change in pore size was observed between the reference sample and NY5. Increasing the yeast extract concentration up to 5 g/l shifted the threshold pore size from 0.06 µm to 1 µm (Figure 6b). While sample NY0 and NY2 with lower yeast extract concentration had two threshold pore sizes. The first threshold has a similar value as the reference sample, while the other has a similar value as NY5. The threshold pore diameter in the mesopore zone occurring in NY2 and NY0 could be due to the ink bottle effect. NY5 has a limited number of small pores, which makes that the ink bottle effect could not be observed. The presence of small pores connected to a large pore leads to a high pressure needed for mercury to penetrate through the small pores into the larger pore (27). It results in the machine recording a high number of small pores instead of the large pores that are actually present.

Summarizing, adding yeast extract with the concentration of 5 g/l significantly changes the pore properties of the hardened mortar. Indeed, the appearance of gas bubbles could improve the flowability of fresh mortar, which was disturbed by the addition of calcium. However, when the water in the mortar mixture evaporates, the entrapped gas bubbles will become pores. The presence of a high number of pores was confirmed with the low bulk density of hardened mortar with high yeast extract concentration that will need further discussion in the next section.

3.5 Bulk Density and Compressive Strength

The bulk density of the resulting mortar was well correlated with its 28 days compressive strength (Figure 7). Increasing the yeast extract concentration proved to decrease the bulk density and the compressive strength of the resulting mortar. Introducing 5 g/l yeast extract into the mixture decreased the compressive strength of the resulting mortar by almost 50% compared to the reference sample. The strength was decreased by about 16% when the yeast

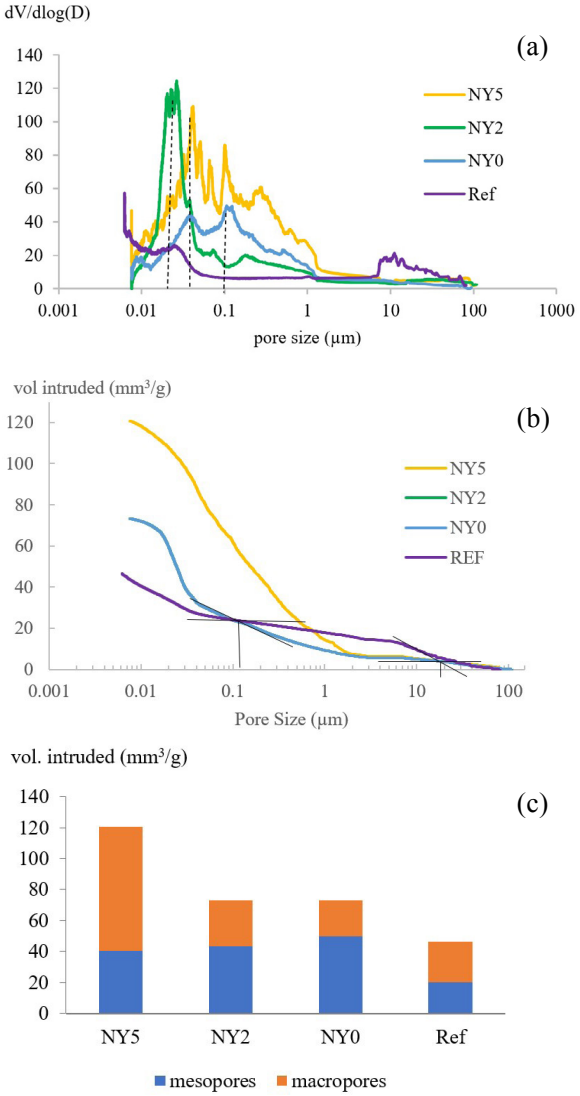

FIGURE 6. (a) Pore size distribution of mortar; (b) Cumulative intruded volume of mortar; (c) Types of pores in mortar. The dashed vertical line in figure 5a represents the highest peak.

extract level was decreased to 2 g/l and no significant effect could be seen when yeast extract was omitted from the mixture, but calcium source and urea were still present.

The low workability of NY0 did not lead to a significant decrease in strength and density. It seems that the presence of calcium in NY0 that should improve the strength was neutralized by the presence of urea. The urea was reported to decrease the hydration heat in the system and slow down the hydration reaction (19). The urea and calcium have an opposite role in the hydration process, which could neutralize their effects when combined in hardened mortar.

The mechanical properties of hardened mortar were also correlated with its pore characterization data generated from MIP. The sample with a high yeast extract concentration (NY5) delivers a high porosity, a low bulk density, and compressive strength.

The slightly higher number of mesopores and lower number of macropores in NY0 leads to higher compressive strength of resulting mortar compared to NY2 sample.

In the literature, the addition of urea yeast extract medium (YE 0.63% of paste weight) into cement paste leads to a 26% strength decrease of paste relative to the reference sample (28). In mortar application, adding treated aggregate with NBU medium (yeast extract, peptone, urea, sodium chloride, and calcium chloride) proved to decrease the 28 days mortar compressive strength up to 15% (24). Compared to results obtained in a mortar application by Joshi et al., the strength reduction of NY2 mortar is similar.

The decrease in bulk density due to the pore formation from protein hydrolysis of yeast extract was the main reason for the strength decrease in the mortar samples with a high concentration of yeast extract.

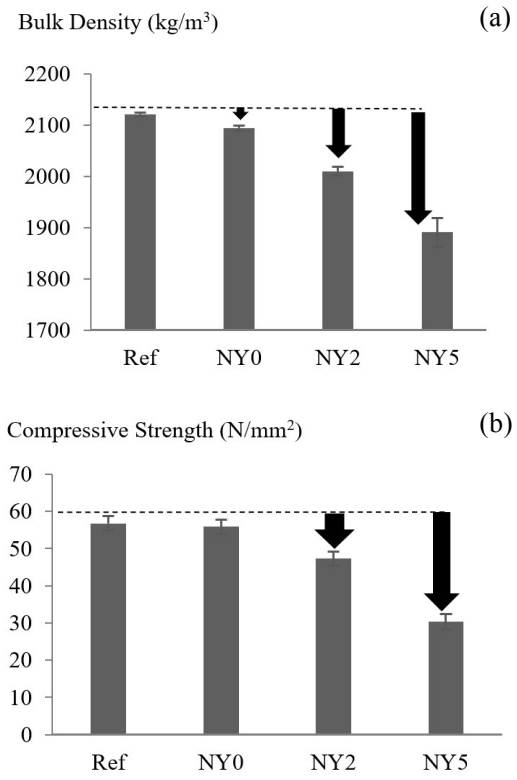

FIGURE 7. The effect of yeast extract concentration on the (a) bulk density and (b) compressive strength of lightweight mortar. The dashed line corresponds to the average value of the reference sample, while the error bars represent the standard deviation of the mean value, n=3.

3.6 Ureolytic activity of cells and spores

Based on the decomposed urea data presented in Figure 8, it can be seen that all urea in the medium solution (20 g/l) could be fully decomposed in 3 days. In the spores group sample, from 0 until 24 hours, only limited urea could be decomposed by the bacteria, but at 72 hours, all the spores were likely germinated into vegetative cells and actively decomposed the urea. Interestingly, the decomposition rate between spores cultivated at 2 g/l and 5 g/l yeast extract concentration was almost the same. It is an indication that the yeast extract medium with a concentration of 2 g/l was sufficient to support the germination of spores into vegetative cells in 72 hours (Figure 8). This result is in line with previous findings by Wang et al., which revealed that in a medium containing 2 g/l yeast extract, spores of *Bacillus sphaericus* could germinate into urease positive cells in 3 days (5).

In the vegetative cells sample, the urea decomposition process ran faster compared to the spore sample group, as the bacteria were already in the active state. All the urea in the solution could be completely decomposed within 8 hours in a vegetative cells sample without the presence of yeast in the medium. Thus, it could be concluded that when vegetative cells were used as a healing agent, the yeast extract could be omitted from the mixture, as the vegetative cells could still decompose urea in the solution without the presence of yeast extract.

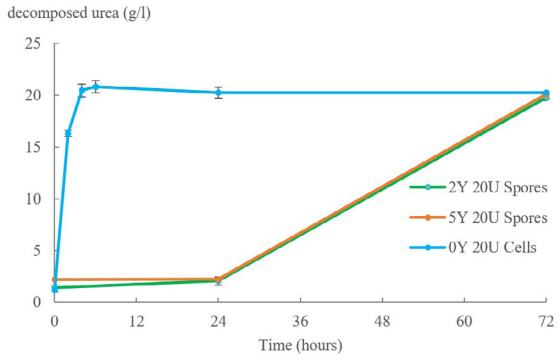

FIGURE 8. The ureolytic activity of spores or vegetative cells of *B. sphaericus* in medium containing 0, 2, or 5 YE, and 20 g/l urea. The error bar represents the standard deviation (n=3).

3.7 Crack closure observation

Autogenous healing provided by the presence of EC LWA was observed in the reference sample, which showed more healing than the control sample without LWA (Figure 9, 10, and 11). The healing ratio for cracks with a width of 0.2-0.3 mm was 71 %. In comparison, in the same crack width range, the control sample only showed a healing ratio of 33%. The ability of EC LWA to retain water in their pores and release it at a later age to react with unhydrated cement could contribute to the crack healing in the reference samples (29).

Most of the existing literature on optimization on yeast extract dosage has focused on bacterial activity monitoring in different yeast extract concen-

trations (24, 26, 30). Only limited reports could be found on the effect of yeast extract concentration on the healing ability of the resulting mortar. Wang et al., using diatomaceous earth as carrier for vegetative cells of *B. sphaericus*, reported that higher yeast extract concentration resulted in a faster urea decomposition process (30). However, no results regarding the healing performance of mortar with bacterial cells and different yeast extract concentrations was reported.

The presence of vegetative cells or spores in the mixture tends to improve the healing performance in the resulting mortar. In the vegetative cells sample (16 VY0) cracks with a width range of 0.3-0.4 mm could be 96 % healed, while in the spores sample (16 SY2) the average healing ratio observed in the same crack width range was 68%. In comparison in the reference sample, in the same crack width range, the average healing ratio was only 51%. The maximum crack width that could be completely healed was 0.23, 0.33, and 0.32 mm for reference, vegetative cells, and spores sample group respectively (Figure 9). This result has a good agreement with the ureolytic activity of free vegetative cells and spores presented in Figure 8, which shows that without the presence of yeast extract, cells are still viable and actively decomposed urea in the solution and that the addition of yeast extract with the concentration of 2 g/l is enough for spores to germinate.

The healing performance obtained in this study is comparable with results reported in the literature. Using the spores of *B. alkalinitrilicus* as a healing agent loaded into expanded clay, a crack with a width up to 0.46 mm could completely be closed after immersion in water for 100 days (8). When the vegetative cells of *Diaphorobacter nitroreducens and Pseudomonas aeruginosa* were loaded into expanded clay, the maximum crack width that could be completely closed was 0.35 mm after a mortar was subjected to wet-dry cycles in water for 28 days (14).

A decrease in healing performance of the spores sample compared to the vegetative cells sample was observed. In the vegetative cells samples, the bacteria were already in an active state, while when spores were employed as a healing agent, more time is needed for germination. Moreover, during this germination process, favorable conditions such as access to nutrients need to be ensured (31). However, in the literature, the performance of vegetative cells was decreased when cracks were created at a later age (3). Still from the same literature, when cracks were fabricated at the age of 6 months, cracks in the crack width range of 0.3-0.4 mm showed an average healing ratio of around 75% after being immersed in water for 28 days. Bundur et al., also reported a decrease in the number of viable vegetative cells of *S. pasteurii* in mortar mixtures, only around 50% of cells were still viable after 49 days in the curing chamber (32). Thus, the vegetative cells could be suitable to induce crack healing in young mortar, while spores would be recommended for use as a healing agent when cracks will occur at a later age.

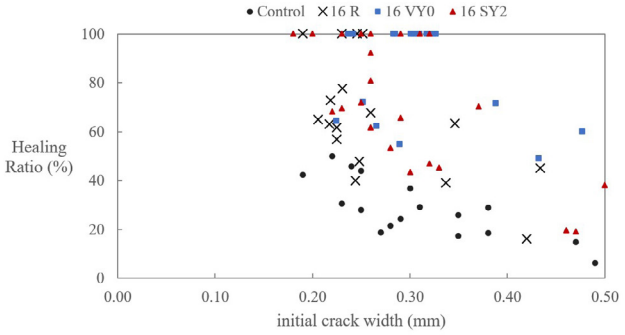

FIGURE 9. The healing ratio of each crack in control, reference, and bacteria samples after being healed for 30 days in wet-dry cycles. Cracks were created at the age of 28 days.

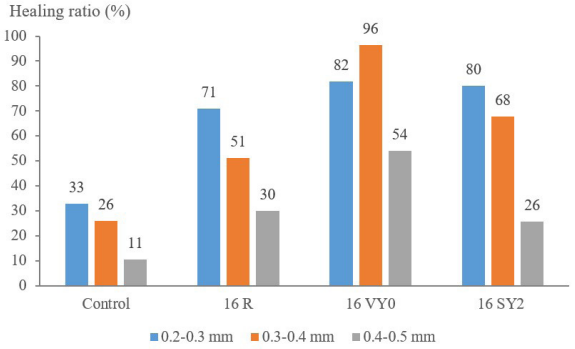

FIGURE 10. The average healing ratio of control, reference, and bacteria samples after being healed for 30 days in wet-dry cycles. Cracks were created at the age of 28 days.

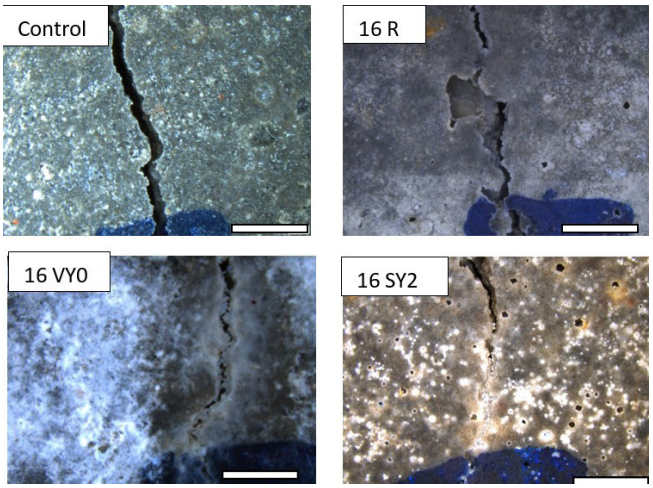

FIGURE 11. Microscope images of cracked control, reference, and bacteria samples after being healed for 30 days in wet-dry cycles. Cracks were fabricated at the age of 28 days, and the average initial crack width was in the range between 0.35-0.37 mm. The white scale represents 2 mm.

4. CONCLUSIONS

The presence of yeast extract significantly affected the properties of hardened bio-mortar. The number of macropores was significantly increased by increasing the yeast extract concentration. The increase in macropores content consequently decreased the density and the compressive strength of 28 days old mortar.

Due to the presence of calcium in the mixture composition, the retarding effect of yeast extract was alleviated. There was a slight delay in setting time when the yeast extract concentration was increased compared to a no-yeast extract sample. However, compared to the reference samples without calcium nitrate and urea, samples containing yeast extract still had a shorter setting time. This was corresponding well with the hydration kinetic results. The hydration peak of samples with yeast extract was similar to that of the reference sample. More heat was released in the sample with the addition of calcium source and urea, compared to that of the reference sample.

A workability issue was found in the no-yeast extract samples containing only calcium nitrate and urea. With the absence of yeast extract which induced the formation of bubbles during its hydrolysis, the workability of no-yeast extract samples dropped up to 25%.

Summarizing, if spores are used as the healing agent, where yeast extract is required for the germination process, 2 g/l yeast extract into the mixture would be the best option, as the spores could germinate and actively decomposed the urea in this medium. Moreover, by adding yeast extract with a concentration of 2 g/l, the properties of the resulting mortar decreased to a still acceptable level. The healing performance of mortar with 2 g/l YE with spores as a healing agent is also improved. However, in the condition where the presence of yeast extract is not mandatory, for example when vegetative cells are employed as a healing agent, omitting yeast extract from the mixture is advised as the presence of only calcium nitrate and urea did not significantly affect the properties of resulting mortar. For cracks formed at young age, the best healing performance of mortar can be obtained with no yeast and vegetative cells as a healing agent.

ACKNOWLEDGEMENTS

The first author would like to thanks State University of Malang (UM) through RKI funding research for their financial support.

AUTHOR CONTRIBUTIONS:

Conceptualization: P. Risdanareni, J. Wang, N. De Belie. Data curation: P. Risdanareni, L. Ma. Formal analysis: P. Risdanareni. Funding acquisition: P. Risdanareni, N. De Belie. Investigation: L. Ma. Methodology: P. Risdanareni, J. Wang, N. De Belie. Project administration: N. De Belie. Resources: L. Ma. Supervision: J. Wang, N. De Belie. Validation: J. Wang. Visualization: P. Risdanareni. Writing, original draft: P. Risdanareni. Writing, review & editing: L. Ma, J. Wang, N. De Belie.

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