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Physico-chemical, Mechanical and Mycological Properties of Cement Pastes Incorporating Vinasse

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Abstract Vinasse is the wastewater produced during the manufacture of ethyl alcohol by fermentation of sugar containing residues. The current research reports an experimental study on the properties of cement paste incorporating vinasse that was treated by the addition of H_2O_2 at different percentages to prevent fungal growth. In this study, four cement-based mixes (blank, with vinasse and with two different vinasse ratios) were molded, water cured up to 3 months and characterized using different means as SEM and IR. The cubic pastes of the studied mixes were evaluated in point of views of physico-chemical, mechanical and mycological tests and analyses. It is revealed that, the studied physico-mechanical as well as mycological properties of the studied mixes was relatively enhanced with the addition of treated vinasse at the addition of 3% H_2O_2. Also, according to the test results and SEM images that 3% H_2O_2 addition has successfully inhibited the fungal growth and further addition (5%) is not recommended. Furthermore, the obtained results encourage the using of cement incorporating vinasse treated by 3% H_2O_2 (CTV3 mix) in sewage systems and water canal works.

Keywords Vinasse, Portland cement, Cement paste, Physico-chemical and mechanical properties, Mycological test

1. Introduction

To be competitive and sustainable, the trend worldwide moves the sugar industry towards one of renewable biomass where the molasses produced are generally converted into ethanol. However, production of ethanol from molasses generates a waste effluent, namely vinasse, which used for many useful applications [1]. Vinasse is a major environmental problem for the ethanol industry. This type of waste possesses a high polluting power as a result of its high organic load, pH and color [2-3]. No one has found a convenient and economical disposal solution for this black-reddish and viscous wastewater. The disposal of vinasse represents a major environmental problem due to its high BOD. This material can cause damage to aquatic life, especially when dumped in large volume in rivers, streams, and landfills [4].

Vinasse may contains negligible amounts of heavy metals [5], the dissolved organic matter (DOM) that is present in the vinasse is known to bind to heavy metals during its movement through the soil and impair the quality of the groundwater [6] due to the formation of stable, aqueous complexes with heavy metals such as Cu, Ni, Zn and Cd. This DOM may thus enhance metal mobilization and transportation towards groundwater [7].

Vinasse has been reported to be used for irrigation and fertilization due to its high nutrient and organic matter content. Nevertheless, the presence of phytotoxic, antibacterial and recalcitrant compounds such as phenols, poly phenols and heavy metals has been observed to cause negative effects on micro-organisms and plants in discharge areas. It is therefore necessary to subject this waste to a conditioning treatment prior to its disposal in the environment [8].

Usage of molasses in concrete as a water reducing and retarding admixture was emphasized [9, 10] and comparison is made between molasses with 40% purity grade and lignsulphonate with respect to improvements in properties of concrete. The study used molasses as a plasticizer in concrete and the achieved results are satisfied the American Society for Testing and Materials for water reduction criteria [11].

Sugar cane molasses contribute significantly in initial setting time and water absorption [12]. It is also reported that, sugar cane juice (SCJ) retarded the setting time of concrete. The workability, compressive strength and tensile strength of the concrete initially decreased as the content of SCJ was increased at optimum (SCJ) content 5 % [13].

Reports may differ in their findings on the concentrations of H_2O_2 needed for the inhibition of bacterial growth for bactericidal action, probably due to differences in bacterial strains used in other environmental factors such as pH and temperature [14].

The antibacterial effects of hydrogen peroxide have been extensively investigated due to its possible involvement in a number of important biological events in which bacterial cells are either killed or their growth inhibited. Hydrogen peroxide is extensively used as a biocide depending on microbical type, particularly in applications where its decomposition into non-toxic by-products is important. Investigations of DNA oxidation suggest that the oxidizing radical is the ferryl radical formed from DNA-associated iron, not hydroxyl. Investigations of protein oxidation suggest that selective oxidation of certain proteins [15-17].

2. Materials and Methods

2.1. Materials

Three different materials were used in the study: Ordinary Portland cement (OPC), vinasse (V) and hydrogen peroxide (H_2O_2). The Portland cement used was of SEM (I) 42.5 type, locally manufactured and supplied by the Hellwan cement company, Egypt. The used vinasse was delivered as diluted liquid from sugar and integrated industries company, Al-Hawamdiyah city, Giza governorate, Egypt. The used H_2O_2 in this investigation was of 50% purity and its molecular weight 34.01. It was produced by Diachem chemicals company, Egypt.

2.2. Methods

Different analytical methods and techniques were used on the starting raw materials and hardened cement pastes. Physico-chemical analyses (temp, pH, color, conductivity, moisture contents, ash, protein , carbohydrates, TDS, TSS, TS,COD, BOD, Cu , Fe, Ca, Mg, Mn, Al, NO_3, P and N) were conducted on the studied vinasse according to relevant standard [18]. Also, mycological analyses were carried out on the studied vinasse and mixes according to relevant aspect [19]. Scanning electron microscopy (SEM) was used to determine microstructure and morphological features of hardened specimens of each mix cured at 90 days. This was carried out using SEM model Quanta 250 FEG (field emission gun) attached with accelerating voltage 30 K.V. FEI company (Netherlands). The formed phases in the cured hardened cubes were identified using a Jasco-6100 Fourier transformed infrared spectrometer (FTIR; Varian model, Excalibur FTS 3000MX, Paolo Alto, CA, USA). The tested samples were prepared using the KBr pressed disc technique. The analysis was done between 400- 4000 cm^{-1}.

2.3. Mix composition and cement paste preparation

In order to investigate the effect of the vinasse used, four mixes namely C (the reference mix), CV, CTV3, CTV5 were designed for the study, as given in details in Table 1. The vinasse used in CV mix was of untreated type while the vinasse in mixes CTV3 and CTV5 was of treated type with 3% and 5% H_2O_2, respectively.

Table 1: Mix composition and proportion of the studied mixes

Mixture	Cement (g)	Water (ml)	Vinasse (ml)	Vinasse with 3% H_2O_2 (ml)	Vinasse with 5% H_2O_2 (ml)
C	700	195	___	___	___
CV	700	190	2.8	___	___
CTV3	700	190	___	2.8	___
CTV5	700	190	___	___	2.8

The mixing process of the studied mixes was started by thoroughly mixing the ingredients of each mix in a laboratory electrical mixer for at least 3 minutes. Through the mixing process water is added gradually to obtain a homogenous and workable mix. Then, the prepared paste was casted in 2.5 cm side length steel cube. The paste was vibrated manually during molding. The cubes of each mix were de-molded after 24 hours. Then after they were labeled, and immersed in water in plastic containers for curing until the testing times of 3, 7, 28 and 90 days. Setting time tests of the fresh cement pastes of different mixes were determined [20]. At the end of curing times, a series of tests were carried out to determine the physico-mechanical properties of the hardened cured cubes in terms of water absorption, bulk density and compressive strength. The studied properties were conducted according to the American Society for Testing and Materials (ASTM) [21-22].

2.4. Mycological survey and fungal isolation

The used vinasse samples were examined for the presence of fungi by total fungal count using potato dextrose agar at 28 °C for 5-7 days. It was carried out using the dilution plate method for treated and untreated vinasse. Five sets of H_2O_2 percentages (1%, 2%, 3%, 4%, and 5%) by volume were used in the experimental study to select the most promising percentage to be add in the cement paste for the applied work. Pure cultures were obtained by subculturing inoculum from young colonies developing on the initial medium [18].

2.4.1. Preparation of media and fungal cultures

Potato dextrose agar medium was prepared and pH was adjusted to 7 then it was sterilized by autoclaving at 15 lbs pressure 121 °C for 15 min [18]. Then after, the isolated fungi from the vinasse were purified and were kept in 4 °C for antifungal activity test.

2.4.2. Antifungal assay

Petri plates containing 20 ml of autoclaved and solidified potato dextrose agar medium were inoculated by 1 ml of prepared fungal spore's suspension then were plated onto agar surface then the hardened cubes of different mixes were impregnated within. It was gently pressed down to ensure contact and incubated at 28 °C for 28 days. Each plate was checked at the end of incubation time and the obtained results were reported as the rating described in Table 2. The results were visually detected for each fungal isolates based on relevant aspects [23-24].

Table 2: Rating description of fungal growth

Rating	0	1	2	3	4
Observed growth on specimens	None	Traces of growth (less than 10 %)	light growth (10 – 30 %)	medium growth (30 – 60 %)	Heavy growth (60 – 100 %)

2.4.3. Identification of fungal isolates

Identification of isolated fungi in the lab was carried out using the morphological characteristic as colony diameter, color of conidia, extracellular exudates, pigmentation color of reverse mycelium and microscopic features. Also, conidial heads, fruiting bodies, degree of sporulation and the homogeneity characters of conidiogenous cells were examined by optical light microscope (10x90) Olympus CH40 [25-29]. For penicillium species, fungal isolates were grown onto potato dextrose agar medium at 28°C for several days (5-7) then the culture were then kept in 4°C. The pure isolated fungi from the used vinasse were studied for determination of antifungal activity for studied mixes incorporating different ratios of H_2O_2 (3% and 5%).

3. Results and Discussion

3.1. Characterization of the used vinasse

Physico-chemical results of vinasse liquid that was measured at 28.3˚C are shown in Table 3. The most characteristic results of vinasse are, it is an acidic liquid with pH value of 4.5, it shows high chemical oxygen demand (COD), high biological oxygen demand (BOD) and total dissolved salts (TDS) contents. The inorganic solids contain considerable amounts of nutrients such as total phosphorus, total nitrogen, nitrates, magnesium and calcium.

Table 3: Physico-chemical characteristics of the used vinasse

Item	Result per unit
pH	4.5
Color	74061 M.A.U
Conductivity	22.90 µ s/cm
Moisture contents	82.27 %
Ash	10.60 %
Protein	6.20 %
Carbohydrate	0.93 %
TDS	10500 mg/l
TSS	4633 mg/l
TS	15133 mg/l
COD	100000 mg/l
BOD	55000 mg/l
Calcium	1376 mg/l
Magnesium	1745 mg/l
Copper	76 mg/l
Iron	17 mg/l
Manganese	14 mg/l
Aluminum	0.01 mg/l
Nitrate	600 mg/l
Total nitrogen	930 mg/l
Total phosphorus	700 mg/l

3.2. Mycological results

Vinasse sample used without H_2O_2 addition (blank) was examined visually and the fungi growth was observed on the surface of liquid after 7 days at room temp. as shown in Fig. 1.

a) Fresh sample (Side-view) **b) Sample after 7 days (Side-view)** **c) Sample after 7 days (up-view)**

Figure 1: (a) Vinasse fresh sample, (b) Worms appeared after 7 days (c) Fungal growth on surface of vinasse

Different mixes of the used vinasse were studied by using H_2O_2 at 1%, 2%, 3%, 4% and 5% by volume replacement for vinasse sample. The Fungal isolates from the blank sample used are *Aspergillus flavus*, *Aspergillus niger*, *Penicillum notatum*, *Candida ablicans* and *Saccharomyces cereiviaece*. It was observed that there is no evidence towards fungi growth inhibition when $H_2O_2$was used at ratios of 1% and 2% as shown in Fig. 2. It is worthy to note that, up to 2% H_2O_2addition Aspergillus niger is only appeared and grown. Further additions of H_2O_2 3-5%, all isolates have no growth. As hydrogen peroxide ratio increased the fungal growth decreased.

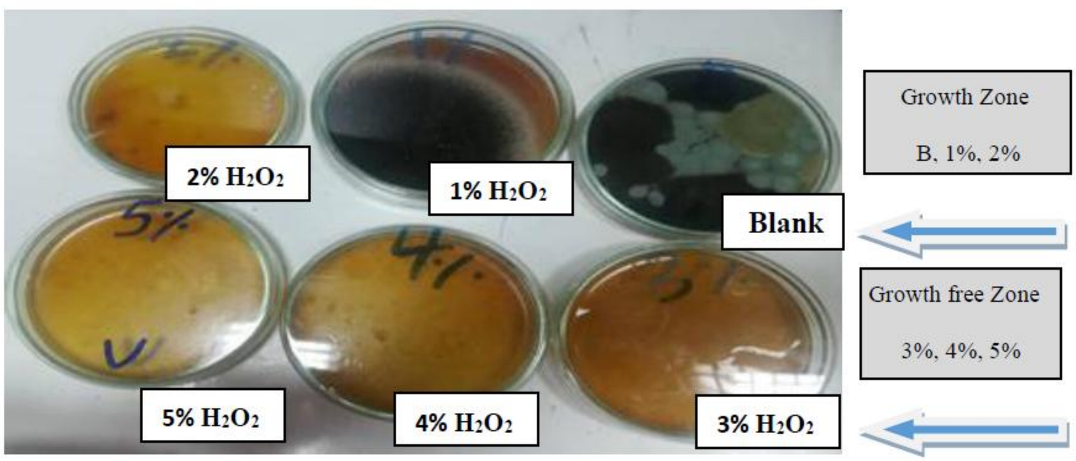

Figure 2: Fungi isolated from vinasse sample used before and after treatment with different ratios of H_2O_2.

The positive effect of hydrogen peroxide is clearly found in the addition of H_2O_2 at 3%, 4% and 5%. From these results, 3% and 5% H_2O_2 ratios were selected for the experimental work (preparation of cement paste cubes). The used samples in this stage are named as treated samples.

The ratios of hydrogen peroxide used (3% and 5%) by volume percent replacement for vinasse were used for preparation of two types of treated vinasse mixes (TV3, TV5) as chemical admixture for Portland cement. These two pastes were used for antifungal activity test to study the effect of treatment on cement paste properties. Three pure fungal isolates were isolated in this test namely *Aspergillus flavus, Aspergillus niger, Penicillium notatum*. The hardened cement cubes from different mixes were cut to slabs of 0.8 cm thick and examined based on antifungal activity test.

3.2.1. Results of *Aspergillus flavus*

The results showed that slab of C mix has heavy growth of 60% with rating 4 followed by the slab of CV mix of 40% (medium growth) and rating 3, then the slab of CTV3 mix of 10% (light growth) and it has rating 2. The inhibition zone appeared has 8mm diameter. Finally, the slab of CTV5 mix obtained rating 1 with traces of growth (less than 10%).

3.2.2. Results of *Aspergillus niger*

The results showed that slab of C mix has medium growth of 90% with rating 4 followed by the slab of CV mix (70%) of medium growth with rating 4, then the slab of CTV3 mix of medium growth of 50% with rating 3 (medium growth). Finally, the slab of CTV5 mix obtained rating 1 (traces growth) with growth ratio less than 10%.

3.2.3. Results of *Penicillium notatum*

The results showed that slab of C mix has medium growth of 90% with rating 4 followed by the slab of CV mix (50%) of medium growth with rating 3, then the slab of CTV3 mix of light growth of 30% with rating 2. Finally, the slab of CTV5 mix obtained rating 1(traces growth) with growth ratio less than 10%.

Non treated A. niger at a nonlethal concentration confers greatly enhanced resistance to kill by H_2O_2 at lethal concentrations in the early exponential phase of growth. This adaptation involves the induction of a number of antioxidant enzymes, predominantly catalase. Enhancement of the activities of these antioxidant enzymes prevents accumulation of H_2O_2inside the cells, as supported by the increased decomposition rate of external H_2O_2 by intact cells, and higher gradient of H_2O_2 concentration between intracellular and extracellular milieu estimated from a mathematical model [30].

Results of mycological tests may be attributed mainly to the biocide reactions yielded different types of oxidative structural change and different degrees of oxidation to amino acids and proteins, and differences in activity against a microbial enzyme. In particular, there was a marked difference in the interactions of liquid H_2O_2 and gaseous H_2O_2 with the macromolecules, the latter causing greater oxidation; these results explained the dramatic differences in antimicrobial efficacy between liquid and gas peroxide [30].

The pH value of the used diluted vinasse was 4.5 but after the treatment the value was decreased to 3.9 and 3.8 for 3% and 5% H_2O_2additions, respectively.

It was shown from the antifungal activity results that the most resistant isolates was Aspergillus niger, and the most effective concentration was 5% H_2O_2 but also at 3 and 4% the antifungal solution works properly.

3.3. Results of SEM for the cured cubes

The SEM plays an important role in the examination of cured cubes of different mixes. The hydration process of cement and development of cement paste structure can be presented in three stages. In the first period of hydration of cement paste, the first forms of hydrated calcium silicates are created. In the second stage of cement hydration, the calcium hydroxide (portlandite) is formed. Its formation is a result of the hydrolysis of tricalcium (C_3S) and dicalcium (C_2S) silicates. It appears as massive and hexagonal crystals. In the third period of paste hydration, the occurred pores are filled by short fibers or lamellar phases of hydrated calcium silicates. The duration of this stage is a period of several days to several months, and covers almost complete hydration of the cement. A characteristic feature of this stage is transformation of calcium aluminate trisulfate $3CaO{\cdot}Al_2O_3{\cdot}3CaSO_4{\cdot}3H_2O$ (ettrigite) to calcium aluminate monosulfate $3CaO{\cdot}Al_2O_3{\cdot}CaSO_4{\cdot}12\ H_2O$ [31, 32]. It is usually commonly found as white, needle-like crystals.

The SEM images in Fig. 3 showed that, the most common cement hydrated phases are formed in the studied hardened cement cubes of all mixes. This means that, the addition of vinasse (blank or treated) doesn’t affect negatively on the formation of cement hydrated phases. Also, it was observed that the structure compactness is decreased with the increase of H_2O_2 amount as revealed clearly in mix CTV5 (5% H_2O_2). Furthermore, the SEM image of the mix CTV5 show the less compacted structure and that may be is attributed to the negative effect of H_2O_2 addition (corrosive action) which leads to create macro open pores in the body structure. The adverse effect is associated with high content of H_2O_2 as supported before [33].

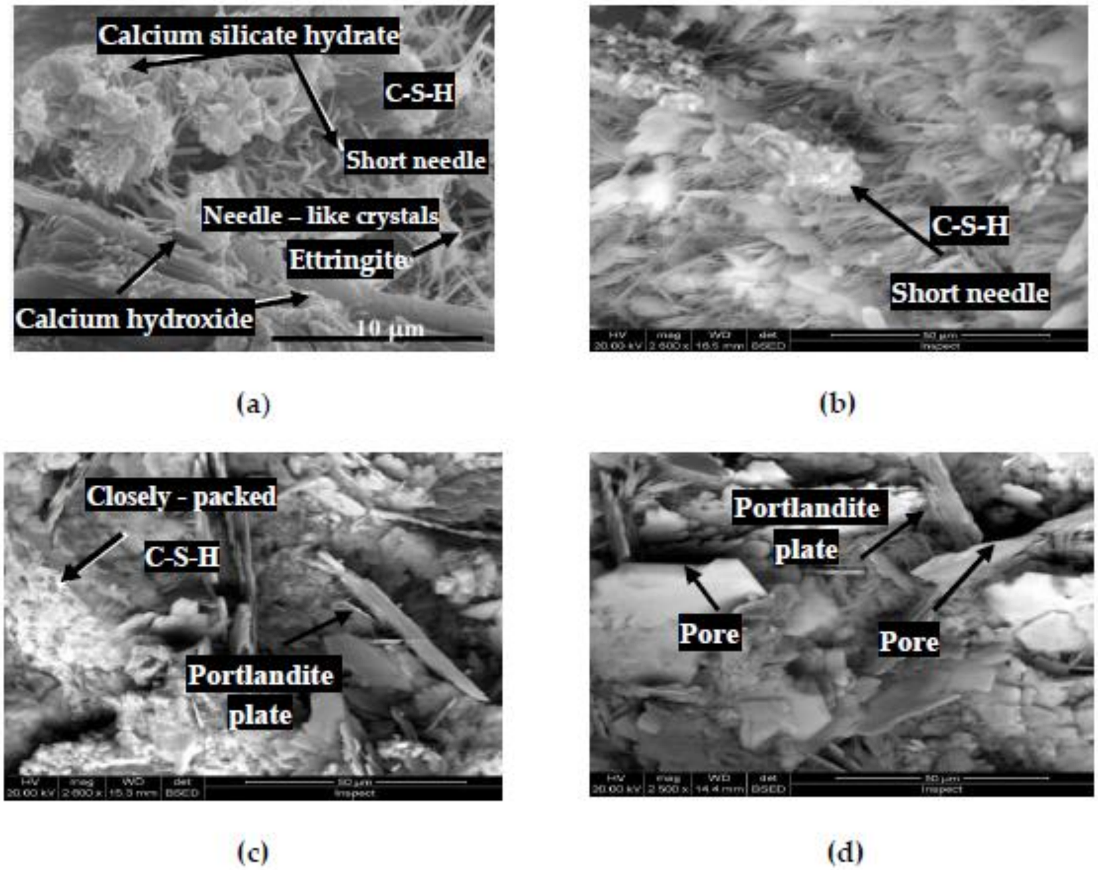

Figure 3: SEM images of hardened cubes of the studied mixes cured for 3 months a) C mix; (b) CV mix; (c) CVT3; (d) CVT5 mix.

3.4. Results of Fourier transformed infrared spectrometer (FTIR) analysis of cured cubes of different mixes

Fig. 4 depicted the FTIR spectra of the cubes of different mixes that were cured for 90 days. The matching in the band locations is obvious and the difference is only in the band intensity. The detected bands were interpreted according to published papers [34-36]. The prominent sharp peak located at 3640 cm^{-1} is due to the presence of OH^- stretching of portlandite crystalline phase present in cement hydration. The broad band at 3500 cm^{-1} can be attributed to the stretching vibration of the OH^- group in the H_2O. The band located at 1450 (large and symmetric peak), small bands at 1800 and 2500 cm^{-1} can be assigned to the vibration corresponding to the CO_3^{-2} group of calcite. This band decreased as H_2O_2 addition increased. The bands at region between 700- 1000 cm^{-1} are due to the complexity and associated vibrations sometimes indicating a low crystalline grade of calcium silicate hydrate gel. All of the tested samples of different mixes had complex intensive silicate bands, Si-O, Si-O-Al and Si-O-Si stretching and bending bands. Difficulties were found to interpret this region by studying FTIR spectra only, since many forms of silicates give rise to several peaks, causing lots of overlaps. Also, the broad band located between 3000-3500 cm^{-1} region is attributed to OH^- stretching vibration of water molecules of calcium silicate hydrate gel. The broad band of calcium silicate hydrate is decreased with the increase of H_2O_2 addition reflecting its negative effect as appeared in SEM images especially for CTV5 mix. The weak peak presented at 1150 cm^{-1} is indicative for ettringite structure and stretch vibration modes of SO_4 tetra hydra. The extra increase of H_2O_2 addition (5%) as appeared in CTV5 mix leads to decrease of portlandite and increase of ettringite phase as well as lowering the intensity of C-S-H as the following chemical equation.

1. Ca $(OH)_2$ + SO_4 → $CaSO_4$ ------- 1
2. $CaSO_4$+ C_3A → Ettringite -------- 2

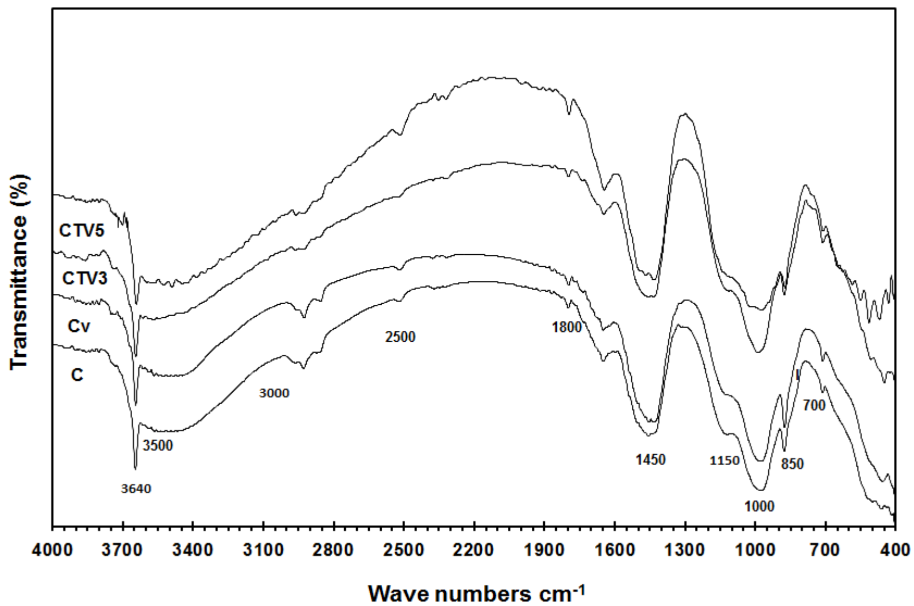

Figure 4: FTIR spectra of the studied mixes cured for 3 months

3.5. Physico-mechanical properties of the cured cement pastes

Figures 5 show the results of initial (I. S.) and final setting (F. S.) times, water absorption, bulk density and compressive strength of the cement cubes of different mixes cured for different curing days.

The results of Figure 5 show that, the setting times decreased as the amount of H_2O_2 increased. Also, it was noticed that the lowest setting times was found for CTV5 mix of 5% H_2O_2 addition. Moreover, the addition of treated vinasse on Portland cement accelerated the initial and final setting times. This may be due to decrease the mix pH as H_2O_2 increases as measured in the research (3. 2. 3).

Results of final setting time (hours) of the studied mixes shows 3.4 (CV), 3.3 (C), 3.2 (CTV3) and 2.5 (CTV5) in decreasing order.

In terms of bulk density Fig. 5 of the cubes of different mixes, the results increased with curing time (days) as well as H_2O_2 decreasing. The density of mix CV (untreated vinasse) showed the highest value compared to other mixes. The result of CTV3 mix cube has a higher bulk density than CTV5 mix at all curing days. The bulk density increase is indicative of the pozzalnic reaction progressing (cement phase formation and vice versa). The highest value was achieved by the cubes of CV mix where the lowest one was for the CTV5. In other words, as H_2O_2 content decreased, the bulk density increased giving evidence of densification of the paste cubes.

Fig. 5 points to the water absorption increase with the prolongation of the curing times and H_2O_2. The results of water absorption show that the CTV5 mix cubes have the highest water absorption value (7%) and the CV mix cube have the lowest value (5%) at 90 days of curing. The lowest value was for the mix of CV (untreated) followed by the C mix, then CTV3 mix (3% H_2O_2) However, when the H_2O_2 dosage was too high (5%), the water absorption gained was too high.

In terms of compressive strength, figure 5 illustrates the relationship between the compressive strength of OPC pastes of different mixes as function of curing times up to 90 days. It was observed that, the strength increases with curing time increases and H_2O_2 content decreases. The increase of strength with curing times is a function with the increase of the formed hydrated phase (binders). The results of the compressive strength reveal that addition of vinasse (untreated) increases the compressive strength (CV) rather than the treated vinasse as shown in CTV3 and

CTV5. The results also show that the mix of 3% H_2O_2 addition (CTV3) has superior results than the mix of 5% H_2O_2 addition (CTV5) especially at the later stage of curing. The worst results that achieved for the CTV5 cube mix may be due to open pores occurred in the body structures as shown in the SEM images of hardened cubes of the CTV5 mix cubes (Fig. 3d). It was obvious that, the adverse effect of H_2O_2 was found at high H_2O_2 content (5%).

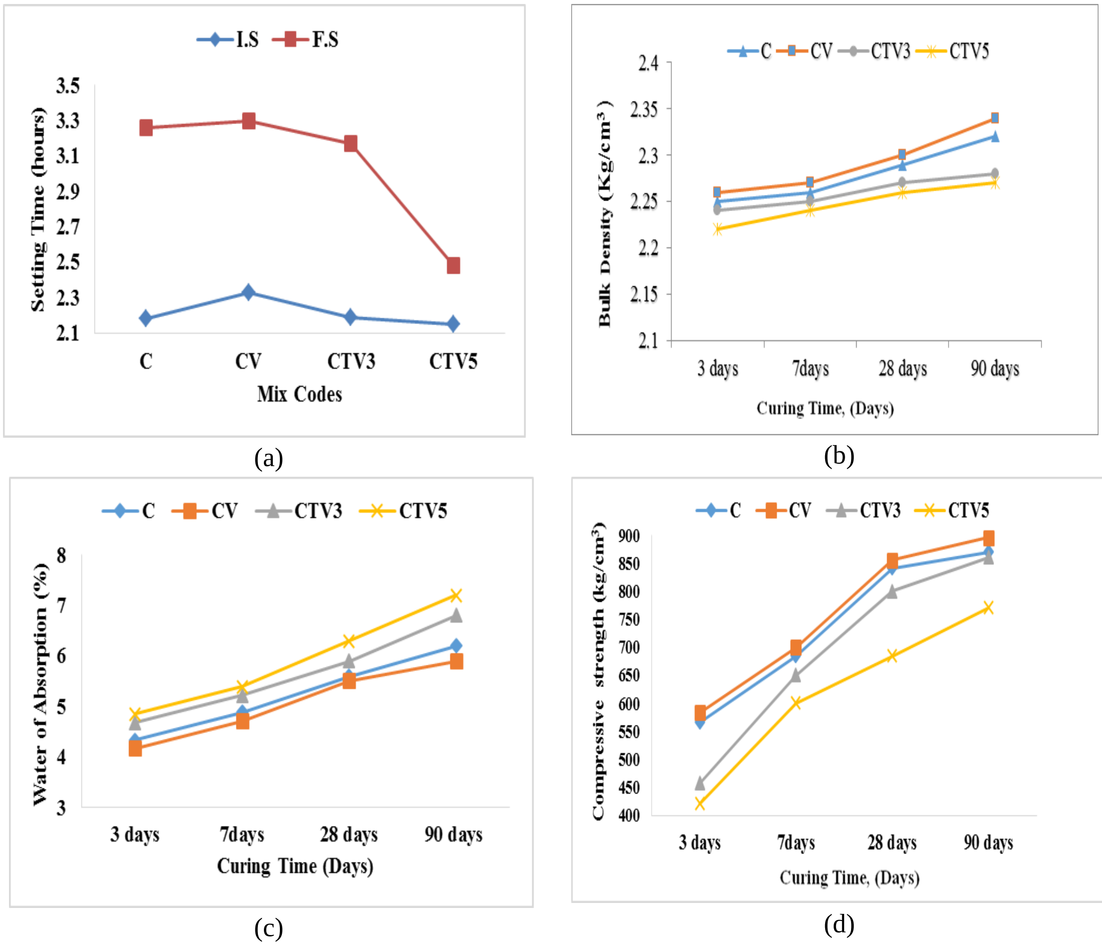

Figure 5: a) Initial and final setting times; b) Bulk density; c) Water absorption; d) Compressive strength of different mixes

4. Conclusions

The research studied the physico-chemical, mechanical and mycological properties of cement pastes incorporating vinasse either treated at different ratios of H_2O_2 or untreated. From the test results, the following conclusion can be drawn:

- To avoid the fungal growth, the H_2O_2should be used. The recommended amount of H_2O_2 used was 3% by volume replacement for vinasse, any further addition is prohibited.
- The addition of treated vinasse to Portland cement pastes accelerated the initial and final setting times.
- The physico-mechanical properties enhanced as curing time increased and H_2O_2content decreased.
- Although the Portland paste of untreated vinasse has the superior result than the treated ones but it is not recommended to use without treatment according to the obtained results from mycological test analysis (fungi growth).
- The SEM results show that, the addition of vinasse has no effect on the formation of cement phases. Also, the addition of 5% of H_2O_2promotes open pores formation in the microstructure in the cement paste.
- It is encouraging to use CTV3 mix as a mortar in the environment of sewage or water canals works.

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