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Original Article

Investigation of the microbial effect of wastewater on concrete performance

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ABSTRACT

Concrete subjected to wastewater environment deteriorates faster than its counterpart in an ordinary environment. The investigation also showed that the deterioration effect could also be counteracted using bacteria. This study investigated the deterioration and healing effects of bacteria isolated from wastewater on concrete properties like weight loss, compressive strength, modulus of elasticity and SEM analysis. The result at 28 days of curing showed that the greatest reduction in weight (4.2%) and compressive strength (16.69%) compared with control was observed in concrete inoculated with *S. epidermidis*; while the least decrease in weight (0.8%) and an increase in compressive strength (1.79%) was observed with the concrete cast with potable water and cured in nutrient broth medium. The healing effect of *B. subtilis* on the concrete was also considered and analyzed, the result showed that *B. subtilis* improved the strength of the concrete exposed to *S. epidermidis*. Scanning Electron Microscopy analysis showed that an increase in the pores within the concrete leads to a reduction in compressive strength.

1. Introduction

Concrete is one of the strongest materials used in various continents all over the world. Concrete structures belonging to these are usually considered indestructible mainly because of their long service life compared with other construction materials. However, it can get destroyed for a variety of reasons including materials limitation, construction practices and poor quality control, as well as exposure to microorganisms in the environment [1-4].

Concrete undergoes biodeterioration when exposed to contact with microorganism environments such as soil, water, sewage, agricultural product and waste materials. Biodeterioration of concrete structures is caused by the interaction of microorganisms with concrete surfaces as well as the movement of those microorganisms via micro-cracks or through the capillaries of the concrete [5]. Also, the action of microorganisms affects the concrete mainly by contributing to the erosion of the exposed concrete surface, reducing the protective cover depth, increasing concrete porosity, and increasing the transport of degrading materials into the concrete that can accelerate cracking, spalling, and other damage and reduce the service life of the concrete structure [6]. Laboratory analysis of concrete samples has shown that many microorganisms such as fungi (yeasts, *Cladosporium* etc.), bacteria (*Actinomycetes*, *Thiobacillus* among others), algae (the most popular are diatom algae) and even protozoa, can be found within the concrete matrix [6]. The consequences of microorganisms within the microstructure are different. Although there is insufficient experimental evidence, it has been observed that the action of microorganisms on the concrete matrix increases concrete porosity, which in turn can change the diffusivity of the concrete [6]. Higher porosity values can also lead to higher surface wear, reducing the depth of the protective

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concrete cover over the reinforcement. Higher diffusivity and lower concrete covers can facilitate other deterioration processes such as corrosion of the reinforcement. Bertron *et al* [7] and Diercks [8] also discovered in their work that bacteria and microscopic fungi are the main microorganisms influencing the concrete bio-deterioration by producing biogenic organic acids (acetic, lactic, butyric and the like) and carbon dioxide, which can be extremely corrosive towards the concrete.

However, a critical review of the literature indicated that approaches used by researchers are categorized into three, namely - the use of admixtures, self-healing concrete technology and microbiological prevention [1, 9-12]. This has led to the development of a way of improving the strength of the concrete with a model laboratory bacterium, *Bacillus subtilis*. Therefore, this research aims to consider the healing effect of *B. subtilis* in concrete exposed to *S. epidermidis*.

2. Materials and Methods

2.1. Material

Ordinary Portland cement conforming to IS 12269-1987 was used. Locally available clean, well-graded, natural sharp sand conforming to IS 383-1970 was used as fine aggregate. Crushed granite aggregate of size 20 mm nominal size having AIV (Aggregate Impact Value) of 19.21% and ACV (Aggregate Crushing Value) of 27.7% was used. Locally available potable water conforming to ACI (American Concrete Institute) 318 – 2008 was used. *B. subtilis*, model laboratory bacterium and *S. epidermidis* isolated from wastewater were used.

2.2. Test on wastewater samples

Wastewater samples were collected in a sterilized universal bottle from three different sources which were labelled as samples A, B and C respectively. A five-fold serial dilution of the wastewater samples was carried out. Nutrient Agar was prepared according to the manufacturer's specification and sterilized in an autoclave at 121 ℃ for 15 mins. The pour plate technique was adopted and solidified inoculated media was incubated at 37 ℃ for 24 hours. Colonies were counted and sub-culturing was carried out to obtain pure isolates after which morphological and biochemical characterization of distinct bacterial colonies isolated from the wastewater samples were determined. Morphological and biochemical characterization of the organisms was carried out on pure isolated cultures. This involves the observation of the colour, shape, elevation, edge and arrangement of the agar plate. Likewise, Gram staining, catalase test, indole test, citrate test and sugar fermentation tests were carried out for the identification of pure isolates.

2.3. Culture of bacteria

The pure culture of *S. epidermidis* was isolated from the wastewater sample and is constantly maintained on nutrient agar slants. It forms irregular white colonies on nutrient agar. When required, a single colony of the culture is inoculated into a nutrient broth of 8 g in 1 litre and the growth condition is routinely maintained at 37℃. The same procedure was repeated for the culture of *B. subtilis.*

2.4. Maintenance of stock culture

Stock cultures of the bacteria were maintained on nutrient agar slants. The cultures were streaked on nutrient agar slants with a loop and the slants were incubated at 37 ℃. After 24 hours of growth, the slant cultures were preserved under refrigeration (4) until further use. Contamination from other bacteria was periodically checked by streaking on nutrient agar plates.

2.5. Compressive strength test

To study the compressive strength of the concrete, *S. epidermidis*, and a consortium of *B. subtilis* plus *S. epidermidis* were grown in NBU (Nutrient Broth Medium) separately. The cement-to-sand-to-granite ratio was 1: 2: 4, and the bacteria culture-to-cement ratio was 0.55. Standard cubes of 100mm x 100mm x 100mm were used, as per IS 4031-1988. Sand, cement and granite were thoroughly mixed, adding along with the grown culture of *S. epidermidis* and a consortium of *B. subtilis* plus *S. epidermidis* then cured in NBU (Nutrient Broth Medium) respectively. Sand, cement and granite were also thoroughly mixed with water and cured in NBU (Nutrient Broth Medium) which is the control. Cubes were cured after casting and demoulding at room temperature until compression testing at intervals of 7, 14, 21 and 28 days. Compression testing was carried out using an automatic compression testing machine of 1000KN capacity as per IS 515:1959. Scanning Electron Microscopy (SEM) analysis was made on the broken sample of a 28-day concrete cube specimen. The weight loss of the concrete specimen was also determined and presented in Figure 1.

2.6. Modulus of elasticity

The modulus of elasticity of the concrete cubes was determined. It indicates a material's resistance to being deformed when stress is applied to it. According to ACI 318-08 section 8.5 [13], the modulus of elasticity is based on the relationship between the density of concrete and the compressive strength of concrete. The modulus of elasticity for concrete is expressed as shown in Equation 1

$$E_c = w_c^{1.5} \text{x} 0.043 \sqrt{f_c} \text{N/mm}^2 \quad (1)$$

Where;

E_c is the Modulus of elasticity for concrete

W_c is the Density of concrete

f_c is the Compressive strength of concrete at 28 days

3. Results and Discussion

3.1. Microbiological analysis of wastewater samples

3.1.1. Isolation and Identification of Microorganisms in Wastewater

This study shows the total plate counts (TPC) of bacteria growth in colony-forming units per 100 ml (CFU/100ml) isolated from each wastewater sample. Sample C had the highest number of bacteria growth, having a total count of 1.05 x 10^9 CFU/100ml, followed by sample A having count of 1.16 x 10^8 CFU/100ml and sample B had the least number of bacteria growth with a count of 2.30 x 10^7CFU/100ml as shown in Table 1. Three different bacteria isolates were identified in sample A which were designated A1, A2 and A3, having total plate counts of 5.80 x 10^7, 1.80 x 10^7 and 4.00 x 10^7 respectively. Three different bacteria isolates were also identified in sample B which are designated B1, B2 and B3, having total plate counts of 1.64 x 10^7, 6.00 x 10^5 and 6.00 x 10^6 respectively. Also, three different bacteria isolates were identified in sample C which is designated C1, C2 and C3, having total plate counts of 1.90 x 10^5, 1.00 x 10^5 and 1.05 x 10^9 respectively as shown in Table 1[14-15]. Biochemical tests carried out on the bacteria isolated from the wastewater samples showed that all bacteria isolated from samples A and B are Gram-positive, two bacteria from sample C are Gram-positive and one bacterium is Gram-negative as shown in Table 2.

Table 1. Total Plate Count (TPC) of Bacteria in CFU/ 100 ml

SAMPLE	TPC (CFU/ 100 ml)	Colony count (CFU/ 100 ml)
A	1.16 x 10^8	A1 = 5.80 x 10^7
		A2 = 1.80 x 10^7
		A3 = 4.00 x 10^7
B	2.30 x 10^7	B1 = 1.64 x 10^7
		B2 = 6.00 x 10^5
		B3 = 6.00 x 10^6
C	1.05 x 10^9	C1 = 1.90 x 10^5
		C2 = 1.00 x 10^5
		C3 = 1.05 x 10^9

Table 2: Identification of Isolated Microorganisms

Isolate code	Shape	Arrangement	Gram Reaction	Catalase	Coagulase	Maltose	Glucose	Lactose	Sucrose	D. Mannitol	Fructose	Galactose	Probable organism
A1	cocci	cluster	+	+	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
A2	cocci	cluster	+	+	-	+	+	+	+	-	+	+	*Staphylococcus. epidermis*
A3	short rods	chains	+	+		A	A	-	A		A	-	*Bacillus* species
B1	cocci	cluster	+	+	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
B2	long rods	chains	+	+		A	A	-	-	+	A	-	*Bacillus* species
B3	cocci	cluster	+	+	-	+	+	+	+	-	+	+	*Staphylococcus epidermidis*
C1	short rods	singly	-	+		AG	A	A	A	+	A	-	*Klebsiella* species
C2	cocci	cluster	+	+	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
C3	cocci	cluster	+	+	-	+	+	+	+	-	+	+	*Staphylococcus epidermidis*

3.1.2. Effect of Concrete on Staphylococcus epidermidis

This study shows that the growth of *S. epidermidis* isolated from wastewater samples inoculated into a broth culture medium increased when counted at 7 days intervals, and decreased after 21 days. The exponential increase in the growth of the bacteria at 21days is a result of the presence of available nutrients in the broth medium and the decrease in growth after 21days is due to depletion in the available nutrients and accumulation of toxic waste products in the medium which align with the works of Bridges *et* al [16] that DNA damage is responsible for many of the mutations arising in the genomes of stationary phase or starving bacteria. The total counts of the bacteria were 35.00 x 10^3, 82.00 x 10^3, 260.00 x 10^3 and 60.00 x 10^3cfu/100 ml after 7, 14, 21 and 28 days respectively (Table 3). The growth of *S. epidermidis* inoculated into a broth medium containing concrete decreased when counted at 7-day intervals. The total counts of the bacteria were 29.00 x 10^3, 24.00 x 10^3, 10.00 x 10^3 and 1.00 x 10^2 after 7, 14, 21 and 28 days respectively as shown in Table 3. This can be due to the presence of toxic chemical substances in the concrete which inhibit the growth of the bacteria in the medium [17].

Table 3: Total Plate Count (TPC) of Bacteria in CFU/ 100 ml

Date	Concrete Colony count (CFU/ 100 ml)	No Concrete Colony count (CFU/ 100 ml)
25/09/2019	29.00 x 10^3	35.00 x 10^3
3/10/2019	24.00 x 10^3	82.00 x 10^3
10/10/2019	10.00 x 10^3	260.00 x 10^3
17/10/2019	1.00 x 10^2	60.00 x 10^3

3.2. Properties of concrete

3.2.1. Weight loss

The result of the average weight loss of the concrete specimen is shown in Figure 1. The percentage of weight loss is plotted against age. It can be seen from the graph that there is an increase in the percentage loss of the concrete specimen with age. The percentage weight loss of the concrete specimen increases with age which is in line with the report of Jorge *et al* [18]. The result showed that the greatest reduction in weight loss in all the days occurs in specimen D, having a value of 4.2% at 28 days. This may be due to the fact that *S. epidermidis* must have utilized some of the constituents of the concrete, thereby reducing the average weight of the concrete. The result also shows the healing effect of *B. subtilis* and the destructive effect of *S. epidermidis*. The result as displayed in Figure 1 also shows specimen C containing *S. epidermidis* (destructive microorganism) and *B. subtilis* (healing organism) to lose lesser weight than specimen B containing *S. epidermidis* only. While specimen A and the control having no destructive microorganism show lesser weight loss than both specimens B and D.

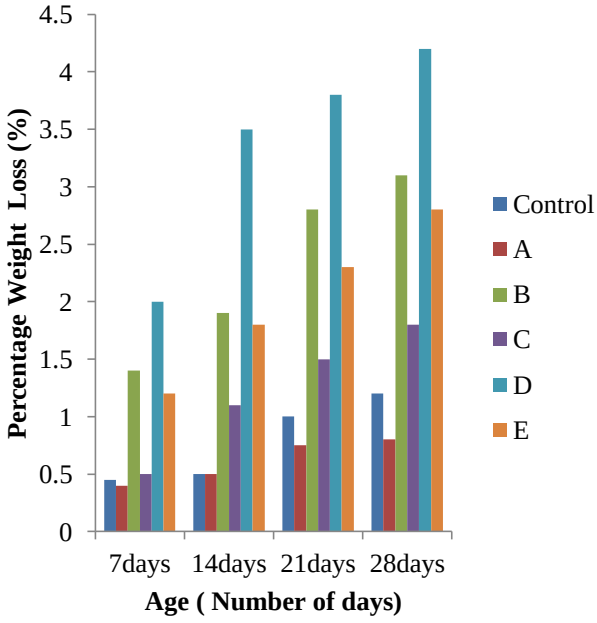

Fig 1. The Variation of Percentage Weight Loss with Age

Key: Control = Concrete cube cast and cured with potable water; A= Concrete cube cast with potable water and cured in nutrient broth; B= Concrete cube cast with potable water and cured in *S. epidermidis* medium; C= Concrete cube cast with potable water and cured in a mixed culture of *S. epidermidis* and *B. subtilis*; D= Concrete cube cast with *S. epidermidis* and cured in nutrient broth; E= Concrete cube cast with a mixed culture of *S. epidermidis* and *B. subtilis* and cured in nutrient broth

3.2.2. Compressive strength

The results obtained from the average compressive strength of the hardened concrete cubes produced are presented in Figures. 2, 3 and 4. It contains the result of the average compressive strength as related to their

various test ages. It could be seen that the compressive strength of the concrete cubes increases with their respective ages for all the concrete specimens.

The variation of the control (concrete specimen cast and cured with potable water) to specimen A (concrete cubes cast with potable water then cured in nutrient broth medium) is shown in Figure 2. It can be seen from the chart that the increase in compressive strength at 28 days for the control specimen and specimen cured in nutrient broth are 18.8 N/mm^2 and 19.14 N/mm2 respectively. Figure 2 also shows that at 28 days, the increase in average compressive strength due to the nutrient broth is 1.79%. This, therefore, justifies the assertion by Varenyam *et al* [19] that the increase in average compressive strength due to nutrient broth is approximately negligible.

The variation of the control specimen compared with specimen B (the concrete cube cast with potable water then cured in *S. epidermidis* medium), specimen C (the concrete cube cast with potable water then cured in the consortium containing *S. epidermidis* plus *B. subtilis)* is shown in Figure 3. The percentage decrease in compressive strength of 10.2% and 3.2% were noticed for specimens B and C respectively at 28 days. From Figure 3, the increase in compressive strength for specimen C compared with specimen B is due to the presence of *B. subtilis* in the nutrient broth medium used to cure specimen C. This result affirms the assertion by Sunil *et al* [12] that *B. subtilis* can increase the compressive strength of concrete.

The variation of the control specimen compared with D (concrete cubes cast *S. epidermidis* then cured in nutrient broth), specimen E (concrete cubes cast with the consortium containing *S. epidermidis* and *B. subtilis* then cured in nutrient broth) is shown in Figure 4. The percentage decrease in compressive strength of 16.69% and 7.1% were noticed for specimens D and E respectively at 28 days. From Figure 4, the increase in compressive strength for specimen D compared with specimen E is due to the presence of *B. subtilis* in specimen D. This result further affirms the assertion by Sunil *et al* [12] that *B. subtilis* has the ability of healing concrete thereby giving it an improved compressive strength when compared with concrete subjected to same condition without *B. subtilis.* As illustrated in Figures. 3 and 4, adding microorganisms to the concrete itself rather than just putting them on the surface causes the microorganisms to have a greater effect on the concrete.

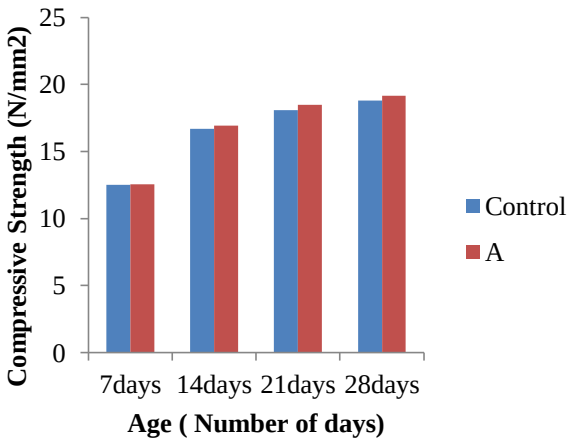

Fig 2. The Variation of the Average Compressive Strength of the Control and Specimen A with Age

Key: Control = Concrete cube cast and cured with potable water; A= Concrete cube cast with potable water and cured in nutrient broth

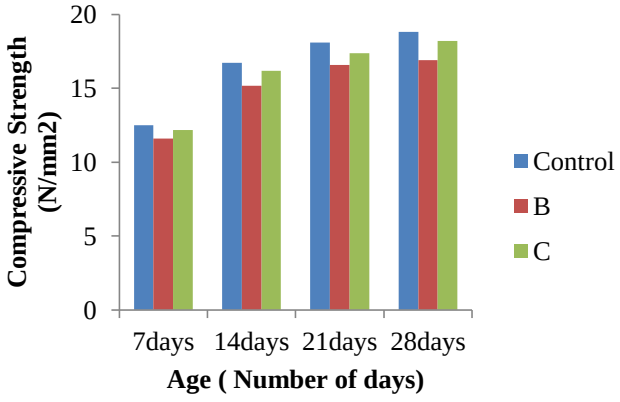

Fig 3. The Variation of the Average Compressive Strength of the Control, Specimen B and C with Age

Key: Control = Concrete cube cast and cured with potable water; B= Concrete cube cast with potable water and cured in *S. epidermidis* medium; C= Concrete cube cast with potable water and cured in a mixed culture of *S. epidermidis* and *B. subtilis*

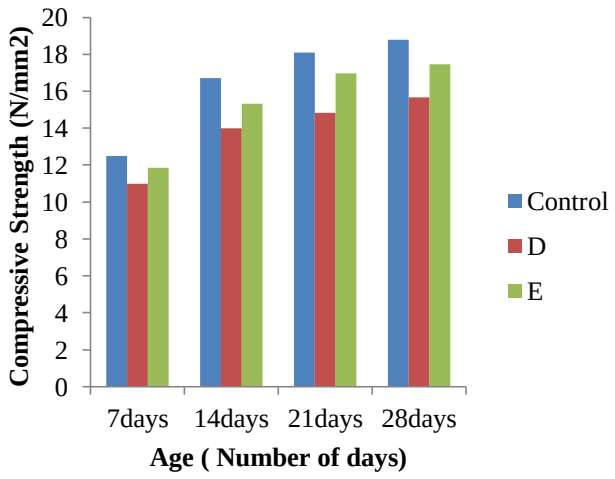

Fig 4. The Variation of the Average Compressive Strength of the Control, Specimen D and E with Age

Key: Control = Concrete cube cast and cured with potable water; D= Concrete cube cast with *S. epidermidis* and cured in nutrient broth; E= Concrete cube cast with a mixed culture of *S. epidermidis* and *B. subtilis* and cured in nutrient broth

3.2.3. Statistical analysis of the compressive strength of the concrete

The result of the LSD post hoc test shown in Table 4 showed that when the OPC was compared with A7 (control), there was no significant difference in the changes that took place on the concrete cubes. This implies that nutrient broth used in curing the concrete does not affect them. The comparison of A7 with B7, C7, D7 and E7 showed significant differences. This is an indication that the microorganisms used in the curing of the concrete cubes have effects on them at 28 days of curing.

3.2.4. Modulus of elasticity of the concrete specimen

The result of the density and modulus of elasticity of the concrete specimen is presented in Table 5. It can be seen from the result that specimen C has the highest modulus of elasticity while specimen D has the lowest modulus of elasticity. It can also be observed that the modulus of elasticity increases with an increase in compressive strength. Therefore, the result shows that the modulus of elasticity of the concrete is in direct proportion to the density of the concrete.

Table 4: LSD Test for Compressive Strength Test for 28-Day Curing.

I	j	MD(i-j)	P	Remark
1	2	-0.34	0.280	NS
	3	1.92*	0.000	*
	4	0.60	0.059	NS
	5	3.14*	0.000	*
	6	1.34*	0.001	*
2	3	2.26*	0.000	*
	4	0.94*	0.007	*
	5	3.48*	0.000	*
	6	1.68*	0.000	*
3	4	-1.32*	0.001	*
	5	1.22*	0.001	*
	6	-0.58	0.067	NS
4	5	2.54*	0.000	*
	6	0.74*	0.025	*
5	6	-1.80*	0.000	*

*Mean Difference is significant at $p < 0.05$, NS= Not Significant, 1-OPC, 2-A7 (control), 3-B7, 4-C7, 5-D7, 6-E7

OPC Concrete cube cast and cured with potable water.

2-A7 (control) - Concrete cube cast with potable water and cured in nutrient broth

3-B7- - Concrete cube cast with potable water and cured in *S. epidermidis* medium

4-C7- Concrete cube cast with potable water and cured in a mixed culture of *S. epidermidis* and *B. subtilis*;

5-D7- Concrete cube cast with *S. epidermidis* and cured in nutrient broth

6-E7- Concrete cube cast with a mixed culture of *S. epidermidis* and *B. subtilis* and cured in nutrient broth

Table 5: Modulus of Elasticity of Concrete Specimen

Parameter	Control	A	B	C	D	E
Density (kg/m^3)	2217	2265	2239	2348	2213	2239
Modulus of Elasticity (N/mm^2)	19,462.38	20,278.78	18,716.98	20,871.39	17,714.81	19,035.83

3.2.5. Scanning electron microscopy analysis of the concrete specimen

The Scanning Electron Microscopy (SEM) of the concrete specimen was conducted with the aid of a Phenom Proxy desktop scanning electron microscope (SEM) in the Materials Analysis and Research laboratory, Ahmadu Bello University, Zaria, Nigeria.

This was used to explain the role and contribution of the bacteria on strength development on the micrographs. The results of the SEM micrographs of the developed samples of control, B and C, D and E are shown in Fig 5.

From Figure 5 (a), (b) and (c), the particles of the control specimen were observed to be closely packed with a minute degree of looseness compared to specimen B and C. Comparing specimen B and C, the particles in specimen C (the concrete cube cast with potable water then cured in *S. epidermidis* medium) as shown in Figure 5 (b) and (c). The increase in pores of Specimen B. S. epidermidis which is in contact with the concrete (specimen B) must have utilized some of the nutrients of the nutrients of the concrete thereby increasing the pores, this invariably leads to the decrease in compressive strength of the concrete as shown in Figure 3. Therefore, the reduction in compressive strength can be attributed to the increase in pores inside the concrete cubes as shown in Figure 5 (b) and (c) respectively which agrees with the result of other research [12, 20, 21] that increase in pores in concrete will reduce the compressive strength of the concrete.

From Figure 5 (a), (d) and (e), the particles in the control specimen were observed to have a minute degree of looseness compared to specimens D and E. Comparing specimens D and E, the particles in specimen E (concrete cubes cast with the consortium containing *S. epidermidis* and *B. subtilis* then cured in nutrient broth) were observed to have a minute degree of looseness compared to specimen D (concrete cubes cast *S. epidermidis* then cured in nutrient broth) as shown in Figure 5 (d) and (e). These results support and explain the results of weight loss and compressive strength. The increase in pores of Specimen D could be a result of the interactions of *S. epidermidis* within the concrete specimen. *S. epidermidis* within the concrete of specimen D must have utilized some of the nutrients of the concrete thereby creating an increase in pores of the concrete compared with the specimen E as shown in Figure 5 (d) and (e). The increase in the compressive strength of specimen E compared with that of specimen D is due to the presence of *B. subtilis* in Specimen E, this justifies the findings of Sunil *et al* [12] that *B. subtilis* enhances the compressive strength of concrete. From Figure 4, it was observed that there is an increase in the compressive strength of Specimen E compared with Specimen D but not up to that of the control specimen. Therefore from Figure 5 (d) and (e), the reduction in compressive strength can be attributed to the increase in pores inside the concrete cubes which are in line with the report of Sunil *et al* [12].

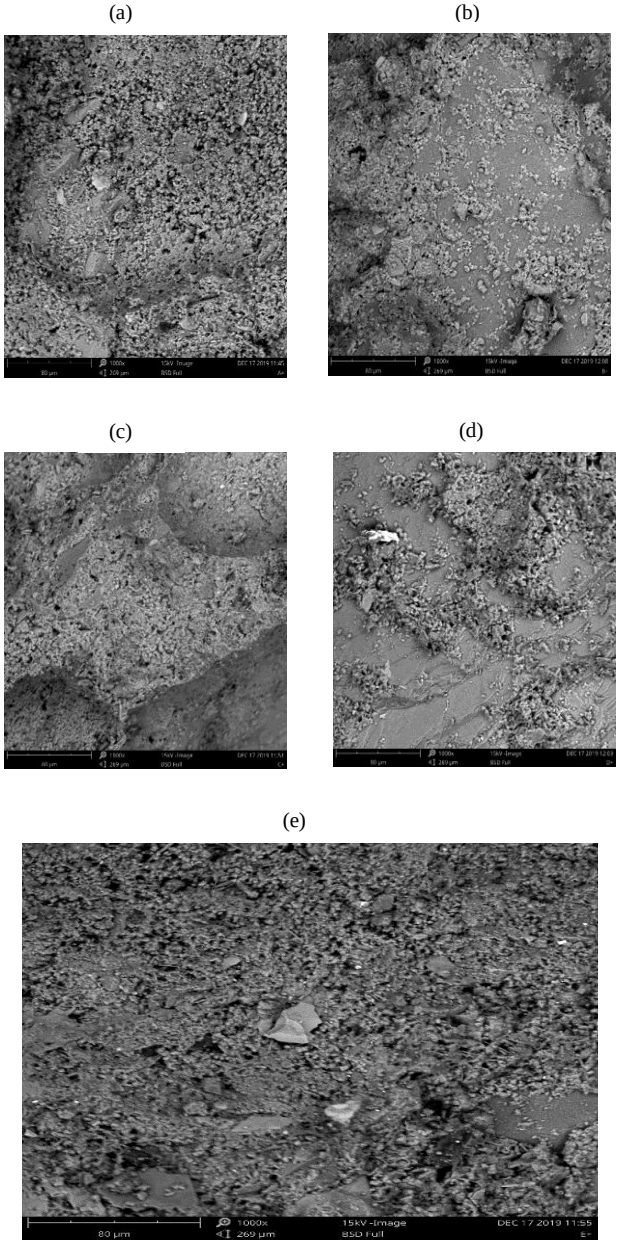

Fig 5. SEM image of Concrete Specimens: (a) Control; (b) Sample B; (c) Sample C; (d) Sample D; (e) Sample E.

4. Conclusion

This research has evaluated the effect of *S. epidermidis* and the consortium containing S. epidermidis and *B. subtilis* isolated from wastewater on concrete performance. This study showed that the bacterium (*S. epidermidis*) had a detrimental impact on the qualities of concrete by decreasing its compressive strength by 16.69% and weight by 4.2% compared to the control at 28 days. The study also showed that B. subtilis improved the strength of the concrete exposed to *S. epidermidis*. Therefore, *B. subtilis* can improve the compressive strength of concrete that has been damaged by negative microorganisms. Scanning Electron Microscopy analysis showed that an increase in the pores within the concrete leads to a reduction in compressive strength. Previous researchers have not compared the effect of microorganisms introduced within and on the surface of the concrete. However, this research has shown that microorganisms have a more significant effect on the concrete when mixed with the concrete rather than introducing it only on the surface of the concrete.

Conflict of Interest

The authors declare that they have no conflict of interest

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