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New Thermoplastic based Composite material for antimicrobial applications

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Abstract: In this work, Nano-Micro composite material is fabricated, from thermplastic plastic powders and treated powder from marble and granite wastes, they were mixed together in the solid state, after drying and sieving. The mixture was heated at the necessary temperature under pressure, based on its components. The cooling processes was done for heated mixture. The new composite material was renowned with the light weight and its capability to tolerate scratch and damping capacity. All experiments were done to achieve very important objective by studying the interaction between the new manufacturing composites and some pathogens reference microorganisms. Pathogenic microorganisms, used in the current study, were obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA). One gram-positive bacteria, two gram-negative bacteria and one pathogenic fungus were applied. The analysis of results were done by SEM and EDX techniques. The effect of different types of micro-organism on the decomposition, chemical composition and structure distribution of both types of composite with marble and granite filler were done. Comparison is done between the effect of microorganisms on the two types of composite were investigated. The research end with conclusions and recommendation for future development in the composite material manufacturing techniques which may increase its resistance to micro-organism during life time and facility its safe decomposition during sanitary landfill.

Keywords: Composite Material, Waste, Antimicrobial, Microorganisms, Pathogen.

1 Introduction

Granite & Marble mining and industrial process are one of the most important business areas for the mining sector with a despicable growth in the world production for about 6 % yearly in the last 10 years [1]. The global exchange is approximately US$ 6 billion / year and about US$13 billion, taken into account tools and equipment [2]. With concentrates a large amount of marble and granite process industries, responsible for producing of hundreds tons of wastes in the environment yearly [3]. Actually, even more serious by increasing production in the last decade, receiving attention from all society with the purpose of disposal wastes. The marble and granite waste in powder form with very fine size in micro and nano scale can be used as filler in composite material with polymer based [4]. The manufacturing of biodegradable composite material with polymer matrix has been increasing in recent years, specifically toward various biomedical applications as these materials not only serve the desired purpose but also get eliminated from the body due to their biodegradable nature [5]. Ideally, a material should possess specific physical, chemical, biological, functional, biomechanical, and degradation properties that fit a particular biomedical application [6]. The importance of biodegradable composite material with polymer matrix in building application in elimination of contamination inside building is very important topic, it should be noted that introducing new polymeric materials such as nano composite with polymer matrix requires extensive development, which may take several years and involve a significant financial investment. Thus, the trend has been to rely on varying compositions and modifications of building elements used biodegradable materials such as floor and wall covering, raised floor and ceilings. Special areas like intensive care and operation rooms needs special types of biodegradable composite materials [7]. The safe sanitary land fill of composite material needs also extensive study for the advancement in construction and superior properties attained in biodegradable constituents. They mostly highlighted on the

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suitability of bio-based polymer mixtures as substitutes for conservative synthetic polymers. The main problem stems from the plastics fact have become one of the major pollutants of recent times. Due to the non-degradable ubiquity of plastics associated with products used in everyday life [8,9].

2 Materials and Methods

2.1 Materials

The matrix is polymethyl methaacrylate (PMMA) $C_5H_8O_2$. It is a thermoplastic prepared monomer from (methyl methacrylate) by addition of polymerization process; polymer was in the granule form with 5 mm diameter.

2.2 Filler (Marble and granite Wastes)

Marble and granite refuses consists wet refuse (sahla) (wet wastes): It is a type of the wet refuse where its percentage of water reaches about 70%. It is sticky and it is produced from the processes of leveling and polishing.

Marble content CaO 30%, SiO_2 30%, MgO 25%, Fe_2O_3 +FeO 3%, Losses

Granite content SiO_2 72 %, Al_2O_3 14%, K_2O, Na_2O, CaO, FeO, losses.

2.3 Composite material Manufacturing

Plastic powders and treated marble and granite wastes were mixed together in the solid state. The manufacturing process is done in special machine designed for this purpose. The machine achieves all manufacturing quality parameters [10].

2.4 Preparation of pathogenic microbe's cultures and making interaction with produced composites

Pathogenic microorganisms, used in this study, were obtained from the American type culture collection (ATCC; Rockville, MD, USA). Two gram-positive bacteria, two gram-negative bacteria, yeast and pathogenic fungus were applied [10,11]. The details of pathogenic microorganisms subjected for this study were represented in Table (1).

Table 1: Pathogenic microorganisms used for evaluation of the developed composites

Type	Microorganisms
Gram +ve Bacteria	*Bacillus cereus* ATCC- 12228
Gram -ve Bacteria	*E. Coli* ATCC- 25922
	Salmonella typhi ATCC 15566
Fungi	*Aspergillus niger* ATCC- 16888

2.5 Culture medium and inoculums

A loopful of bacterial growth from culture slope was inoculated into Minerals salt medium (MSM) broth medium and incubated at 30˚C under shaking conditions (100 rpm) for 48 h. After incubation period, 100 µl of growing cultures were inoculated into flasks (250 ml) containing 100 ml of MSM broth medium and the treatments (surface mechanical attrition treated sheets at times and frequencies, also etched as treated surfaces) were added to flasks [12,13]. The inoculated flasks were incubated at 37˚C for 7 days under shaking conditions with speed (120 rpm). In case of fungal strain, 100 µl of spore suspensions were inoculated into flasks (250 ml) containing 100 ml of MSM broth medium (pH 5.5) and the sheets were added. The flasks were incubated under shaking conditions for 7 days at 30˚C.

The microorganism growth was recorded by two methods: -

1. Weighting the cell biomass daily by digital electrical balance of fungal strain and measuring the changing in cultures optical density using spectrophotometer (Jenway UV/Visible- 2605 spectrophotometer, England) at wavelength 550 nm [45].
2. Measurement of the changing in pH using digital Orion pH meter (model 420A).

Composite material exposed to microorganism in disc shape with 10mm length and 16mm diameter.

2.6 Scanning Electron Microscope (SEM)

The specimens were examined by scanning electron microscope (SEM) operating at a nominal accelerating voltage of 30kv. Specimen preparation is very simply accomplished by cutting a thin slice of the specimen containing the surface of interest, the samples were inserting into the specimen chamber for direct examination of the effects microorganisms on the structure [14].

2.7 Energy dispersive X-ray "EDX"

The quantitative method of elemental analysis of the samples has been examined by SEM JSM-T200 at 25KV acceleration voltage, 20mm working distance, and magnification 200x, (1peak omitted 0.02 KeV). Each value is at least an average of 2 readings [15].

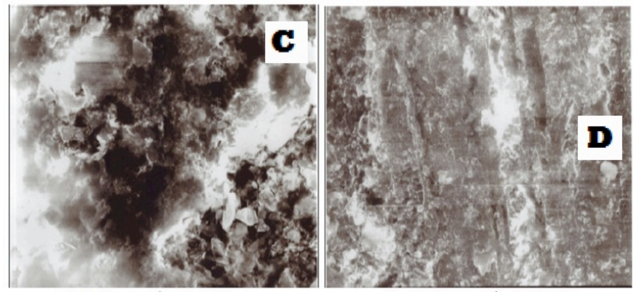
3. Results and Discussion

The interaction between composite structures under consideration and different types of microorganism in the study range (ATCC; Rockville, MD, USA). were evaluated by studying the influence of pH and weight change after soaking of composite in standard media for 7 (seven) days at specific temperature 37° C under shaking conditions with speed (120 rpm). Table (2) shows the interaction between microorganism and composites. From the table, we can notice that slight change in PH value was recorded for both bacteria gram + and bacteria gram negative, the change ranges from 0.2 for bacteria gram - and 0.4 for bacteria gram +. The change in basicity for composite structure due to interaction with fungi (*Aspergillus niger* ATCC-16888) was pronounced the media become acidic and PH is 2.5. The behaviour of composite was investigated along a continuous pH gradient, and the weight change corresponding to the change in PH introduce medium increase for bacteria gram + and bacteria gram - respectively, while swelling leads to increase in weight was pronounced for *Aspergillus niger* fungi [16,17].

Table 2: The interaction between microorganism and composites.

Properties		PH		weight loss(gm)	
Microorganism		Original conditions	After 7 day	Original conditions	After 7 days
Bacteria +	Control	7	6.6	0.91	1.13
	B.C Bacillus cereus ATCC-12228	7	6.7	0.824	1.214
Bacteria -	Control	7	6.6	1.3	1.143
	E.coli ATCC-26922	7	6.7	1.29	1.347
	Control	7	6.8	0.778	1.18
	Salmonella typhi ATCC-15566	7	6.8	0.727	1.114
Fungi	Control	5.5	2.3	0.25	0.7
	Aspergillus.niger ATCC-16888	5.5	2.5	0.21	0.64

The weight increased from 0.21 to 0.64 gm. The change in PH and weight due to the decomposition process, the kinetics of decomposition of composite structure divided into two parts decomposition of matrix PMMA and decomposition of filler ceramics materials Marble and granite [18]. Decomposition was studied by several differing SEM for qualitative analysis and as shown in Figure (1).



C-B.C gram $^{+}$ of Marble D- B.C gram $^{+}$ of Granite

Figure 1: SEM of composite material with PMMA matrix reinforced by marble and granite filler exposed to bacteria gram $^{+}$, *Bacillus cereus* ATCC-12228; (C) Marble filler with *B.C* (*Bacillus cereus*), (D) Granite filler with *B.C.*

EDX elemental analysis for quantitative analysis and for evaluation of elemental change in chemical composition as shown in Figure (2). Table (3) shows EDX elemental analysis composite material with PMMA matrix reinforced by marble and granite filler exposed to bacteria gram $^{+}$.

The change in carbon in carbon content in the structure introduce indication about the interaction between matrix and bacteria gram +, Carbon is the main elements in PMMA structure while both types of filler (marble and/or granite) are free from carbon contents. Change in oxygen content express interaction between B.C bacteria gram + and both of matrix and two types of filler. Decomposition can be make problem on a large or small scale. Organic word describes any material made up of molecules containing carbon and hydrogen atoms [19].

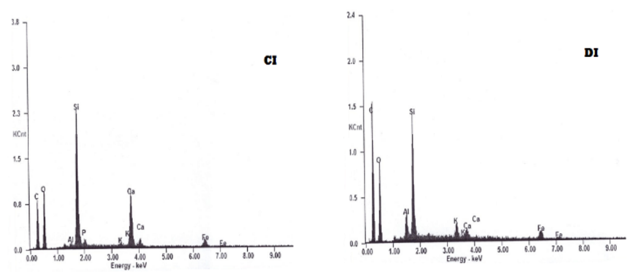


Figure 2: EDX of composite material with PMMA matrix reinforced by marble and granite filler exposed to bacteria gram $^{+}$ *Bacillus cereus* ATCC-12228; CI- Marble filler with *B.C*, DI- Granite filler with *B.C.*

Table 3: EDX elemental analysis composite material with PMMA matrix reinforced by marble and granite filler exposed to *Bacillus cereus* ATCC-12228.

Element	Wt %			At %		
	Control	Marble Treated with *B. cereus*	Granite Treated with *B. cereus*	Control	Marble Treated with *B. cereus*	Granite Treated with *B. cereus*
C	42.99	48.70	61.98	55.55	61.11	71.42
O	36.46	31.43	27.79	35.38	29.61	24.04
Na	00.73	ND	ND	00.49	ND	ND
Mg	01.33	ND	ND	00.85	ND	ND
Al	01.02	00.28	1.56	00.59	00.16	0.8
Si	03.25	11.63	05.95	01.80	06.24	2.93
Cl	00.53	ND	ND	00.23	ND	ND
K	00.70	00.28	00.84	00.28	00.11	00.30
Ca	11.23	05.52	00.54	04.35	02.07	00.19
Fe	01.76	01.65	1.34	00.49	00.45	00.33
S	ND	ND	ND	ND	ND	ND
P	ND	00.52	ND	ND	00.25	ND

The matrix of the composite material under consideration is PMMA, which is organic matter. The decomposition process is completed the number of sub-processes. Consider the decomposition of matrix which is the binding element in the structure, it breaks the matter into smaller pieces in a process called fragmentation. This is an important step, because smaller fragments have more surface area, distortion in structure due to exposure to standard moisture media increase the distance between particles and matrix so swelling was occurred and increase in weight due to water absorption was recorded in the structure [20]. The interaction of composite material with bacteria gram $^-$ is similar to bacteria gram $^+$. The optical density variation which recorded during incubation time by spectrophotometer as shown in Figure (3).

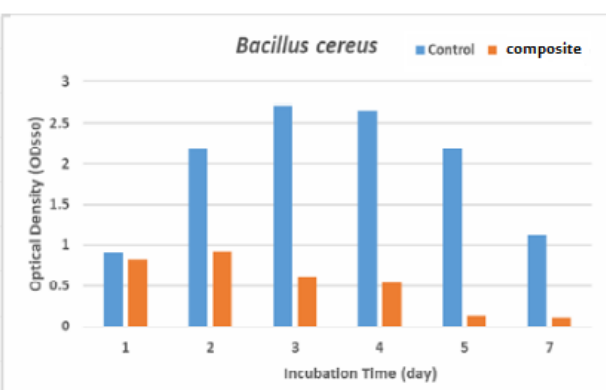


Figure 3: Optical density change during microbial growth for the tested G^+ bacteria

Also, Figure (4) shows SEM of composite material with PMMA matrix reinforced by marble and granite filler exposed to bacteria gram $^-$. While Figure (5) shows SEM of composite material with PMMA matrix reinforced by marble and granite filler exposed to bacteria gram $^-$. Table (4,5) EDX elemental analysis composite material with PMMA matrix reinforced by marble and granite filler exposed to bacteria gram$^-$ (*E-coli*) [21].

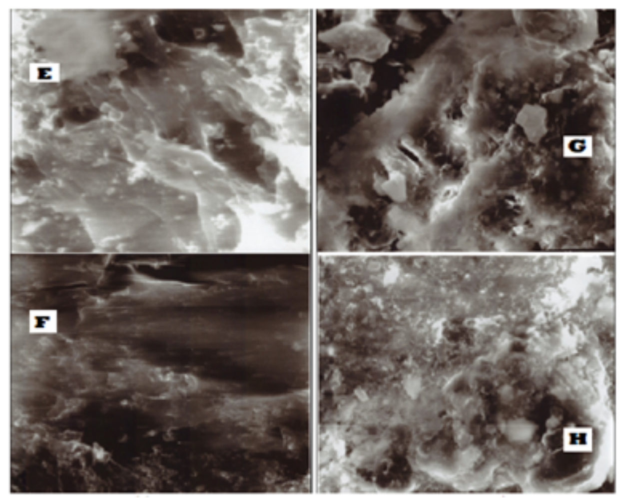


Figure 4: SEM of composite material with PMMA matrix reinforced by marble and granite filler exposed to bacteria gram $^-$ *E. coli* ATCC-25922 -*Salmonella typhi* ATCC-15566; (E) marble filler with *E. coli*, (G) granite filler with *E. coli*; (E) marble filler with *Salmonella typhi*, (G) granite filler with *Salmonella typhi*.

Table 4: EDX elemental analysis composite material with PMMA matrix reinforced by marble and granite filler exposed to bacteria gram -(E. coli ATCC-25922)

Element	Wt %			At %		
	Control	Marble Treated with *E. Coli*	Granite Treated with *E. Coli*	Control	Marble Treated with *E. Coli*	Granite Treated with *E. Coli*
C	42.99	58.57	54.45	55.55	69.33	65.77
O	36.46	29.87	28.44	35.38	26.54	25.79
Na	00.73	ND	1.02	00.49	ND	0.64
Mg	01.33	ND	ND	00.85	ND	ND
Al	01.02	00.35	2.57	00.59	00.18	1.38
Si	03.25	01.28	10.32	01.80	00.65	5.33
Cl	00.53	ND	ND	00.23	ND	ND
K	00.70	00.51	1.29	00.28	00.18	0.48
Ca	11.23	07.14	1.06	04.35	02.53	0.38
Fe	01.76	02.28	0.86	00.49	00.58	0.22

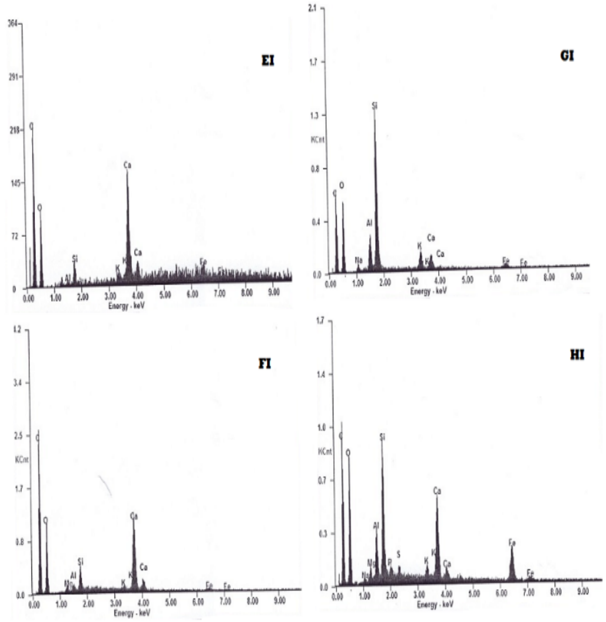


Figure 5: EDX of composite material with PMMA matrix reinforced by marble and granite filler exposed to bacteria gram$^-$ *E. coli* ATCC-25922 and *Salmonella typhi* ATCC-15566; (EI) marble filler with *E. coli*, (GI) granite filler with *E. coli*; (EI) marble filler with *Salmonella typhi*, (GI) granite filler with *Salmonella typhi*.

The condensation under the varying moisture and temperature conditions caused only restrained fungal growth in the materials, it had only a slight effect on the viability of bacteria. The results showed that when water was absorbed by capillary action to the materials, microorganism growth started fast and was abundant in the composite materials. Microorganism contamination caused by its interactions in the composite material is a complex phenomenon because of complex behavior of moisture between the matrix and micro and nano filler from marble and granite filler [22,23]. The variation in optical density for both versions of bacteria gram $^-$ were appear in Figure (6).

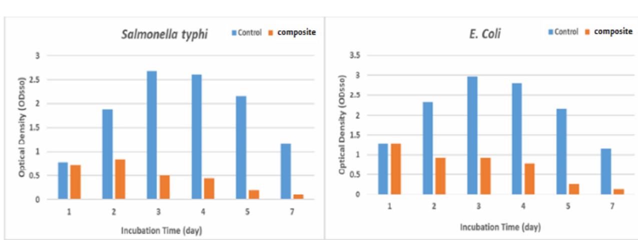


Figure 6: Optical density change during microbial growth for the tested G$^-$ bacteria.

Table 5: EDX elemental analysis composite material with PMMA matrix reinforced by marble and granite filler exposed to bacteria gram$^-$(*Salmonella typhi* ATCC-15566)

Element	Wt %			At %		
	Control	Marble Treated withs. typhi.	Granite Treated withs. typhi.	Control	Marble Treated withs. typhi.	Granite Treated withs. typhi
C	42.99	61.53	53.26	55.55	70.71	65.3
O	36.46	30.25	29.46	35.38	26.10	27.11
Na	00.73	ND	0.4	00.49	ND	0.26
Mg	01.33	00.56	0.83	00.85	00.32	0.5
Al	01.02	00.34	2.27	00.59	00.17	1.24
Si	03.25	01.35	5.2	01.80	00.66	2.73
Cl	00.53	ND	ND	00.23	ND	ND
K	00.70	00.21	0.52	00.28	00.07	0.2
Ca	11.23	05.41	3.83	04.35	01.86	1.41
Fe	01.76	00.35	3.49	00.49	00.09	0.92
P	ND	0.39	ND	ND	ND	0.18
S	ND	0.34	ND	ND	ND	0.16

Certain species of fungi is the engines of the process of decomposition. Fungi is the major organisms decomposing of organic substances, fungi growth is especially affected by fragment size, since fungi can penetrate substances more easily than bacteria or other microorganisms [24]. The drop in the basicity to 2.5 and 2.3 and great increase in weight to 0.7 and 0.64 due to the interaction between composite structure and *Aspergillus niger* ATCC-16888. *A. niger* is cultured for the industrial production of many substances. Various strains of *A. niger* were used in the industrial preparation of citric acid (E330) and gluconic acid (E574) and have been assessed as acceptable for daily intake by the World Health Organization [25]. Figure (7) shows SEM of composite material with PMMA matrix reinforced by marble and granite filler exposed to fungi (*A. niger*), while Figure (8) shows EDX of composite material with PMMA matrix reinforced by marble and granite filler exposed to fungi (*A. niger*). *A. niger* fermentation is "generally recognized as safe" (GRAS) by the United States Food and Drug Administration under the Federal Food, Drug, and Cosmetic Act [26,27]. Many useful enzymes are produced using industrial fermentation of *A. niger*. Some enzymes that breaks down certain complex sugars so the inseams affect the matrix which have carbon hydrogen and carbon oxygen bonds similar to sugar bonds. Another use for *A. niger* within the biotechnology industry is in the production of magnetic isotope-containing variants of biological macromolecules for NMR analysis so its affect both types of filler. *A. niger* is often used as a challenge organism for cleaning validation studies performed within sterile manufacturing facilities [28]. *A. niger* growing from gold mining solution contained cyano metal complexes, such as gold, silver, copper, iron, and zinc which have bond found in fillers such as Silicon oxygen bond and calcium oxygen bonds. The fungus also plays a role in the solubilization of heavy metal sulfides. [29] Alkali-treated *A. niger* binds to silver to 10% of dry weight. Silver biosorption occurs via stoichiometric exchange with Ca(II) and Mg(II) of the sorbent. Table (5) EDX shows elemental analysis composite material with PMMA matrix reinforced by marble and granite filler exposed to fungi (*A. niger*). The decomposition rate cause also changes in optical density during incubation period as shown in Figure (9). In normal conditions degradation due to PMMA material contact with water does not happen for acrylic because it is non-biodegradable material. PMMA is considered as a group 7 plastic which is non-biodegradable grades of plastics. The degradation occurs due to the effect of fungi (*Aspergillus niger*). Development of new technique for recycling of PMMA can be achieved by its interactions with fungi. There are several ways to recycle PMMA. Often these recycling processes involve pyrolysis, in which the PMMA is extremely heated in the absence of oxygen. Another procedure involves depolymerization of PMMA using molten lead to obtain the monomer MMA in a purity >98%. However, this recycling process is not environmentally viable due to use of lead and production of harmful byproducts and therefore limiting the use of the acrylic [30,31].

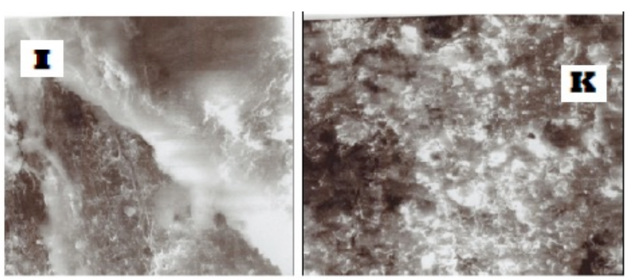


Figure 7: SEM of composite material with PMMA matrix reinforced by marble and granite filler exposed to fungi (*Aspergillus niger* ATCC-16888); (I) Marble filler fungi (*A. niger*), (K) Granite filler with *A. niger*.

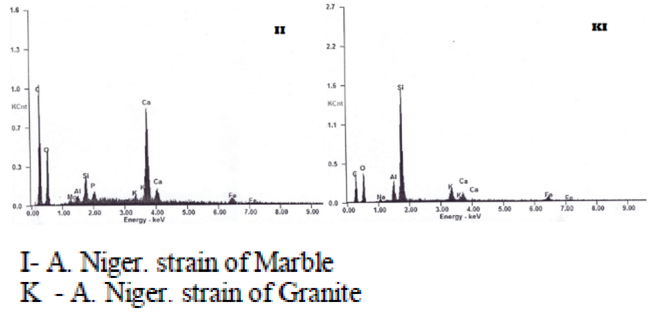


Figure 8: EDX of composite material with PMMA matrix reinforced by marble and granite filler exposed to fungi (*A. niger* ATCC-16888); (II) Marble filler with *A. niger*, (KI) Granite filler with *A. niger*.

Table 6: EDX elemental analysis composite material with PMMA matrix reinforced by marble and granite filler exposed to fungi (*A. niger* ATCC-16888).

Element	Wt %			At %		
	Control	Marble Treated with *A. niger*	Granite Treated with *A. niger*	Control	Marble Treated with *A. niger*	Granite Treated with *A. niger*
C	42.99	58.36	51.45	55.55	69.29	64.78
O	36.46	28.87	24.22	35.38	25.74	22.89
Na	00.73	ND	00.75	00.49	ND	00.50
Mg	01.33	00.41	ND	00.85	00.24	ND
Al	01.02	00.58	2.91	00.59	00.30	01.63
Si	03.25	01.85	15.82	01.80	00.94	08.52
Cl	00.53	ND	ND	00.23	ND	ND
K	00.70	00.36	2.05	00.28	00.13	00.79
Ca	11.23	07.95	1.24	04.35	02.83	00.47
Fe	01.76	01.09	1.56	00.49	00.28	00.42
P	ND	00.53	ND	ND	00.25	ND

Precautions must be considered when using composite material with PMMA matrix with marble and granite filler in any application may contain media have microorganisms. Material development must be done by addition of new compound to become hybrid composite, the new compounds must have toxicity to kill all types of microorganism and prevent its interactions with both matrix and fillers [32].

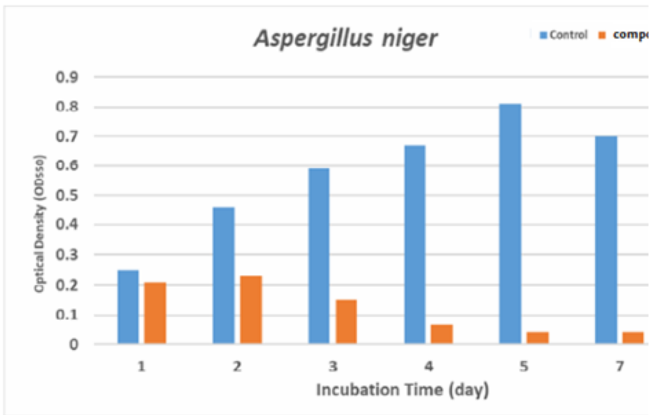


Figure 9: Optical density change during microbial growth for the tested fungi (*A. niger*).

Conclusions

1. Decomposition due to interaction between microorganism and composite material with PMMA matrix in moisture media can introduce new method for recycling of PMMA and its compounds.
2. The new recycling method change PMMA to become biodegradable material, it can be developed by using Fungi in certain media specially *Aspergillus niger* ATCC-16888
3. Interaction between fungi and composite structure can change PMMA from non-biodegradable material to biodegradable one. high demand for biodegradable alternatives has arisen in different fields and industries as an attempt to achieve a more environmentally
4. Both of PMMA matrix and filler from marble or granite powder were interact with different types of microorganism with different rates The interaction with bacteria gram $^{-}$ *E. coli* ATCC-25922 and *Salmonella typhi* ATCC-15566, and bacteria gram $^{+}$ *Bacillus cereus* ATCC-12228 is very slight but can affect the matrix.
5. Using of new material with PMMA matrix and granite and /or marble filler must be limited in the areas have microorganisms which can cause its degradation and change in morphology as results of change in structure and composition.

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