Microbial Formation on Different Biomedia Surface for Wastewater Treatment

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***Abstract*—Even though commercial plastic media such as Cosmoball successfully treated municipal wastewater without clogging, its inadequate surface area prevents its use for high biofilm growth rates. This study compares microbial formation on the activated carbon (AC)-coated and non-coated Cosmoball media by submerging four activated carbon AC-coated Cosmo balls and non-coated Cosmo balls in five-liter beakers containing municipal wastewater. The researchers then analyzed the surface morphology of the biofilms on the coated and non-coated surfaces and applied the next-generation sequencing (NGS) technology to the ecology of microbial-mediated processes. By Day 11, the AC-coated media showed a biofilm coverage of 95%, whereas the non-coated media had a biofilm coverage of about 70%. The research has demonstrated that the coated media has a four times higher surface area, thus saving as much as 50% of the aeration tank volume.**

***Keywords*—microbial growth, biomedia surface, wastewater, bacteria**

I. Introduction

Wastewater is the used water generated by the community from various activities and contains impurities or pollutants in the form of solids, liquids, and gases in concentrations that are harmful if disposed of in the environment. Water quality has been receiving increasing attention globally. People need the best quality water for their everyday lives. Water requires treatment to make it safe for human and living beings consumption. There is also an urgent need for a better and more compact wastewater treatment system. Given the high cost, lack of land availability, and the implementation of secondary treatment standards, it is vital to develop wastewater treatment plants with small footprints capable of producing high-standard effluent while meeting the minimal waste requirements [1]. The need for high biofilm growth rates makes commercial plastic biomedia unsuitable due to their limited surface area.

Previous research has demonstrated that aerobic microorganisms survive primarily on carbon particle surfaces, and most do not thrive in carbon pores [2–4]. Eltawab *et al*. [5] investigated the tertiary treatment of wastewater via filtration technique using sand, anthracite, Granular Activated Carbon (GAC), and rice straw, where the sub-base of the gradual gravel support each layer in terms of its efficiency in removing the different pollutants. The results showed that, even though the granular activated carbon (GAC) filter has a lower efficiency than anthracite, it has a satisfactory removal efficiency for the parameters. Al-Jlil [6] established the performance of sedimentation, aeration, activated sludge, sand filter, and activated carbon in reducing the Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) in domestic wastewater. The result showed a 92.1% and 97.6% mean maximum COD and BOD reduction, respectively. Researchers have successfully used adsorption with activated carbon (AC) in an advanced (tertiary) treatment of municipal and industrial wastewater [7–9]. Surface roughness is a critical factor in the bacterial adhesion process. The roughness of a surface encourages bacterial adhesion and the formation of biofilms. Some authors believe that surface irregularities increase surface roughness, which provides shelter to the bacterial cells by promoting their attachment [10]. Studies have shown that the force of bacterial adhesion increases with higher roughness of the substratum surfaces. Congruent with these studies, Quirynen *et al.* [11] reported no significant effect on microbial composition when the surface roughness is less than 0.2 µm.

The increasing human population has resulted in a higher flow and organic wastewater, making it necessary to build big Sewage Treatment Plants (STP) for treating organic wastewater pollutants, thus causing economic problems. Moreover, the limited surface area of commercial plastic media does not allow their use in cases that require high biofilm growth rates.

An alternative design for compact treatment plants with a high surface area is employing activated carbon to coat plastic media, for instance, Cosmo balls, and using them as biofilm media. This method increases treatment efficiency by increasing bacterial growth on their surfaces. The long residence time for treating organic wastewater pollutants poses economic problems in terms of the large land area required for constructing a large-volume basin. Therefore, it is crucial to develop new, rougher materials that enhance the operational efficiency of wastewater treatment by increasing the area for solid retention area. This approach also has the economic benefit of the smaller land area needed to operate wastewater treatment plants. Therefore, this study compares the microorganism formation on a coated against a non-coated surface as a biomedia surface for municipal wastewater treatment.

II. Material and Methods

A. *Cosmo Ball Biomedia*

Cosmo ball biomedia is widely used in wastewater processes. The principle in using Cosmo balls is that they

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serve as light, floating carrier elements that provide a surface for the microorganisms in a mixed reactor to grow. The suspended biofilm carriers were sourced from Universiti Putra Malaysia (Serdang, Malaysia) [12]. The Cosmo ball elements were fabricated using a strong HDPE polyethylene plastic, which has a density that is 75 kg/m^3 lower than water density. They are shaped like a ball, with a diameter of 85 mm, and have cross and longitudinal fins on the internal and external surfaces of the balls. The Cosmo ball carriers have a specific biofilm-protected surface area of 160 m^2/m^3 bulk volume of carriers with a filling ratio of 65%. Fig. 1 shows the elements of a Cosmo ball biofilm carrier.

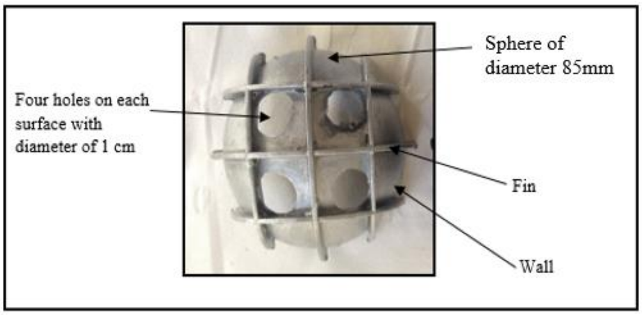


Fig 1. The actual Cosmo ball model.

B. Coating Method

The Cosmo balls were coated with 100–800 μm granular activated carbons. The researchers used distilled water to wash the activated carbon to remove ash that might block their pores and dried it at room temperature for one day before using it as a surface coating. We used the epoxy method to synthesize the AC coating, where the epoxy was brushed onto the HDPE as a base layer for the AC to stick on. After spraying the AC onto the substrate, they were left to dry at room temperature for three hours. The last step was applying a layer of 0.76 mm thick activated carbon to the surface.

C. Analytical Equipment

The Scanning Electron Microscope (SEM) is an electron microscope that scans a sample with a focused electron beam to produce a sample image. The electron generates detectable signals as it interacts with the atoms in the AC sample. The generated signals provide information on the sample's surface topography and composition [13]. SEM is an essential and valuable method for determining the shape and surface topography (surface morphology, homogeneity, and thickness). This research used SEM to determine the shape and coating thickness of the AC.

D. Batch Experiment

This research used two 5000 mL beakers to conduct the aerobic batch experiments. One of the beakers contained four AC-coated Cosmo balls, while the other had four non-coated carriers. The researchers determined the degradation of BOD and COD at 26 ± 2 ºC and observed the bacterial formation and growth on the carriers. We removed any compounds on the Cosmo ball by gently rinsing them with deionized water. Each beaker contained about five liters of domestic wastewater, and an air diffuser at the bottom of the reactor provided aeration. The supernatant samples were taken periodically to determine the BOD and COD concentrations.

E. Bacterial Community Analysis of Biofilm

The biofilm bacterial communities and their percentages were determined using next-generation sequencing technology. The test amplified a particular region (V3-V4) in the (16S) gene present only in bacteria. The procedure comprises four steps: DNA extraction with the MoBio Power Biofilm DNA Extraction Kit, PCR amplification, Illumina sequencing, and data processing with QIIME (version 1.9.1) [14]. This part is a complicated bimolecular analysis that only specialists can do. The samples were sent to a private company for DNA extraction, illumine sequencing and data processing.

III. Results and Discussion

A. Formation of Biofilm on the Coated and Non-coated Packing Media

Surface roughness is an essential property in the bacterial adhesion process. Fig. 2 shows biofilm growth on coated and non-coated Cosmo balls between Day 2 and Day 11. The biofilm formation began after two days; the biofilm on the coated ball is thicker than that on the non-coated ball (the area circled in red indicates the area with biofilm growth). On Day 4, a thin layer of biofilm covered the whole coated Cosmo ball, while some areas only on the non-coated balls (indicated with red circles) had biofilm formation. By Day 6, the biofilm on the coated Cosmo ball was thicker, while some areas on the non-coated Cosmo ball still have not shown biofilm formation (the area without biofilm formation are marked with yellow circles).

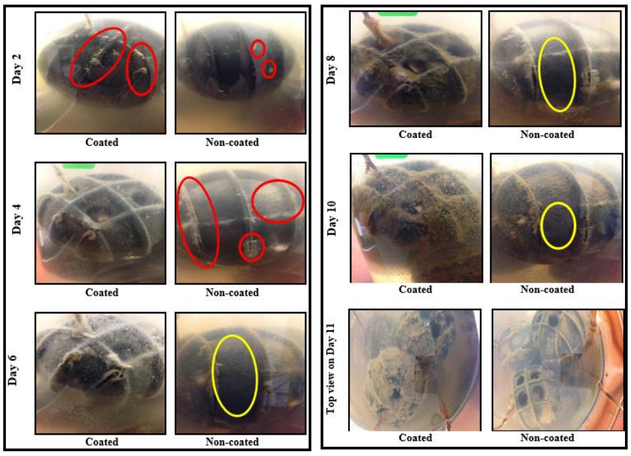


Fig 2. Growth of biofilm on coated and non-coated Cosmo balls.

On Day 8, some areas of non-coated balls were still not completely covered with biofilm, while the coated balls showed an even thicker layer of biofilms. Even after ten days, only a thin biofilm layer covered approximately 70% of the non-coated Cosmo ball. In contrast, a thick biofilm layer covered the entire coated Cosmo ball. After Day 10, visibility has reduced significantly due to biofilm growth on the inner surface of the beaker, making it difficult to view the balls from the side. Therefore, observation was made from the top, and it can be seen from the top view that biofilm has covered 100% of the coated ball and an estimated 70% of the non-coated ball.

A rough surface promotes bacterial adhesion, which in turn results in the formation of biofilm. Some researchers stated that high surface roughness causes irregularities of the surface which provide shelter for bacterial cells, which, in turn, induces bacterial attachment. This is because the surface area is higher on the rougher surface [10]. Several researches have shown that the force of bacterial adhesion increased with the roughness of substratum surfaces. This concurs with the findings of [15], who found that when surface roughness is lower than 0.2 µm, it has no significant effect on microbial composition. In this study, however, roughness increased by more than tenfold and reached 7 µm when AC was applied on the surface of the Cosmo ball.

As can be seen in Fig. 3(a), (b), Scanning Electron Microscopy (SEM) was used to investigate the morphology of biofilms attached to the coated and non-coated surfaces; the coated Cosmo balls with higher surface roughness resulted in the formation of a very crowded, multi-layer bacteria biofilm compared to the single-layer of bacteria attached to the non-coated Cosmo ball. The figure shows that the bacteria species for the coated and non-coated surfaces are very similar. The only exception is concerning the density of the microbial growth. The coated Cosmo ball Fig. 3(b) shows a very crowded distribution of microbes, almost fourfold that on the non-coated surface, as seen in Fig. 3(a). The same observation was reported by previous researchers [15–17].

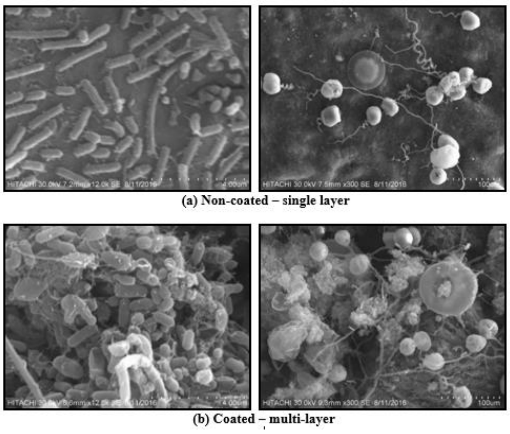


Fig. 3. Bacteria attached to (a) non-coated Cosmo ball and (b) coated Cosmo ball.

B. Microbial Community Analysis

Studies leveraging Next-Generation Sequencing (NGS) technologies provide new insights into microbially mediated processes' ecology. This study identified eight major relative abundance >1% within a biofilm sample of the wastewater. The result of the major phylum assignment showed that the bacterial diversity in both coated and non-coated systems was not different from each other. The same major phyla, namely Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Chloroflex, Saccharibacteria, and Chlorobi, formed on coated and non-coated media, although the ratios for both systems are different. These phyla were similar to that discovered in a study by Meng *et al*. [18] to identify the microbial communities in the parallel coking wastewater treatment systems at the phylum level.

The ratio of phylum for coated and non-coated media is shown in Fig. 4, and the figure shows very clearly that the most dominant bacteria are Proteobacteria (46%) and Bacteroidetes (34%) when coated Cosmo ball was used. In contrast, when non-coated media were used, the ratios were 35% Proteobacteria and 32% Bacteroidetes.

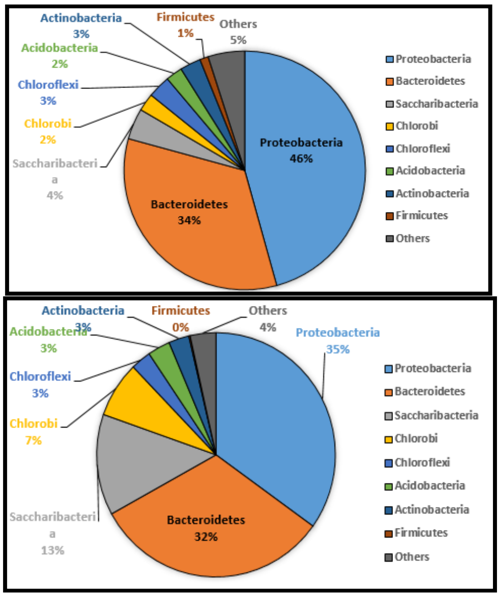


Fig. 4. Bacterial community structures in units at the phylum level, (a) Coated; (b) Non-coated.

In municipal WWTPs, the phylum Proteobacteria predominates (21%–65%), which is the most abundant class and is mainly responsible for the removal of organics and nutrients; the subdominant phyla are Bacteroidetes, Acidobacteria, and Chloroflexi [19–23].

Wan *et al*. [21] investigated the effects of microbial community structure brought by treatment processes for wastewater treatment plant had two sets of processes operating simultaneously, MBR and oxidation ditch. These two systems received identical wastewater, and the samples were collected simultaneously. Their result showed that the most predominant phylum in a sample of MBR was Proteobacteria, which was consistent with the results in other studies [24], followed by Bacteroidetes and Acidobacteria. Notably, the percentage of Proteobacteria was 62.1%. However, the most predominant phylum for sample OD was Proteobacteria, representing 43.9% of the total bacteria. The predominating phyla included Proteobacteria, Bacteroidetes, Acidobacteria, Chloroflexi, Planctomycetes, and Verrucomirobia, which was similar to the microbial community structure in MBR systems

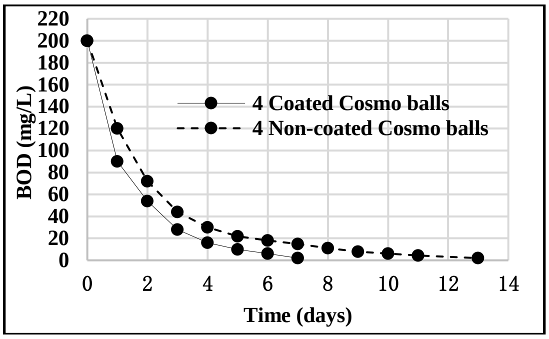
As Proteobacteria and Bacteroidetes are ubiquitous in soil [25], the character of activated sludge appeared similar to that of soil.

The findings of previous studies and this study show that the ratios for Proteobacteria were 46% and 35% for coated and non-coated media, respectively. Hence, the reason for the high performance of coated Cosmo ball in removing organic and nutrients is the high ratio of Proteobacteria which existed on the coated media compared with the low proportion of 10% on non-coated media. In contrast, the other phylum has an insignificant effect on removing organics and nutrients.

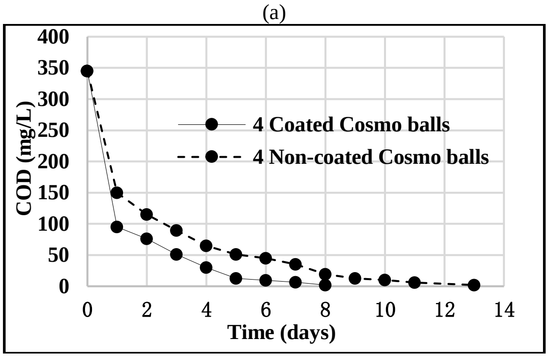
Previous research suggested that microcolonies of Firmicutes were weak and could not resist strong shear imposed on them, unlike Proteobacteria [26, 27]. In these systems studied here, aeration intensities were relatively high. This could explain the observed low abundance of Firmicutes.

C. Degradation of COD and BOD in Batch Experiment

Fig. 5(a), (b) shows the degradation of BOD and COD of organic materials, respectively. The degradation of the BOD and COD of organic material was 15% better on Day 1 for coated Cosmoballs than the non-coated balls. BOD decreased from 200 mg/L to 85 mg/L and 120 mg/L for wastewater treated with coated and non-coated balls, respectively. COD decreased from the 350 mg/L recorded on Day 1 to 90 mg/L and 150 mg/L for wastewater treated with coated and non-coated balls, respectively. Thus, the amount of organic matter degraded by the coated balls was higher than that of the non-coated balls (around seven days for the coated and 13 days for the non-coated balls). Hence, the coated media can reduce the amount of organic materials six days faster than the non-coated media. This is plausible since the surface area of coated Cosmo ball is fourfold that of the non-coated media, while its roughness is more than tenfold that of the non-coated media. This provides shelter as well as more space for the bacteria to grow. This agrees well with the findings made by other researchers where rough surface results in more bacterial growth [10, 16].



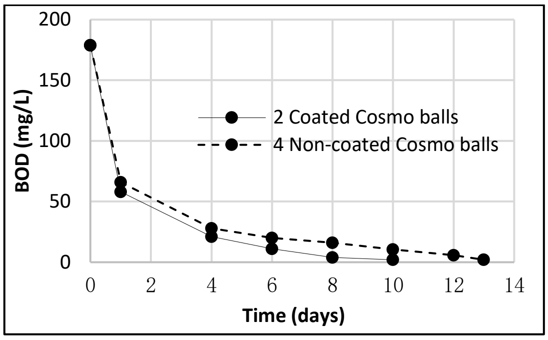
(a)



(b)

Fig 5. Degradation of (a) BOD and (b) COD with time using four coated vs. four non-coated balls.

Fig. 6(a), (b) shows the degradation of COD and BOD for wastewater treated with two coated Cosmo balls in one beaker and four non-coated balls placed in another. On the first day, the degradation of organic matter by the coated balls is slightly higher than that of the non-coated balls, with a reduction of BOD from 180 mg/L on Day 1 to 57 and 63 mg/L for coated and non-coated balls, respectively. COD decreased from 288 mg/L on Day 1 to 67 and 76.8 mg/L, respectively. This indicates that more microorganisms are attached to the coated media than those attached to the non-coated media even when the number of coated media was reduced to half due to the high surface area of the coated balls. Even when comparing the treatment of wastewater by using only two coated balls instead of four, the degradation of organic matter still occurred three days faster.



(a)

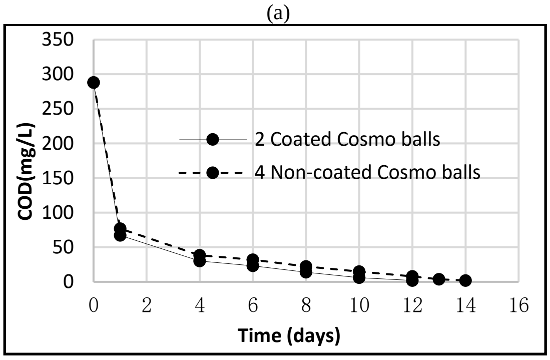
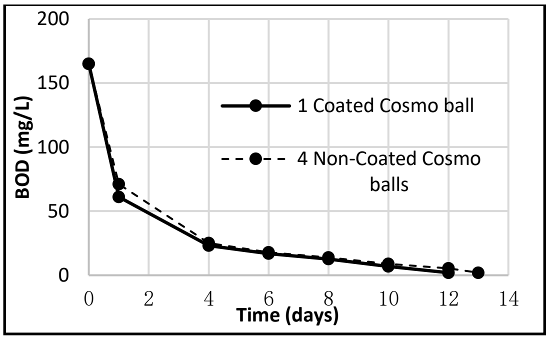
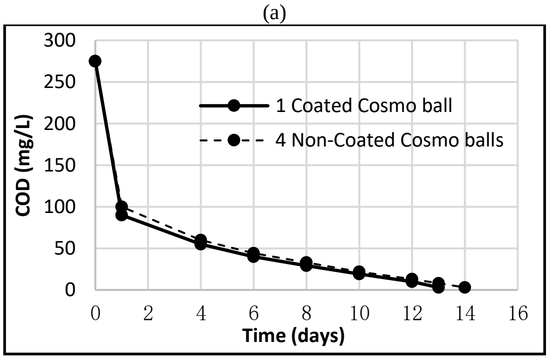


Fig. 6. Degradation of (a) BOD and (b) COD with time using two coated vs. four non-coated balls.

Fig. 7(a), (b) shows that the number of Cosmo balls was further reduced to only one coated ball compared to four non-coated balls. The beaker with one coated ball still shows better wastewater degradation than the container with four non-coated balls, although the amount of degradation was lower than when two coated balls were used. The degradation of organic matters for BOD was from 165 mg/L on Day 1 to 61 and 71 mg/L for coated and non-coated balls, respectively, while the reduction of COD was from 275 mg/L on Day 1 to 90 and 100 mg/L, respectively. Degradation was completed in 12 days when one coated ball was used and 13 days when four non-coated balls were used as the attached media.



(a)



(b)

Fig. 7. Degradation of (a) BOD and (b) COD with time using one coated vs. four non-coated balls.

The result shows that it is now possible to coat the Cosmo ball surface to improve process efficiency (removal of BOD, COD). It also shows that, by coating the Cosmo ball, a smaller number of Cosmo balls is needed to obtain the same result. This finding has significant implications for real treatment plants because future aeration tanks can be made smaller or more compact (lower hydraulic retention time).

Based on these results, the performance of just one coated Cosmo ball could equal the performance of four non-coated Cosmo balls. The surface area of coated balls is estimated to increase fourfold that of non-coated balls. As shown in Fig. 8, the filling ratio of the non-coated Cosmo balls was 65% of the reactor, and based on the increased surface area of the coated balls, this ratio can be reduced by up to 15%, thus saving 50% for upgrading purposes.

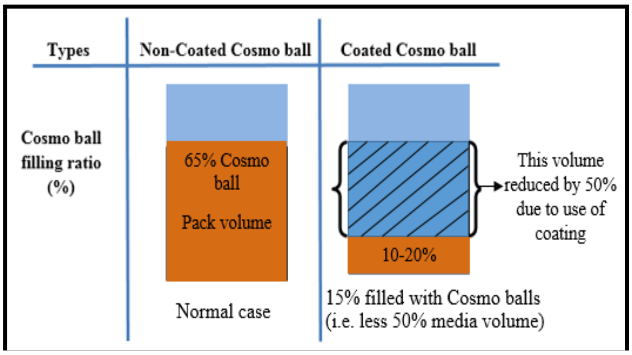


Fig. 8. Highest surface active area.

IV. Conclusion

The study aimed to observe the formation of bacteria on activated carbon-coated and not-coated biomedia for wastewater treatment. The experimental results showed that biofilm had covered more than 95% of the coated balls and an estimated 70% of the non-coated balls. The coated Cosmo ball showed a very crowded distribution of microbes, almost fourfold that on the non-coated surface, and bacterial diversity in both coated and non-coated systems was not different, however, with different ratios. Moreover, the beaker with one coated ball still shows better wastewater degradation than the container with four non-coated balls. This finding has significant implications for real treatment plants because future aeration tanks can be smaller or more compact.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

KH supervised the analysis, supported this work to improve the scientific quality of the manuscript and wrote the paper and RO contributed to analysis under the advice and follow-up of PA who also contributed to data collection. YT supervised the work, proofread and corrected the revised version. All authors had approved the final version.

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