GROWTH OF *Typha angustifolia* AND MEDIA BIOFILM FORMATION IN CONSTRUCTED WETLANDS WITH DIFFERENT MEDIA

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ABSTRACT. *This study was conducted to investigate the effect of various media on: (1) the growth of* Typha angustifolia *in tropical climate and (2) the state of biofilm formation on the media. Five micro-scale constructed wetland units containing five different media, namely pea gravel, oil palm shell, oil palm mesocarp fibre, granular activated carbon and zeolite, with one of each was planted with* Typha angustifolia *plantlets, were fed with sewage and operated at nominal hydraulic retention time of five days. The plant height, shoot count and biomass as well as the pH of each microcosm were monitored throughout the experiment period. At the end of the experiment, the media were taken out and their surface morphologies were examined utilising scanning electron microscope. The results showed that the media affect the growth of* Typha angustifolia *plantlets planted in the media where oil palm shell-based microcosm produced plant biomass gain of 18.0 g* m^{-2} day^{-1} *while pea gravel-, oil palm mesocarp fibre-, granular activated carbon- and zeolite-based microcosms produced plant biomass gains of 12.0, 11.6, 14.2 and 13.8 g* m^{-2} day^{-1}*, respectively. The biofilm formed on the experimented biodegradable oil palm shell and oil palm mesocarp fibre were also thicker and more uniformly distributed than the biofilm formed on the experimented non-biodegradable pea gravel, granular activated carbon and zeolite.*

KEYWORDS. Constructed wetland, media, *Typha angustifolia* growth, biofilm formation

INTRODUCTION

Constructed wetland represents an emerging ecotechnology for water treatment by mimicking the original functions and improving the limitation factors of the natural wetland (Lim and Polprasert, 1998). With proper engineering, the hydraulic conductivity can be improved as well as the efficiency of the treatment system (Shutes, 2001). Recently, constructed wetland has gained interest in South East Asia (SEA) where the tropical climate is an added advantage for plant growth, which is important for the well functioning of the constructed wetland in reference to nitrogen removal (Koottatep and Polprasert, 1997; Lim *et al.*, 2001). Normally, local aquatic plant is chosen due to its natural adaptation with the local climate and availability as well as to mitigate the unnecessary introduction of foreign or new species to the local environment (Calheiros *et al.*, 2008).

Over the years, researchers in SEA have investigated the applicability of constructed wetland technology to remove or treat oxygen demand (Lim *et al.*, 2003a), ammonia (Ahmad *et al.*, 2006a), nitrogen (Ahmad *et al.*, 2006b), phosphorus (Sohsalam and Sirianuntapiboon, 2008; Sim *et al.*, 2008), heavy metal (Chong *et al.*, 2003; Lim *et al.*, 2003b), faecal coliform (Khatiwada & Polprasert, 1999), leachate (Ahmad *et al.*, 2003; Sawaittayothin and Polprasert, 2007), and dye (Tan *et al.*, 2007). Constructed wetland employed for water treatment purposes in SEA is usually the subsurface flow type where cattail *(Typha* sp.) is normally used as the emergent plant because it forms extensive monocultures very rapidly through vegetative reproduction and maintains its dominance with formation of dense rhizomes mats and litter (Motivans and Apfelbaum, 1987).

Another important aspect of constructed wetland water treatment ecotechnology is the selection of appropriate media or substrate (Chong, 2008). Although pea gravel is normally used in SEA (Lim *et al.*, 2003a; Tee *et al.*, 2009), the interest of this study is looking into the possibility of applying alternative media such as oil palm shell, oil palm mesocarp fibre, industrial grade coconut-based granular activated carbon and zeolite, which are available abundantly in this region itself. Nonetheless, the application of alternative media may affect the constructed wetland vegetation as well as biofilm attachment on the media.

In the light of these concerns, the objectives of this work are: (1) to study the effect of various media on the growth of *Typha angustifolia* in tropical climate and (2) to investigate the state of biofilm formation on the alternative media.

MATERIALS AND METHODS

Preparation of media

Samples of spent oil palm shell and mesocarp fibre were collected from an oil palm mill in Nibong Tebal, Penang. Both samples were separately solar dried at 1000 – 1500 hrs for two days consecutively to reduce the moisture content as well as to eliminate unwanted insects and arthropods. Reflective silver canvases were used as the drying base to improve the solar drying efficiency. Solar dried samples that have less offensive smell and pest-free were then stored and further processed as described in the following paragraphs.

Solar dried spent oil palm shell was washed thoroughly to eliminate unwanted soil particles and residual oil under flowing water. It was then soaked overnight, extensively rewashed and rinsed to minimise the residual oil to a steady level. Six days and five nights were spent in accomplishing this task. The cleaned spent oil palm shell sample was then sieved to produce medium in the size range of $1 < Ø \leq 4$ mm. The medium was further rinsed with water to remove the dusty particles resulted during the sieving process. The processed oil palm shell was then dried in the oven at 55°C for 48 h and labelled OPS.

Solar dried oil palm mesocarp fibre was washed thoroughly to eliminate impurities under flowing water. During this washing process, unwanted oil palm shell was removed manually from this sample. Overnight soaking, extensive rewashing and rinsing were performed repetitively until the residual oil was minimised to a steady level. Six days and five nights were taken to accomplish this task. The processed oil palm fibre was then dried in oven for 48 h at 55°C and labelled OPF.

Pea gravel, industrial grade coconut shell-based granular activated carbon and zeolite were bought from a local supplier. These media were sieved to obtain size $1 < Ø \leq 4$ mm. Washing and rinsing were performed to minimise dusty particles of their own parent materials. These media were individually dried at 55°C for 48 h and labelled G, GAC and Zeo, respectively.

Preparation of *Typha angustifolia*

The plant used in this study originated from the wetland area of Penang International Airport. Identification was performed during the fieldwork to ensure samples collected were of *Typha angustifolia*. Twelve plants, in the range of 1.6 – 1.8 m in height were uprooted. Their above-ground portion were cut into 30 cm length (measuring from the roots) and transported back immediately to the university.

The plant samples were then cleaned with flowing water before being transplanted into an 8-ply-fibreglass tank measuring 110 × 235 × 75 cm^3 (width × length × height) which contained a layer of 10 cm G and sewage. The first flowering occurred about four and half months after the transplant.

The seeds from the transplanted plants (first generation) were allowed to germinate in the same nursery tank; however, the entire first generation plants were removed when the second generation plants start to flower. The second generation plants took about six months to flower. The seeds from second generation plants were also allowed to germinate in the same nursery tank.

The third generation plantlets of approximately 30 – 40 cm in length that have exactly five leaves were chosen to be used in the experiments. These plantlets, which are prone to dehydration, were brought back to lab with great caution for documentation.

Excessive water was removed from each plantlet prior to measuring its weight and maximum length of leaf and root. This documentation process was completed in not more than 5 min, as a must, for each individual plantlet in order to prevent dehydration. The documented plantlets were then transplanted into designated microcosms. None of the first, second or third generation plants showed any indication of Typha latifolia, *Typha domingensis or Typha × glauca.*

Preparation of microcosms

The microcosm tank was made of a modified commercial polypropylene (PP) container with the dimension 22.5 × 22.5 cm^2 (top), 21.0 × 21.0 cm^2 (base) and 28.5 cm (height). At the bottom edge of the tank, a tap was securely fitted and nylon mesh was used to cover its opening at the inner side of the tank to prevent media from getting into the tap. Precisely 10 L of water were inserted in each tank and a mark was drawn on the outer wall at this 10 L water level. The water was then drained. The outer part of the tank was later wrapped with aluminium foil to protect it from sunrays as well as to prevent unintended algae growth on the inner side of the tank wall.

Each tank was then loaded with 15.50 kg G, 5.90 kg OPS, 1.85 kg OPF, 6.30 kg GAC and 9.10 kg Zeo to the 10 L marking, respectively. Except for the OPF, other media were of $1 < Ø \leq 4$ mm. The media laden tanks were then filled up with water to the 10 L level respectively and two *Typha angustifolia* plantlets were planted at the diagonal corner opposite to each other. Each plantlet was about 5 cm from the sidewall of each side and their roots were at 6 cm below the 10 L water level. Descriptions about the plantlets used are tabulated in Table 1. The day the plantlets were planted is named Day 0. In the case of OPF- and GAC-based microcosms, the first batch of plantlets died and was replaced with the second batch of plantlets on Day 55.

After planting, all the tanks were then drained and refilled with sewage. The process of draining and filling sewage to the 10 L mark was repeated every five days as the hydraulic retention time of these microcosms was five days. In order to minimise evaporation (Green *et al.*, 2006) induced by environmental albido and to prevent unintended growth of weeds as well as seeds germination, a layer of 6 cm (an equivalent of 3.9 kg) G was topped up to each microcosm at above the 10 L mark. The microcosms were placed in the compound of the university's Desa Aman sewage treatment plant and were exposed to tropical climate throughout the duration of the experiment.

Table 1. Specification of plantlet planted in various media-based microcosms.

Media	Plant		
	Maximum length (cm)		Wet weight (g)
	Leaf	Roof	
G	28.0 36.1	10.1 14.5	3.06 5.03
OPS	33.1 35.3	8.4 11.5	3.62 3.85
OPF	33.1 35.4 34.2* 44.5*	10.3 12.3 11.5* 11.0*	4.16 4.81 4.28* 6.06*
GAC	33.2 35.0 43.2* 38.4*	8.5 9.4 12.2* 9.8*	3.05 3.32 6.00* 4.15*
Zeo	30.2 35.9	8.0 8.3	2.83 3.25

* Second batch plantlet

Monitoring the height and shoots growth of *Typha angustifolia*

The height and shoot count of the plants in each microcosm were monitored from Day 0 to Day 500. Data were normally taken around 1700 hrs. Prior to the first biomass harvest, the heights of both plantlets were measured and the mean was reported. However, after the first biomass harvest, only the height of the tallest plant in each microcosm was recorded. The shoot count recorded signifies the number of shoot that was alive in the microcosm on that particular day.

Monitoring the biomass gain of *Typha angustifolia*

Throughout the experiment, the *Typha angustifolia* biomass in each microcosm was harvested thrice. First biomass harvest was performed on Day 90 for G-, OPS- and Zeo-based microcosms, Day 145 for GAC-based microcosm and Day 185 for OPF-based microcosm. The second harvest was standardised at Day 500. The third harvest, which indicated the end of the experiment, was performed on Day 542 for G-, Day 543 for OPS-, Day 544 for OPF-, Day 545 for GAC- and Day 546 for Zeo-based microcosms, respectively. All the harvesting tasks were performed at 1730 – 1830 hrs.

The harvested above-ground plant biomass of each microcosm was cut into fractions of 15 cm in length and dried at 103°C for 48 h prior to weighing to obtain the dry weight. During the final biomass harvest, the below-ground plant biomass in each microcosm was collected, dried at 103°C for 48 h, further separated from the respective medium and weighed.

Monitoring the pH of microcosms

The water in each microcosm was discharged regularly throughout the experimental period at the interval of five days. The first 1 L (estimate) of this water, which sometimes contains sediments, was left to flow to the ground. After the first 1 L of discharge, 125 mL of sample was collected from each microcosm and the pH was analysed immediately. Samples were normally collected around 0800 hrs. The pH of each microcosm was monitored from Day 0 to Day 180.

Morphological study on the biofilm formed on the experimented media

The medium in each microcosm was collected during the final biomass harvest. Only the medium in between the 1/3 and 2/3 of the microcosm depth (excluding the depth of G cover layer) was collected respectively from each microcosm. Fresh medium sample from each microcosm was vapour fixed, freeze dried and sputtered with 5 – 10 nm layer of Au before having the surface morphology examined utilising scanning electron microscope (SEM) respectively.

RESULTS AND DISCUSSION

Plant height

Figure 1 shows the height growth of the plants in the microcosms from Day 0 to Day 500. The growth of plants in each microcosm (and even the death of the first batch plantlets in the OPF- and GAC-based microcosms) was influenced by the micro-ecosystem of their respective microcosm (Chong *et al.*, 2005). Despite the variation of media used, it is found that the plants have a stabilised dynamic height in the range of 122 – 143 cm towards the late stage of the experiment.

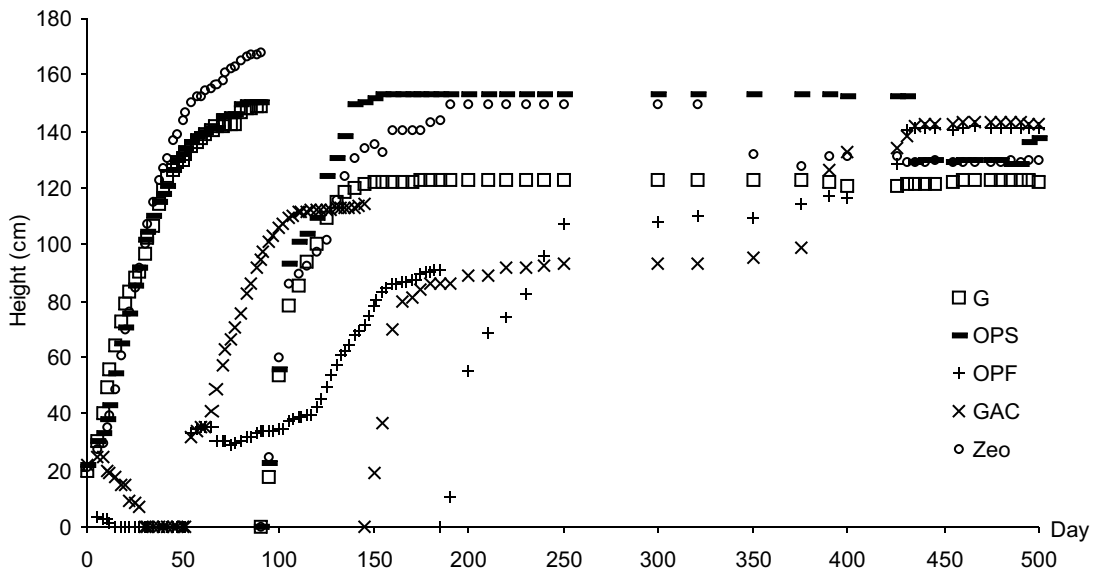

Figure 1. Plants height in the microcosms (Days 0 – 500).

Shoots density

By plotting the shoot count per plantlet planted in 1 m^2 against the number of days the plantlets were planted in the microcosm, the rate of shoot generation can be obtained from the slope of the plot. Figure 2, which in agreement with the finding of Sim *et al.* (2003), shows the plots for the G-, OPS-, OPF-, GAC- and Zeo-based microcosms, respectively. Noting that the first batch of plantlets in the OPF- and GAC-based microcosms died and was replaced with the second batch of plantlets, the plots for these two microcosms were based on the growth of the second batch plantlets; therefore, the full growing time was 445 days and not 500 days as in the G-, OPS- and Zeo-based microcosms. The mathematical equations derived from the plots and their R^2 values are tabulated in Table 2. These mathematical equations describe the shoots density in the microcosms as a function of growing time with the R^2 values of 0.86 – 0.97. In reference to the plantlets that experienced full 500 days growth period, the OPS-based microcosm gave the highest shoot generation rate and R^2 value.

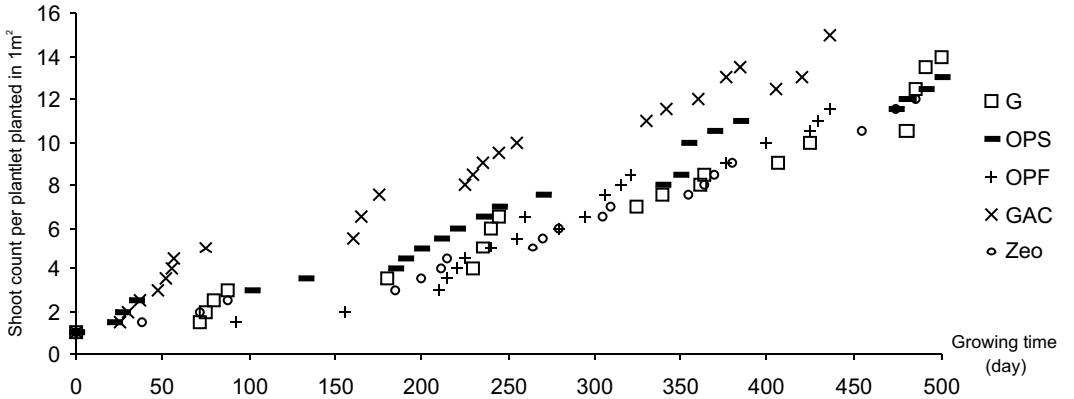

Figure 2. Growth in shoots density of the various media-based microcosms.

Table 2. Mathematical expression of shoots density in various media-based microcosms.

Media	Growth period (days)	Equation	R^2
G	0 – 500	y = 0.0214x + 1 …(1)	0.938
OPS	0 – 500	y = 0.0231x + 1 …(2)	0.973
OPF	55 – 500	y = 0.0204x + 1 …(3)	0.858
GAC	55 – 500	y = 0.0315x + 1 …(4)	0.965
Zeo	0 – 500	y = 0.0195x + 1 …(5)	0.933

y: shoot count per plantlet planted in 1 m2
x: duration of plantlets growing time in the microcosm (day)

It is noticed that the R^2 value for the OPF-based microcosm was lower than others due to the slow shoot generation rate where the micro-ecosystem of its microcosm was less encouraging for rhizomatous growth in the first 200 days.

Nonetheless, its mathematical shoots density equation (3) is quite similar to equation (1) and equation (5) that of the G- and Zeo-based microcosms, respectively.

It is also noted that the shoot generation rate of the GAC-based microcosm was exceptionally higher than others. If this mathematical equation (4) were to be taken to describe the overall growth of *Typha angustifolia* in the GAC medium, it would be misleading because there are factors such as size of leaves and height that should be incorporated in defining the overall plant growth, which normally biomass gain is regarded as a better parameter to measure overall plants growth (Luo and Rimmer, 1995).

Dry plant biomass gain

The plant biomass gain in each microcosm is best described in terms of dry biomass gained per metre square of planted area per day (g m^{-2} day^{-1}) as tabulated in Table 3. The rate of dry biomass gain was dependent on the medium in the microcosm and the stage of plant growth. The biomass gain in the OPS-based microcosm was the highest with an average rate of 18.0 g m^{-2} day^{-1} while others were in the range of 11.6 – 14.2 g m^{-2} day^{-1}.

Table 3. Rate of dry plant biomass gain in various media-based microcosms.

Media	Dry plants biomass gain (g m^{-2} day^{-1})					
	Above-ground				Below-ground	Overall
	1st harvest	2nd harvest	3rd harvest	Average	Average	Average
G	10.4	4.8	8.4	6.0	6.0	12.0
OPS	15.4	7.4	6.6	8.8	9.2	18.0
OPF	2.2	6.6	8.2	5.6	6.0	11.6
GAC	15.2	6.4	7.6	8.2	6.0	14.2
Zeo	16.0	5.6	6.4	7.4	6.4	13.8

pH of waterbody

The pH of the microcosms (Figure 3) were initially influenced by the pH of their media; however, as the experiment progressed, these pH changed towards dynamic pH as a result of the media, sewage and micro-ecology adapting and stabilising themselves towards equilibrium. The time taken to reach the dynamic pH was medium dependent. According to Rook (2004), the cultural pH requirement for *Typha angustifolia* is pH 3.7 – 8.5. Noting that the first batch of plantlets in the GAC- and OPF-based microcosms died (as shown in Figure 1); were pH of the waterbodies the cause?

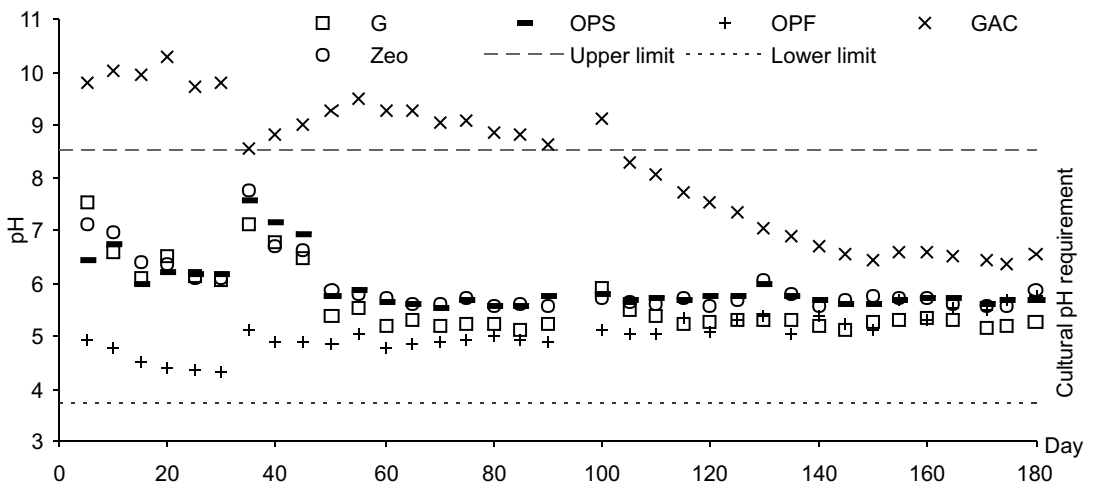

Figure 3. Cultural pH requirement and pH changes in various media-based microcosms.

In reference to the GAC-based microcosm, its pH remained above the upper limit of pH 8.5 until Day 105 where it fell to pH 8.3. During this period, the first batch of plantlets died but the second batch of plantlets survived. If the first batch of plantlets were killed by pH factor, this probably has happened at pH above 9.3 (Day 60) as this is the highest pH recorded after the second batch of plantlets was planted. Nonetheless, the researchers believe that the plantlets would have also been killed by the effect of malnutrition as the GAC would have effectively sorbed the essential nutrient. As the experiment progressed, the GAC became more saturated with nutrient and its affinity for nutrient sorption was reduced, only then the plantlets were able to compete and consume the essential nutrient.

Referring to the OPF-based microcosm, its pH has always been inside the cultural pH requirement range but its first batch of plantlets died by Day 15. This death was due to the presence of residual palm oil in the OPF medium in the microcosm (Chong *et al.*, 2004). As the experiment progressed, the degree of residual palm oil presence in the microcosm has dropped as an effect of dilutions where the microcosm fluid was replaced every 5 days. This explained the reason that the second batch of plantlets survived.

Surface morphology of the experimented media

Figure 4a – e show the SEM micrographs of each experimented medium. For comparison purposes, the SEM images were standardised at 200 ×.

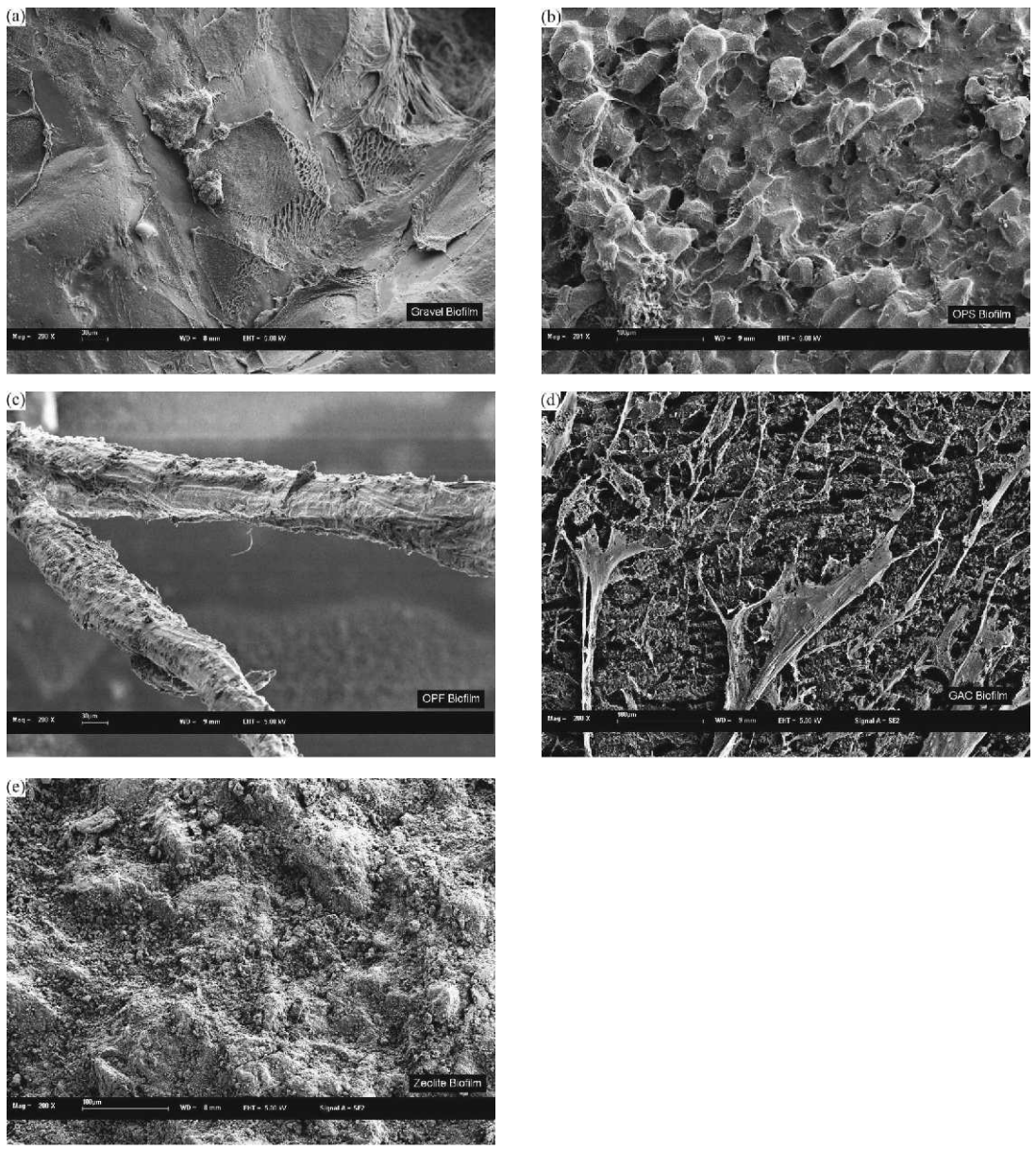

Figure 4. SEM images of the experimented (a) G, (b) OPS, (c) OPF, (d) GAC and (e) Zeo media.

In Figure 4a, it is observed that the biofilm layer shrunk and cracked because the biofilm formed on the experimented G medium was very thin and unable to keep itself intact during the freeze drying process. The biofilm formed on the experimented biodegradable OPS and OPF (as shown in Figure 4b and 4c, respectively) media seem to be thicker, mucus-wrapped and evenly coating their media surfaces; similar observation was previously reported by Underwood *et. al.* (1995). The biofilm formed on the experimented GAC medium has changed its surface morphology to the stage where the original GAC surface structures were no longer detectable and layer of mucus was seen across its surface (as shown in Plate 1d). However, no clear biofilm presence was detected on the surface of the experimented Zeo medium due to its silty characteristic that does not favour biofilm attachments (see Figure 4e).

CONCLUSIONS

Based on the results of this study, the following conclusions can be made:

1. The media used in the microcosm affects the height, shoot generation rate and dry biomass gain of the *Typha angustifolia* planted in it. The OPS has performed better than the conventional constructed wetland medium (G) and therefore it is a better medium for constructed wetland application in special reference to the growth characteristics of *Typha angustifolia.*

2. The surface morphologies of the biofilm formed on different media were different from one another. It is found that biodegrading media such as the OPS and OPF seems to have thicker biofilm formation, non-biodegrading media such as the G and GAC seems to have thin mucus biofilm and silty media such as the Zeo does not favour biofilm attachment on their respective media surfaces.

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