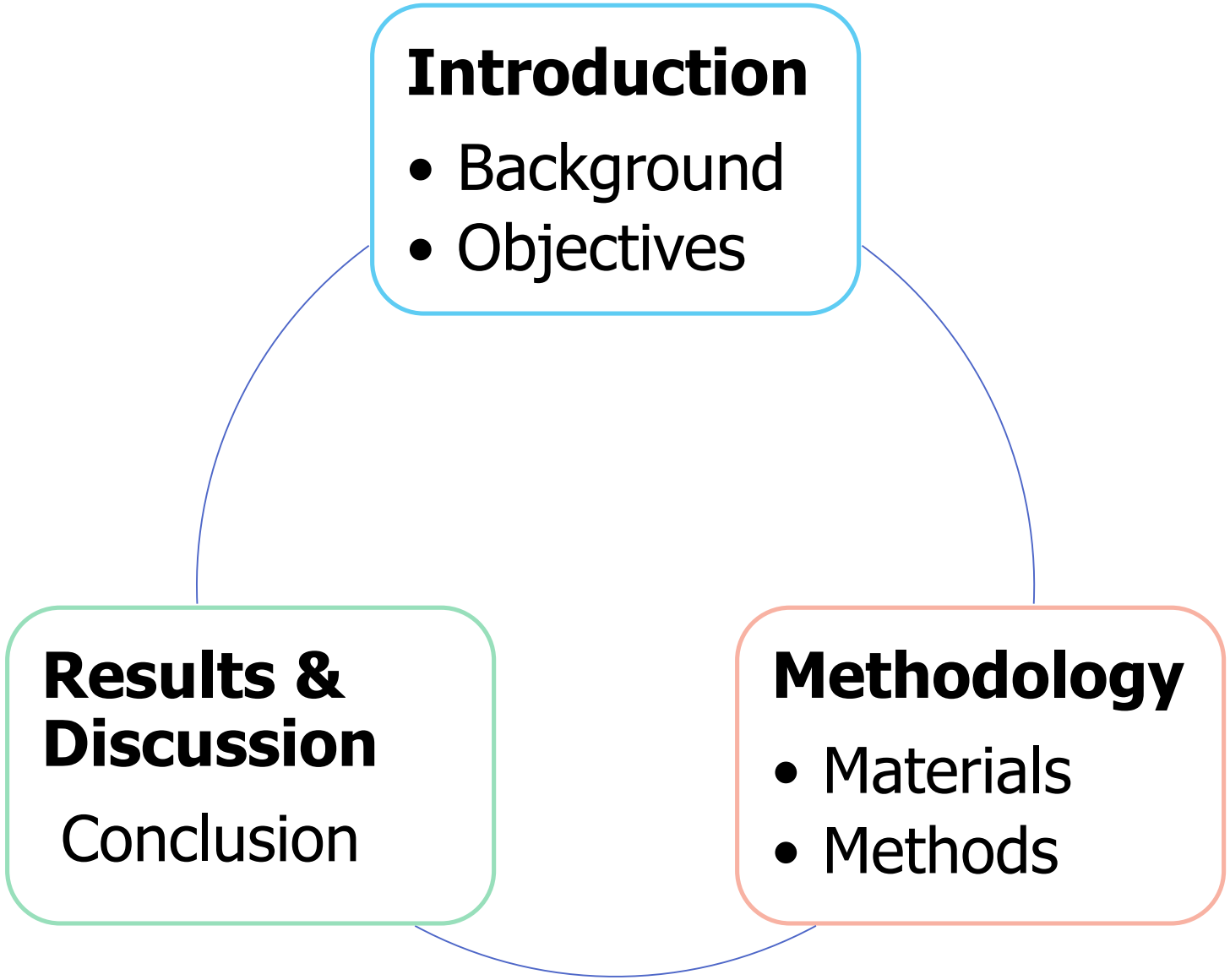
Investigation of the percent contributions to 17α-methyltestosterone depletion in a lab-scale Salvinia-based reactor

Sudtida Pliankarom Thanasupsin*
(M.Sc., M.Eng, Ph.D.)

Chemistry for Green Society and Healthy Living (ChGSH), Department of Chemistry, Faculty of Science,
King Mongkut's University of Technology Thonburi (KMUTT), THAILAND


Introduction
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Emerging contaminants (ECs)

pesticides, pharmaceuticals and personal care products (PPCPs), Endocrine Disruption Compounds (EDC) etc.

EDCs substances

- An exogenous substance that interfere endocrine system and have adverse effect to living organism

Steroid Hormones

- Organic substances
- Toxic both natural and synthetic compounds

17α- Methyltestosterone (MT)

- Synthetic androgen hormone
- Introduce to pharmaceutical and fish aquaculture application

What is 17α-Methyltestosterone (MT)?

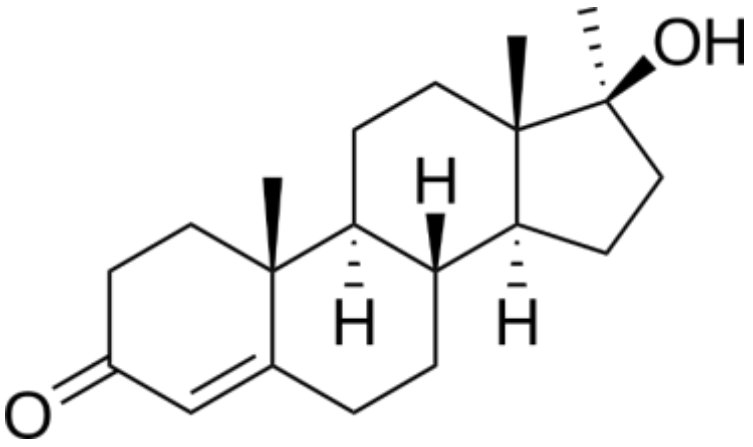
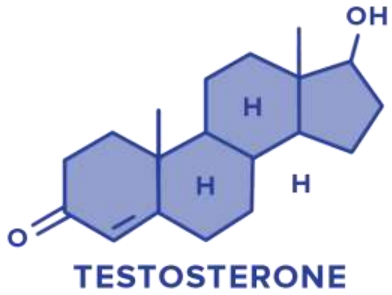


Figure 1. 17α-methyltestosterone ($C_{20}H_{30}O_2$)

Table 1. Chemical and physical properties of 17α-methyltestosterone [1]

Molecular Formula	$C_{20}H_{30}O_2$
Molecular Weight	302.45
Color/Form	White crystal powder
Odor	Odorless
Melting Point	161–166°C
Solubilities	Methanol, ethanol, acetonitrile In water; 34 mg/L at 25°C
Stabilities	In low pH level
pKa/log K_{ow}	15.13/3.4

- Type: Anabolic steroid hormone
- Function: Hormonal growth promoter
- Close structural similarity to testosterone



What are the applications of MT?

17α- Methyltestosterone (MT)

- More muscles
- Stronger bones
- Improve mood
- Healthy heart and blood

- Reverse sex (all males)
- Increase production yield
- Fast growth rate
- Dosage: 60 mg 17MT/kg fish feed (Straus et al., 2013)

- Banned to used in sport activities
- Still used in fish aquaculture application

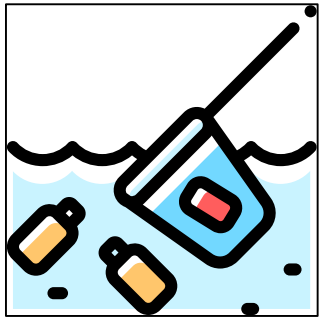
What are the effects of using MT?

Interfere endocrine systems

- Decreased testicular size
- Decrease sperm counts
- Increased aggressive behavior
- Breast enlargement (Combalbert et al., 2012)

Alter the endocrine in system of fish such as :

- Transcriptome (Gao et al., 2015)
- Gonadal gland
- Embryo development (Rivero-Wendt et al., 2016)



MT enter the aquatic environment via :

- Aquaculture discharge
- Domestic sewage
- Industrial discharge (Heidarimoghadam et al., 2016)

MT Levels of which caused effects

Table 2. MT levels in water of which caused effects on the tested animals

MT levels (µg/L)	Tested animals	Effects	Reference
0.1-1	 Ramshorn snail	Affect the male sex organs	(Schulte-Oehlmann et al., 2004)
1.0	 *Gasterosteus aculeatus* L.	Cause intersex in both male and female of three-spined stickleback	(Hahlbeck et al., 2004)
0.01-0.5	 *Zoarces viviparous*	Decrease female characteristic in female eel spout for 10 days	(Korsgaard, 2006)
0.046	 medaka fish	Decrease the fecundity and fertility	(Kang et al., 2008)
4	 zebrafish	Inhibit vitellogenin (VTG) in zebrafish LC_{50}= 10.09 mg MT/L within 96h	(Rivero-Wendt et al.,2016)
0.25-5	 *Biomphalaria glabrata*	Affect hatchling survival and growth rate of snail	(Kaur et al., 2016)

Water and Wastewater Treatment Technology for MT

Technology	Pros	Cons
• biological treatment, • conventional sewage treatment	• Successfully remove soluble biodegradable organic contaminants from water	• However, it cannot completely remove steroid hormones and MT
• Advanced treatment technology (i.e. Advanced Oxidation Process)	• Successfully remove organic contaminants from water	• However, metabolites or intermediate products from complex reactions can lead to other questions regarding their toxicity to the environment
• Natural biological treatment system incorporating aquatic plants	• More effective and economical for the treatment of contaminated water with very low concentrations (parts per billion) of compounds such as surfactants, hormones, polar microcontaminants, and pharmaceutical compounds	• However, performance in the removal of organic contaminants can be highly variable and is influenced by many factors, such as environmental factors and operational parameters. • Little is known about the removal mechanisms involved

OBJECTIVES

- To the best of our knowledge, the potential use of *Salvinia* as a phytoremediator of MT has not been studied thus far.
- The results of our previous study showed that an active *Salvinia*-based reactor could be used for the removal of MT from contaminated water with a more than 90% removal efficiency.
- It was found that sorption was one of the key processes that accounted for the disappearance of MT from the water phase

  (Adnan & Thanasupsin, 2016).

To search for a scientific explanation regarding the removal efficiency of MT in a lab-scale *Salvinia*-based reactor.

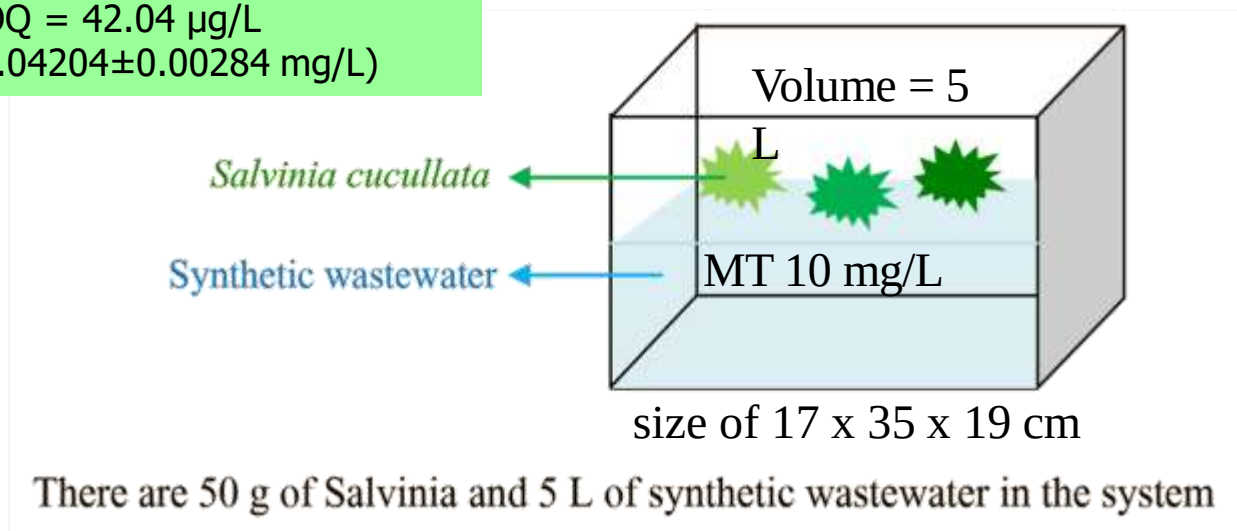
MATERIALS AND METHODS

Determination of MT by HPLC-UV

- The procedure for analyzing the water samples was modified from the protocol reported in (I. R. Barbosa et al., 2013),

LOD = 12.61 µg/L (0.01261±0.00085 mg/L)

LOQ = 42.04 µg/L (0.04204±0.00284 mg/L)



HPLC conditions

- The column used for the chromatographic separation of MT was a carbon 18 reversed-phase (RP-C 18) column (ACE, 5 µm particle size, 250 x 4.6 mm).
- The injection of analytes was performed both automatically and manually manner at 25°C.
- The ratio and flow rate of the mobile phase used for MT detection (acetonitrile and DI water) were 70: 30, v/v and 1 mL/min

A composite sampling of 24 mL.

- Each sample was composed of samples collected from the top (8 mL), middle (8 mL), and bottom (8 mL) of the reactor.
- Water samples were collected in amber glass bottles and kept in the laboratory refrigerator

Table 2 Details of the experimental trials.

- Seven batch experiments, including 4 controls and 3 trials, were performed in this research.
- Each experimental trial was conducted in triplicate.

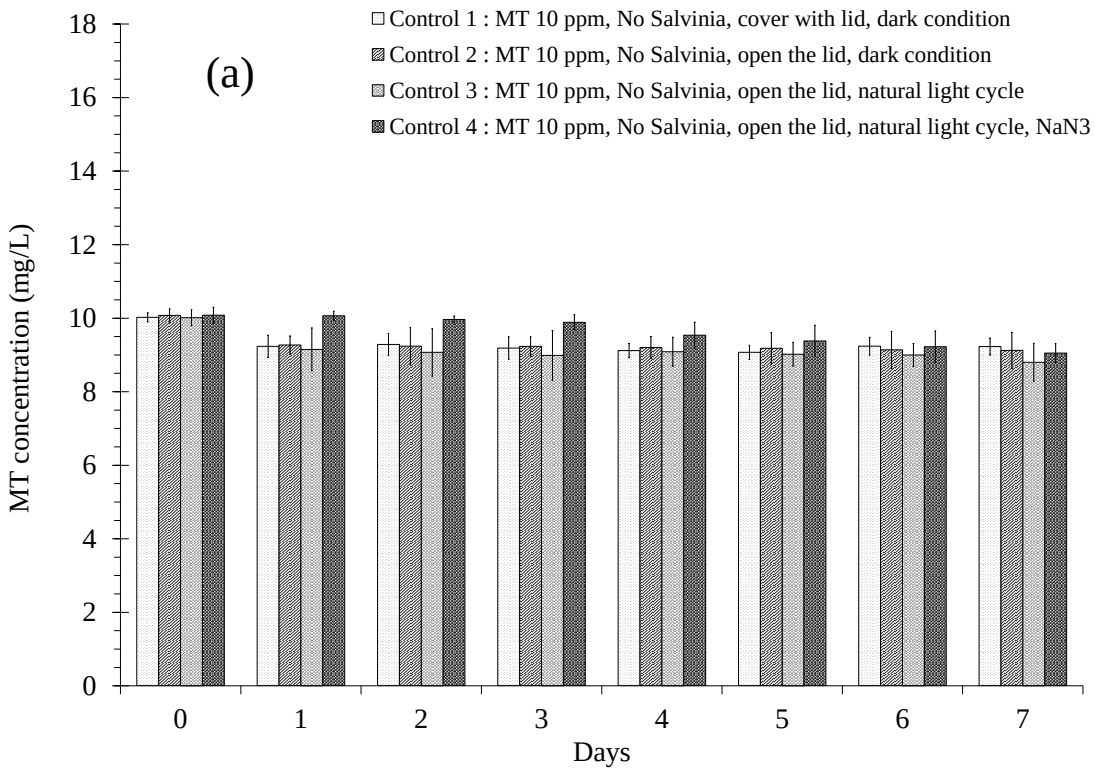
Conditions	Ref.	Control 1	Control 2	Control 3	Control 4	Exp.5	Exp.6	Exp.7
MT 10 mg/L	(Adnan & Thanasupsin, 2016)	/	/	/	/	/	/	/
Plant nutrients	(Shi et al., 2010)	/	/	/	/	/	/	/
NaN_3 (1% w/w)[a]	-	/	/	-	/	/	/	-
Salvinia (50 g)	-	-	-	-	-	/ [b]	/	/
Top of the reactor covered	-	/	-	-	-	-	-	-
Natural light	-	- [c]	- [c]	/	/	/	/	/

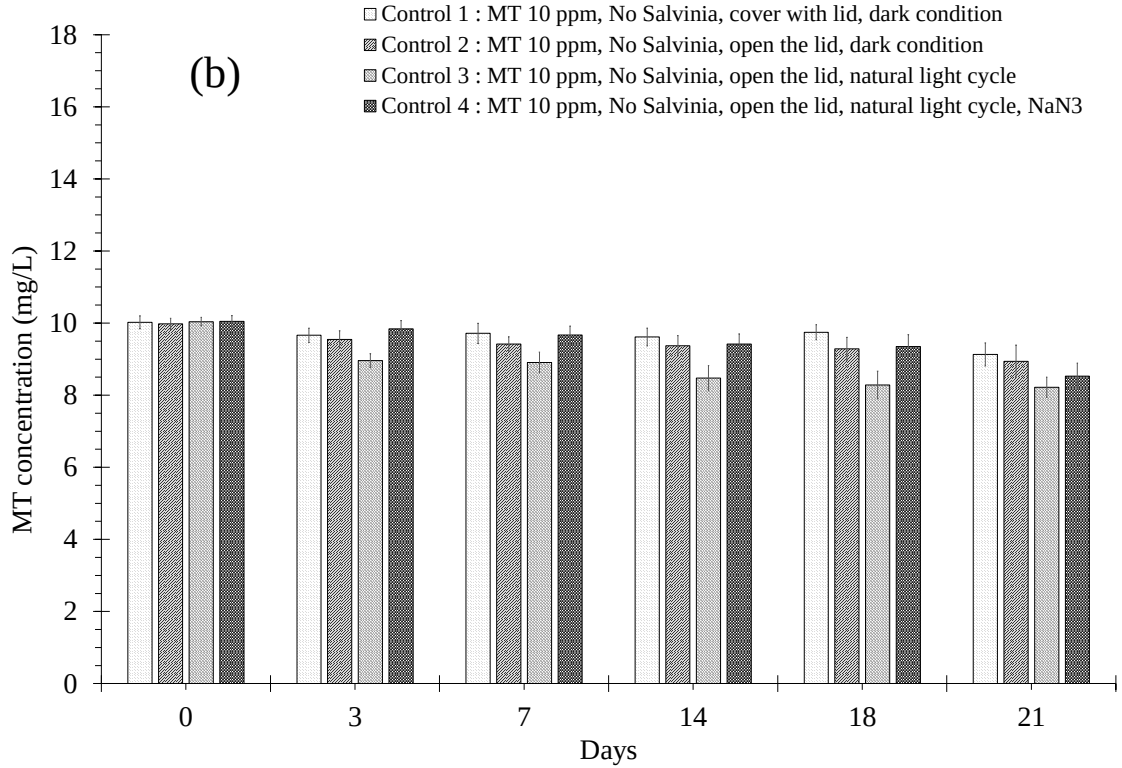
[a] NaN_3 was used for microbial activity inhibition,
[b] homogenized Salvinia
[c] the sidewalls of the reactor were covered with foil, and the reactor was placed in a dark closed cabinet

RESULTS AND DISCUSSION

1. MT removal in the reactors

Figure 2 MT concentrations remaining in the reactors in each control experiment for; (a) a retention time of 7 days or (b) a retention time of 21 days.

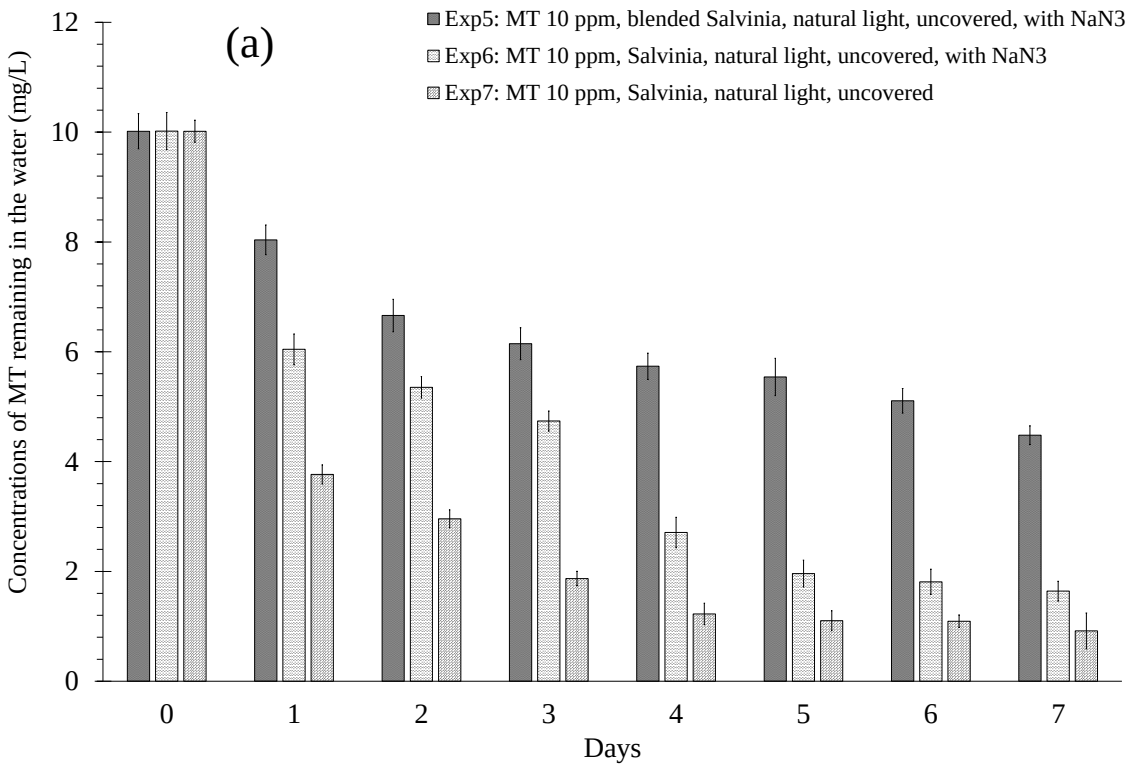


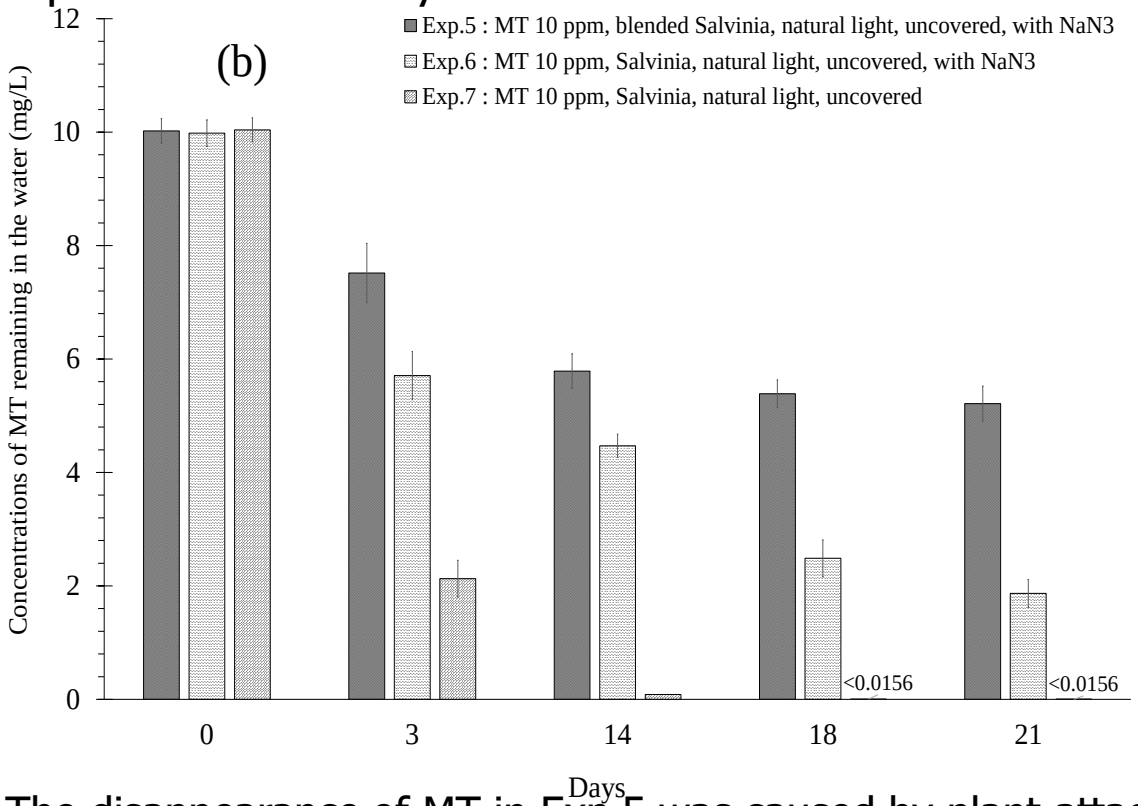


The loss of MT in controls 1, 2 and 4 was related to physicochemical activities such as the attachment of MT to the glass, volatilization, and photodegradation, while microbial activity resulting from post-contamination from the air might have been the reason for the loss of MT (12.6%) observed in the control 4 reactor.

RESULTS AND DISCUSSION

Figure 3 MT concentrations remaining in the reactors in each experimental trial; (a) operated for 7 days or (b) operated for 21 days.





- The highest percentage of MT removal observed in the active Salvinia reactor (Exp.7) might have been affected by photodegradation, live plant activities, and microbial activities.
- In Exp.6, a slightly lower value than that in Exp.7 was probably caused by the inhibition of microbial activity.
- The disappearance of MT in Exp.5 was caused by plant-attached microbial communities and the sorption of MT onto Salvinia biomass.
- Increasing the retention time would improve the sorption and transformation of MT via the integrated activities of live plant and microbial communities.

RESULTS AND DISCUSSION

Table 3 & Table 4 show the MT degradation rates on day 7 and day 21 and half-lives in the reactors

Control/experimental trials	Degradation rate (k) at day-7, (day^{-1})	Degradation rate (k') at day-21, (day^{-1})	Half-life of MT, (day)
Control 1 - No Salvinia + covered top of the reactor + dark conditions	0.000	0.000	-
Control 2 - No Salvinia + dark conditions	0.000	0.000	-
Control 3 - No Salvinia + natural light	0.000	0.000	-
Control 4 - No Salvinia + natural light + NaN_3	0.001	0.000	-
Exp.5 - Blended Salvinia + natural light + NaN_3	0.004	0.013	No microbial activity
Exp.6 - Salvinia + natural light + NaN_3	0.010	0.060	No microbial activity
Exp.7 - Salvinia + natural light	0.014	0.442	1.57

- From Table 3, the biodegradation of MT did not occur within 7 days.
- Increasing the retention time to 21 days would allow the biodegradation process to occur.
- In this study, the biodegradation rate (0.442 day^{-1}) at 21 day was higher than found by the previous research (Homklin et al., 2009).

Medium	Conditions	Half-life of E2	Half-life of E1	Half-life of EE2	Half-life of MT
Water	aerobic	0.2-1.7 day[a]	0.2-1.7 day[a]	17 day	1.57 day (This study)

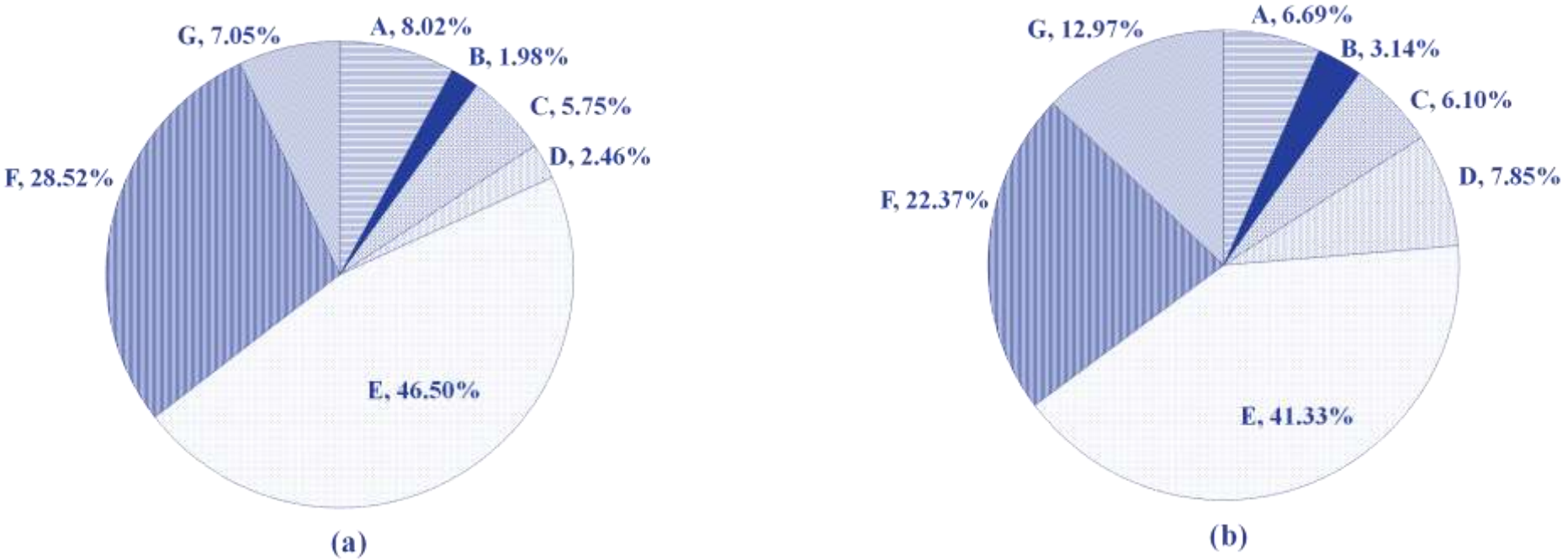
- The half-life of MT fell within the same range as those of E1 and E2.
- This can be explained by the higher similarity of the structure and physicochemical properties of MT to E1 and E2 than to EE2.

Table 5 Contributions of MT depletion in the reactors at retention times of 7 and 21 days.

Contributions to MT removal from the water phase	Calculation of the MT mass fraction	% contribution Day-7	% contribution Day-21
(1) Sorption on glassware	$Mass_{initial\ MT} - Mass_{control\ 1}$	8.02±2.76	6.69±1.89
(2) Volatilization	$Mass_{control\ 2} - Mass_{control\ 1}$	1.98±1.97	3.14±1.78
(3) Photodegradation	$Mass_{control\ 3} - Mass_{control\ 2}$	5.75±1.86	6.10±1.36
(4) Microbial activity (both that originally in the water and post-contamination)	$Mass_{control\ 4} - Mass_{control\ 3}$	2.46±1.09	7.85±1.12
(5) Sorption on plant biomass and plant-attached microbial activity	$Mass_{control\ 4} - Mass_{Exp.6}$	46.50±3.80	41.33±2.73
(6) Intact live plant activity (i.e., plant uptake, sorption on live plant roots and attached microbial activity)	$Mass_{Exp.6} - Mass_{Exp.5}$	28.52±1.19	22.37±1.25
(7) Role of suspended microbial activity	$Mass_{Exp.7} - Mass_{Exp.6}$	7.05±3.60	12.97±2.84
Total	(1)+(2)+(3)+(4)+(5)+(6)+(7)	100.29±0.48	100.45±0.52

- The role of live plant (*Salvinia*) and overall microbial activities was determined by taking the mass of MT partitioned in the water between control 4 and Exp.7 into account. The contributions (%) on day 7 and day 21 were approximately 82.76% and 82.24%, respectively.
- The mass contribution was rechecked by the summation of the MT mass removed from (5) + (6) + (7).
- The result for the retention time after 7 days was 82.07%, which was very close to the experimental data (82.76%).
- Therefore, it can be concluded that the symbiotic action of microbial activities and Salvinia plays a key role in the depletion of MT in the active reactor.

Figure 4 Contribution fractions to MT depletion in the active Salvinia-based reactor; (a) retention times of 7 days and (b) 21 days.



A = Glassware, **B** = Volatilization, **C** = Photodegradation, **D** = Microbial activity (both that originally in the water and post-contamination), **E** = Sorption on plant biomass and plant-attached microbial communities, **F** = Intact live plant activity (i.e., plant uptake, sorption on live plant roots and attached microbial activity), **G** = Role of suspended microbial activity

To determine the major contributions (%) to MT depletion, a target contribution percentage of 75% was established.

- For a retention time of 7 days, the major contributors were sorption on plant biomass and attached microbial activity (46.50%) and intact live plant activity (28.52%).
- For a retention time of 21 days, there were three major contributors: sorption on plant biomass and assigned microbial activity (41.33%), intact live plant activities (22.37%), and suspended microbial activity (12.97%).
- These findings indicated that an increasing retention time has a significant effect on the increase in suspended microbial activity.

CONCLUSION

- *Salvinia* can be used as a phytoremediator for the removal of MT from contaminated water.
- Active *Salvinia* reactors can effectively remove MT from aqueous mixtures.
- As a moderately hydrophobic organic contaminant, MT is likely to be taken up and translocated inside plant tissues.
- For a retention time of seven days, the top contributors to the depletion of MT in the active *Salvinia* reactor are sorption on plants and intact live plant activities
- while for a retention time of 21 days major contributors to the depletion of MT in the active *Salvinia* reactor are sorption on plant biomass and assigned microbial activity, intact live plant activities, and suspended microbial activity.
- Microbial activities in the reactor can be enhanced by increasing the retention time.
- Increasing the retention time to 21 days seems to allow sufficient time for the growth of aerobic microbial communities.
- The biodegradation rate and half-life values obtained in this study revealed that aerobic microbial communities are likely to be a key player in the biodegradation of MT in the active Salvia reactor.
- The symbiotic activity between intact live plant activities and microbial activities can enhance the biodegradation rate and shorten the half-life in the removal of MT.

Thank you for your kind attention