4.7 Effect of Nutrient Broth and Inoculum on Ethylbenzene Removal by *P. aeroginosa*

This treatment had three controls for comparison of the efficiency of ethylbenzene removal, sterile plant (Control I), sterile plant with NB (Control II), and sterile plant with *P.aroginosa* (Control III). The sterile plant (Control I) and sterile plant with NB addition (Control II) in the first cycle showed no significantly different (Table 4.7). This treatment could confirm that ethylbenzene insoluble in NB. In first cycle, control III, treatment I and II not significantly different, they could remove ethylbenzene completely in 36 hours (Table 4.7). Second cycles, found that could remove ethylbenzene slowly than control III about 12 hours (Table 4.8). Third cycles, result of both treatment and control not significance difference (Table 4.9).

The result could explain that in the condition which has enrichment nutrient was easily to selected first by *P.aroginosa* to carbon source instead of ethylbenzene result to no ethylbenzene decrease, and because of high humidity in the chamber, it could effect on plant uptake (Tani et al., 2007). After experiment, number of *P. aeroginosa* on leaf was count by comparing characteristics of colony before treatment. Number of *P. aeroginosa* on leaf had high but no effect on ethylbenzene removal, so this result could confirm that the presence of enrichment nutrient on leaf, microorganisms would select this nutrient easily to use before complex nutrient. Thus, enrichment microorganisms and nutrient in first cycle is a good technique (Table 4.10).

Table 4.7 Percentage of ethylbenzene uptake by *Z. zamiffolia* and *P.aeroginosa* - associated leaf of *Z. zamiffolia* after 1st cycles

Treatment	Time (Hour)				
	12	**24**	**36**	**48**	**60**
Sterile Plant **(Control I)**	38.11^{a}	61.12^{a}	74.21^{a}	84.17^{a}	100
Sterile Plant + NB **(Control II)**	36.58^{a}	$65.02^{a,b}$	75.21^{a}	81.25^{a}	100
Sterile Plant + P(1^{st}) **(Control III)**	64.50^{c}	77.18^{c}	100^{b}	100^{b}	100
Sterile Plant + P(1^{st}) **(Treatment I)**	59.96^{b}	75.44^{c}	100^{b}	100^{b}	100
Sterile Plant + P (1^{st}) **(Treatment II)**	58.20^{b}	$71.55^{b,c}$	100^{b}	100^{b}	100

*P is *P.aeroginosa*, *NB is Nutrient broth

Data are list as average ±SD for three replications. Duncan's multiple range tests with 95% confident level was used to classify the group of data. Values in the same column with the superscripted same letter are not significantly different (a = 0.05)

Table 4.8 Percentage of ethylbenzene uptake by *Z. zamiffolia* and *P.aeroginosa* - associated leaf of *Z. zamiffolia* after 2nd cycles

Treatment	Time (Hour)			
	12	**24**	**36**	**48**
Sterile Plant **(Control I)**	56.22^{a}	74.71^{b}	91.94^{a}	100
Sterile Plant with NB(1^{st})+ NB(2^{nd}) **(Control II)**	56.98^{a}	64.79^{a}	91.57^{a}	100
Sterile Plant with P (1^{st}) **(Control III)**	62.36^{b}	92.31^{e}	100^{b}	100
Sterile Plant with P (1^{st}) + P (2^{nd}) **(Treatment I)**	70.84^{c}	88.46^{d}	91.58^{a}	100
Sterile Plant with P (1^{st}) + NB (1^{st}) **(Treatment II)**	75.74^{c}	86.95^{c}	91.58^{a}	100

*P is *P.aeroginosa*, *NB is Nutrient broth

Data are list as average ±SD for three replications. Duncan's multiple range tests with 95% confident level was used to classify the group of data. Values in the same column with the superscripted same letter are not significantly different (a = 0.05)

Table 4.9 Percentage of ethylbenzene uptake by *Z. zamiffolia* and *P.aeroginosa* - associated leaf of *Z. zamiffolia* after 3rd cycles

Treatment	Time (Hour)			
	12	**24**	**36**	**48**
Sterile Plant **(Control I)**	72.92^{b}	85.38^{b}	90.96^{b}	100
Sterile Plant with NB(1^{st}) + NB(2^{nd}) + NB(3^{rd}) **(Control II)**	64.39^{a}	73.63^{a}	86.54^{a}	100
Sterile Plant with P (1^{st}) **(Control III)**	$69.93^{a,b}$	93.11^{c}	100^{c}	100
Sterile Plant with P (1^{st}) + P (2^{nd}) + P (3^{rd}) **(Treatment II)**	$76.80^{b,c}$	92.30^{c}	100^{c}	100
Sterile Plant with P (1^{st}) + NB (1^{st}) + NB(2^{nd}) **(Treatment II)**	80.55^{c}	$85.86^{b,c}$	100^{c}	100

*P is *P.aeroginosa*, *NB is Nutrient broth

Data are list as average ±SD for three replications. Duncan's multiple range tests with 95% confident level was used to classify the group of data. Values in the same column with the superscripted same letter are not significantly different (a = 0.05)

Table 4.10 Number of *Pseudomonas aeroginosa* on the leaf of *Z. zamiifolia* before and after treatment of ethylbenzene for 3 cycles

Condition	Microorganisms	
	Before CFU/ml	**After 3 cycle CFU/ml**
Sterile Plant with P (1^{st})	1×10^{2}	4×10^{5}
Sterile Plant with P (1^{st}) + *P* (2^{nd}) + P (3^{rd})	1×10^{2}	4×10^{21}
Sterile Plant with P (1^{st}) + NB (1^{st}) + NB(2^{nd})	1×10^{2}	2×10^{13}

*P is *P.aeroginosa*, *NB is Nutrient broth

4.8 Efficiency of Microorganisms for Ethylbenzene Removal

In 24 hours, *P. aeroginosa* and *B. cereus* ZQN5 could remove 59% and 31% of ethylbenzene (with initial concentration of 520 ppm), respectively (Figure 4.7). During 0-12 hours, *Pseudomonas aeroginosa* and *Bacillus cereus* ZQN5 could remove ethylbenzene at a very high rate, about 26.09 ppm/hr (50.33%) and 19.01 ppm/hr (29.68%), respectively so this duration is a log phase of microorganisms, which are more active than during the stationary phase (Table 4.11). These microorganisms were able to grow in ethylbenzene conditions, and removed ethylbenzene in the air, and may also produce some enzymes to degrade ethylbenzene as it was reported by Hamdy A. Hassan (2014) showed that *Pseudomonas* sp. HA10 could degrade ethylbenzene by producing dioxygenase enzymes, which plays a central role in the degradation of a variety of aromatic compounds.

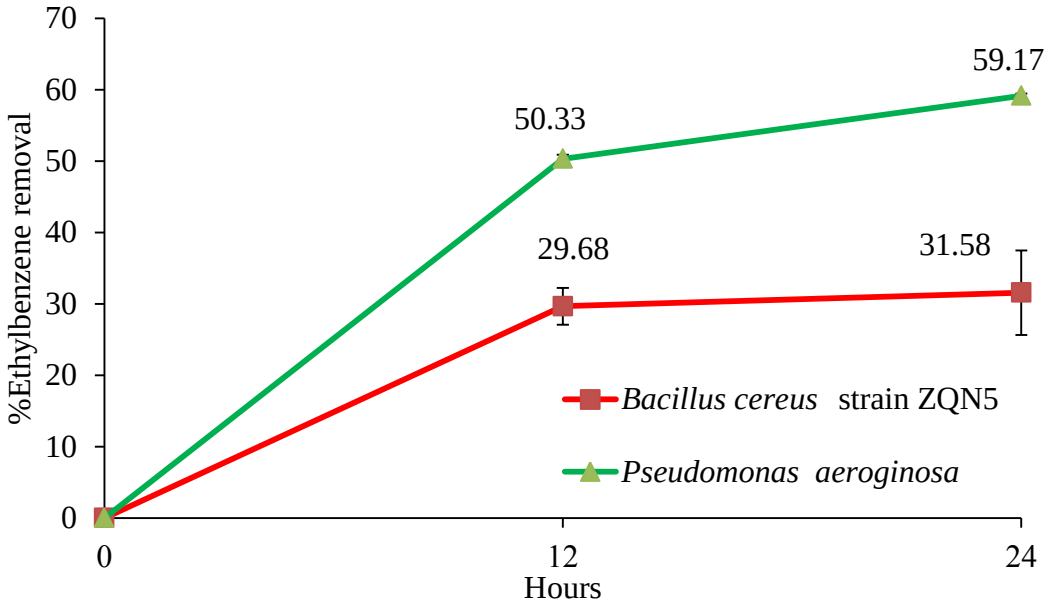

Figure 4.7 Comparison of ethylbenzene removal by two microorganisms grown in nutrient broth with the addition of ethylbenzene

Table 4.11 Rate of ethylbenzene removal by two strains of microorganisms

Microorganisms	Ethylbenzene removal (ppm)			Rate of ethylbenzene removal (ppm/hr)		%Ethylbenzene removal	
	0	12	24	0-12	12-24	12	24
B. cereus strain ZQN5	0	229.15	252.06	19.10	1.91	29.68	31.58
P. aeroginosa	0	313.12	384.66	26.09	5.96	50.33	59.17