CHAPTER 4
RESULTS AND DISCUSSION

4.1 Benzene removal by living plants
4.1.1 Benzene phytoremediation efficiency

Although phytoremediation is not novel process to clean up the environment, there are not many examples of the use of plants to clean up air pollution. Gaseous benzene distribution and degradation *in planta* was first investigated in 1996. Radioactive measurements suggested that plants can uptake benzene though both stomata and cuticle, and that benzene absorbs easily into cells. Passive diffusion is known to be the diffusion mechanism in this case. After benzene diffuses into plant cells, cytochrome P450 mono-/di-oxygenases and phenoloxidases could transform benzene to benzene metabolites such as catechol and phenol. Benzene may be completely degraded to carbon dioxide. However approximately 95% is converted to organic and amino acids of the TCA cycle. Most benzene and benzene metabolites accumulates in the cytosol (Ugrekhelidze, *et al*, 1997; Kvesitadze, *et al*., 2009). These experiments demonstrate that benzene can be taken up and transformed by plants which can be applied to develop highly effective methods for indoor gaseous benzene treatment. There are many experiments showing efficient benzene removal from air by plants, the first being the screening of 50 plant species for removal of 20 ppm benzene in a static system in 1989. The NASA scientists found 10 species to remediate benzene with particularly high efficiency, including *Gerbera jamesonii, Chrysanthemum morifolium, Hedera helix, Sansevieria laurentii, Dracaena deremensis warneckei, Spathiphyllum, Aglaonema, Dracaena marginata, Chamaedorea seifrizii,* and *Dracaena deremensis* (Wolverton, *et al*., 1989). In 2004, Orwell, et al. then proposed the use of plants to remove benzene as a sustainable technology. In China, 72 plant species were screened for benzene removal in 2007. Ten species particularly efficient in benzene removal were identified in this screening (Liu, *et al*., 2007). In this study, 8 indoor ornamental plant species were exposed to 20 ppm benzene in a modified chamber for 72 h, and the remaining benzene in the experimental system was measured (Fig 4.1). The result shows that *D. sanderiana* has the highest benzene removal efficiency of the species investigated. Benzene uptake was 10.00±1.04 µmole of benzene at 72 h. Benzene uptake was also standardized by leaf area; standardized by leaf area, *D. sanderiana* was still the most efficient at benzene uptake, taking up 59.66±6.67 nmole cm^{-2} leaf area at 24 h of treatment and taking up 74.65±11.55 nmole cm^{-2} leaf area at 72 h of treatment. The benzene uptake of *S. trifasciata* increased with time, reaching 92.55±11.67 nmole cm^{-2} at 72 h. Orwell, *et al.* (2004) previously reported that *Dracaena* sp. can take up high amounts of benzene in 2004. The experiment had been done under 25-50 ppm of benzene, and benzene uptake by 7 plant species had been investigated. In this experiment *Dracaena marginata* took up 297 nmole cm^{-2} after 24 h. However, Wolverton, *et al*. (1989) reported benzene removal of only 51.2 nmole cm^{-2} by *D. marginata*, and 21.8 nmole cm^{-2} by *D. deremensis* after 24 h at 20 ppm of initial benzene concentration. In real world, benzene accumulation in ambient condition such as home or office can be around 0.0021 ppm. To remove this concentration of benzene within 24 h in the 6x4x3 m^3 room, only 1 living *D. sanderiana* plants should be enough (see appendices calculation data). The difference in benzene removal efficiency suggests that there are several factors affecting benzene uptake by plants, for example growth conditions, species of plant, etc. From previous experiment, not only benzene but also other VOCs such as xylene, toluene, and ethylbenzene can be also removed by plant. However following Fick's first law (Eq 4.1), molecular size of pollutant had been found as an important factor on phytoremediation of air pollution. Where J is the diffusion flux (mole m^{-2} s^{-1}), and D is the diffuse coefficient (m^2 s^{-1}), and ∅ is the concentration in dimensions. x (m) is position.

$$J = -D\frac{\partial \emptyset}{\partial x} \qquad \text{(Eq 4.1)}$$

Low molecular weight compounds, plant can uptake better than high molecular weight compounds, so benzene, which had lowest molecular weight when it is compared with other BTEX, should be uptake well by plant (Sriprapat and Thiravetyan, 2013).

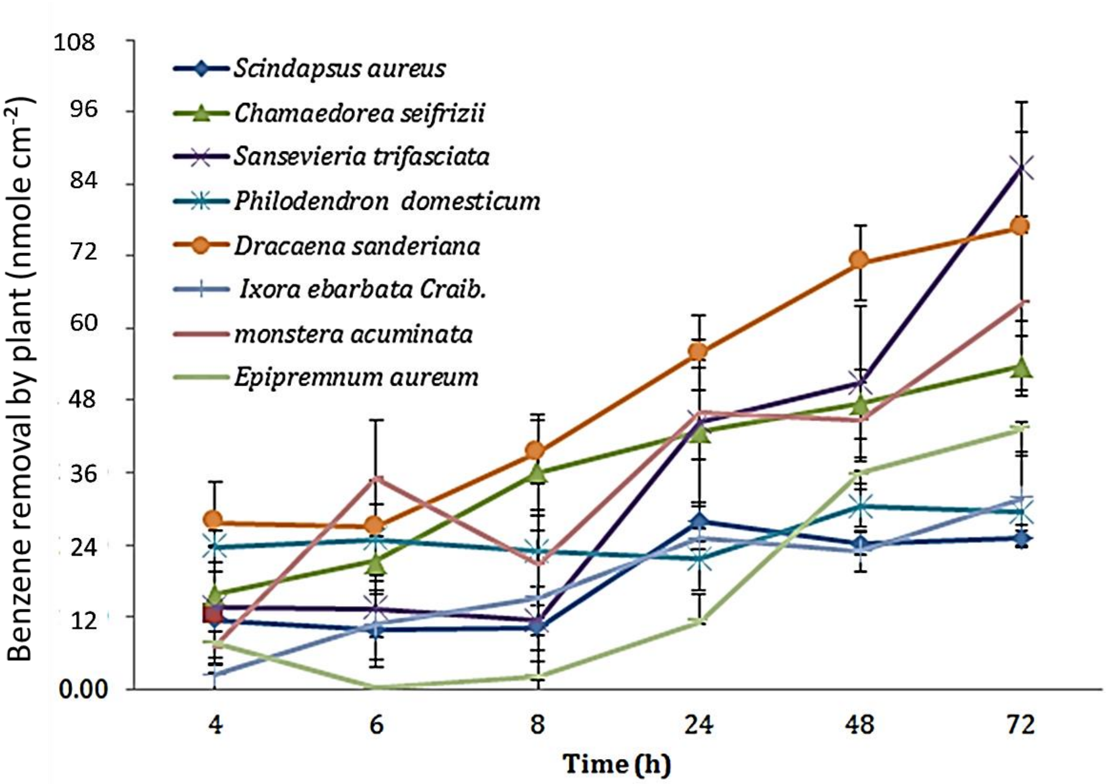

Figure 4.1 Benzene uptake by 8 ornamental plants

4.1.2 Stomata number, wax quantity, photosynthesis, and benzene removal efficiency in 8 ornamental plants

Factors which may affect benzene uptake efficiency were analyzed. Table 4.1 shows the number of stomata, amount of crude wax, photosynthesis rate, and the percentage of benzene removal after 72 h for each plant species investigated. *S. trifasciata* and *D. sanderiana*, the most efficient species at removing benzene, contain high quantities of crude wax. These results suggested that quantity of wax could affect benzene uptake efficiency. However correlation of wax quantity and benzene removal was investigated further. In *D. sanderiana*, a particularly high stomata density was also observed, while *S. trifasciata* had relatively low stomata density when compared with the other species, and so the relationship between stomata density and benzene uptake was not investigated further. Moreover, shape of stomata might also affect benzene uptake efficiency as well. Photosynthetic performance was measured as the ratio between variable fluorescence and maximal fluorescence (Fv/Fm) after 5 min in darkness. This represents the photochemical quenching capacity of photosystem II and operation of photosynthesis. No relationship between photosystem II activity and benzene uptake was found.

Table 4.1 Number of stomata, crude wax, photosynthesis, and percentage of benzene removal at 72 h of various plants.

Plant species	leaf area (cm^2)	Stomata number (Number of stomata cm^{-2})	Crude wax (μg/ leaf area cm^{-2})	Photosynthesis (Fv/Fm)	Range of % benzene removal at 72 hours
S. aureus	$149.67 \pm 25.5^{b,c}$	$3,750 \pm 901^{b,c}$	203.85 ± 5.44^{b}	0.81 ± 0.01^{c}	43-50
C. seifrizii	$131 \pm 30.05^{a,b}$	$5,917 \pm 382^{d}$	16.15 ± 0.00^{a}	0.79 ± 0.02^{c}	51-68
S. trifasciata	$110 \pm 10^{a,b}$	$1,167 \pm 382^{a}$	890 ± 162.09^{c}	$0.57 \pm 0.29^{a,b}$	54-77
P.domesticum	97.67 ± 25.42^{a}	$4,667 \pm 191^{c}$	33.46 ± 3.81^{a}	0.80 ± 0.00^{c}	50-62
D.sanderiana	180 ± 0.00^{c}	$10,583 \pm 1,134^{e}$	769.23 ± 0.00^{c}	0.45 ± 0.04^{a}	60-77
I. craib.	$131.67 \pm 25.07^{a,b}$	$18,444 \pm 509^{f}$	11.92 ± 1.63^{a}	$0.74 \pm 0.01^{b,c}$	65-72
M. acuminata	240 ± 36.06^{d}	$6,083 \pm 505^{d}$	5.77 ± 0.54^{a}	0.84 ± 0.05^{c}	52-62
E. aureum	$128.67 \pm 24.85^{a,b}$	$3,750 \pm 250^{b,c}$	230.77 ± 0.00^{b}	0.78 ± 0.03^{c}	56-59

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.

4.1.3 Benzene removal by living plant under light and dark conditions

To study the sustainability of this technology and the effect of light, *D. sanderiana*, which is high benzene removal plant from screening study, had been grown under 24 h light and dark conditions and benzene removal had been analyzed. For sustainability of technology, the experiment had been designed as 4 cycles with 20 ppm of initial benzene concentration in each cycle. *D. sanderiana* growing under 24 h light condition clearly showed higher benzene uptake efficiency than growing under 24 h dark conditions at 2nd, 3rd, and 4th cycles (Fig 4.2). This result similar to Wood's study in 2001 that *Anubias barteri,* exposed to 25 ppm of benzene in light, removed benzene faster than in the dark. Sustainability of phytoremediation of gaseous benzene had been reported in this experiment (Wood, *et al*., 2001). In 2007, sustained benzene uptake by *Zamioculcas* and *Aglaonema* had been also reported (Tarran, *et al*., 2007). For benzene degradation by plant cytochrome P450 enzymes, electron donors such as NADH and NADPH are normally required (Kvesitadze, *et al*., 2009). These 2 electron donor molecules could be generated by photosynthesis of plant that light is commonly required. The exposure of 24 h light might increase quantity of electron donor and improve benzene removal. In addition, stomata had been considered as important for gaseous benzene uptake (Ugrekhelidze *et al*., 1996), and in most plant species stomata open under light conditions, so stomata of *D. sanderiana* in each cycle had been observed by general light microscope. At the 2nd, 3rd, and 4th cycles under 24 h dark conditions, the closed stomata were observed (Fig 4.3). The result suggests that benzene could be removed better under light conditions than in the dark, due to both the opening of stomata and electron donor generation by photosynthesis.

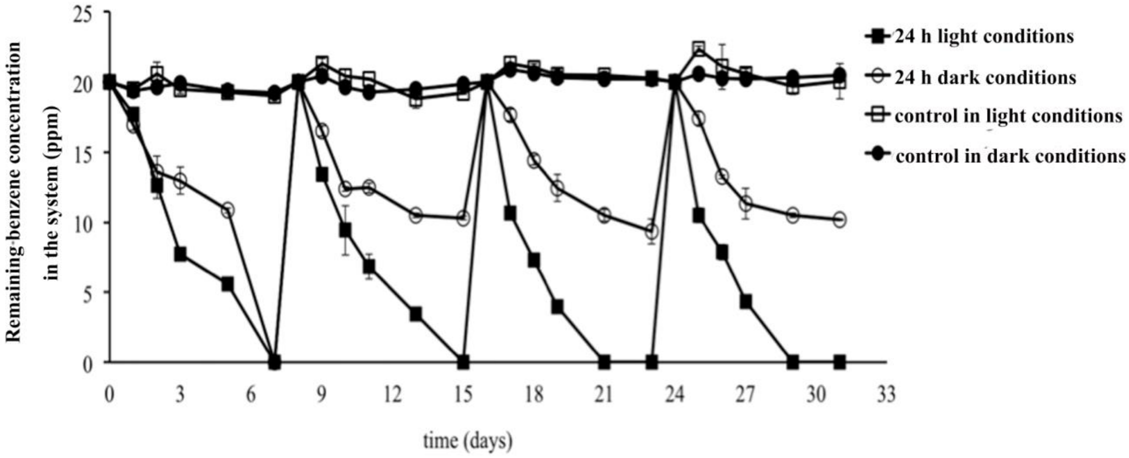

Figure 4.2 Remaining benzene concentration (ppm) in the system of 1st, 2nd, 3rd, and 4th cycle under 24 h light and 24 h dark conditions.

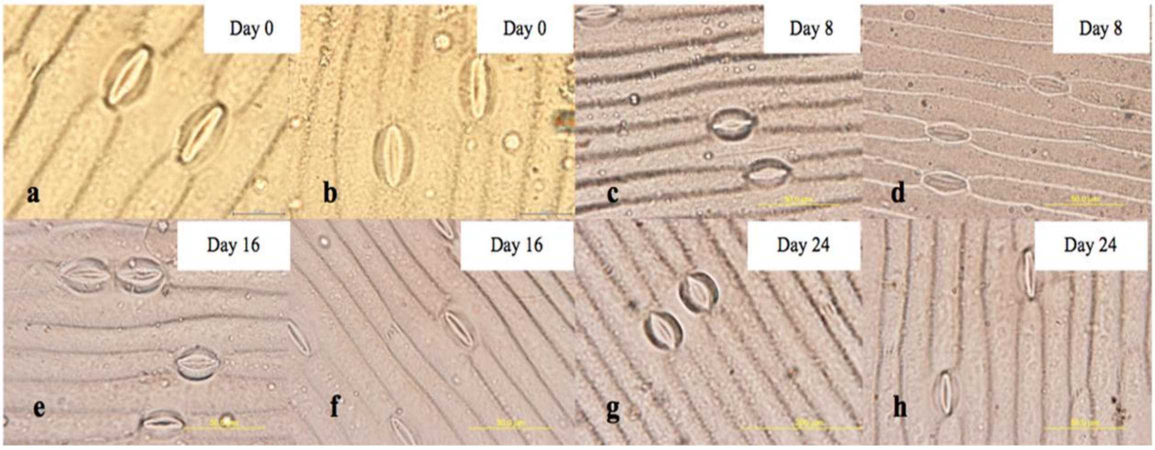

Figure 4.3 Stomata observation on the leaf of *D. sanderiana* under 24 h light conditions (a, c, e, g) and 24 h dark conditions (b, d, f, h) when exposure with 20 ppm of initial benzene concentration.

4.1.4 Benzene uptake by wax of *D. sanderiana*

Kvesitadze, *et al*. (2009) reported the possibility of benzene uptake by stomata and cuticle. The result suggested that although stomata had been known as a main pathway for benzene uptake by plant, some species can also take up high amounts of benzene through the cuticle. Not only research on environment, but also research on food, found the accumulation of benzene on crop leaf surfaces close to industrial locations (Gorna-Binkul, *et al*., 1996; Slaski, *et al*., 2000; Tsiros, *et al*., 1999; Collins, *et al*., 2000; Poborski, 1988; Reiderer, 1990; Kylin, *et al*., 1994). For example, in 1996, Gorna-Binkul, *et al.* (1996) reported benzene accumulation, including cucumber orange, parsley, cucumber (Gorna-Binkul, *et al*., 1996), apple and blackberry (Collins, *et al*., 2000). In theory, benzene that has 2.13 of log K_{ow} could transport easily through plant epidermises (Kamath, *et al*., 2004). The benzene-absorption efficiency of plant waxes was investigated to identify possible compounds for industrial application. In a preliminary experiment, crude wax of *D. sanderiana*, was extracted and used to adsorb benzene 72 h was required for the wax to reach saturation with benzene. The results showed that wax from 130 cm^2 of *D. sanderiana* leaf exposed to 20 ppm benzene, can uptake around 46 % of benzene, as shown in Fig 4.4. This result implied that the use of leaf material from some plant species for benzene adsorption is possible. This method has advantages of low cost and easy secondary disposal.

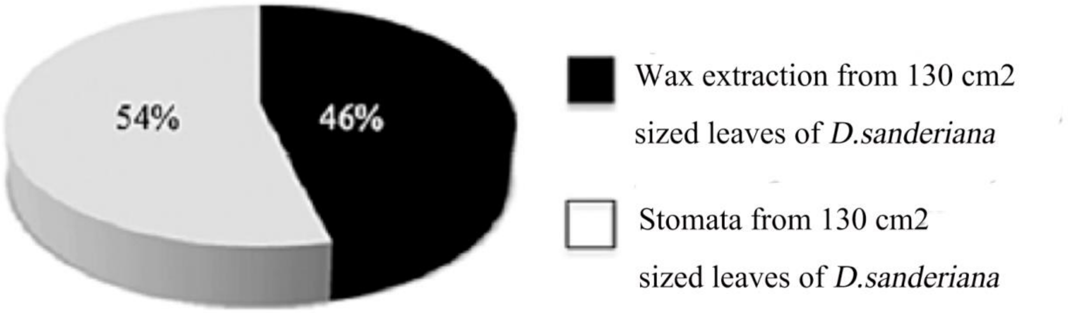

Figure 4.4 The ratios (%) of benzene uptake by wax and stomata of *D. sanderiana*, calculating base on 72 h exposure.

4.2 Benzene adsorption by biomaterials

4.2.1 Benzene adsorption by biomaterials in a static system

Since the cuticle of *D. sanderiana* adsorbed 46% of total benzene in a preliminary experiment, the leaves of other plant species might also adsorb high amounts of benzene, and for industrial application, the benzene adsorption by 21 plant materials was investigated. Benzene adsorbed per g of adsorbent was calculated and shown in Table 4.2. The dried leaf powders of *L. chinensis, D. picta, A. aureum, F. religiosa, L. macrocarpa, A. scholaris,* and *D. sanderiana* were the most efficient in benzene adsorption at day 3 of the experiment, although *L. chinensis* had relatively low benzene adsorption at day 1. Therefore, biomaterials from *D. picta, A. aureum, F. religiosa, L. macrocarpa, A. scholaris,* and *D. sanderiana* were suitable for application in a continuous system.

Table 4.2 Benzene adsorption efficiency by various dried leaf powders.

Plants Species	Benzene adsorption efficiency (µmole g^{-1} of adsorbent)		
	Day 1	**Day 2**	**Day 3**
Homalomena rubescens	0.92±0.01^{a}	0.94±0.02^{a}	1.10±0.28^{a}
Citrus hystrix	7.64±0.33^{d,e}	8.66±8.70^{c,d,e,f}	9.19±2.63^{b,c}
Musa paradisiaca	9.01±1.99^{d,e,f}	15.20±3.29^{g,h}	15.05±1.14^{e}
Mangifera indica	7.67±3.45^{d,e}	7.56±3.54^{b,c,d,e}	6.79±3.05^{b,c}
Catura metet	1.42±0.19^{a}	3.75±0.52^{a,b}	3.58±0.04^{a}
Lagerstroemia inermis	3.80±0.16^{b,c}	6.25±3.63^{b,c,d}	9.45±0.88^{c}
Cananga odorata	4.67±0.23^{b,c}	5.59±1.32^{b,c}	6.36±0.24^{b}
Cassia siamea	3.52±0.34^{b,c}	4.63±1.11^{a,b,c}	7.66±0.17^{b,c}
Bougain villea	3.38±0.37^{b}	5.89±0.30^{b,c,d}	7.06±1.17^{b,c}
Litchi chinensis	3.29±0.59^{b}	10.94±0.25^{e,f,g}	23.46±2.10^{h}
Coccinia grandis	4.62±1.84^{b,c}	4.37±2.28^{a,b,c}	6.64±0.33^{b,c}
Dieffenbachia picta	10.73±0.25^{f,g}	15.77±0.17^{h}	19.37±0.25^{f,g}
Attacus atlas	3.48±0.37^{b,c}	17.49±0.46^{h}	17.08±0.21^{e,f}
Polyalthia longifolia	6.34±0.91^{c,d}	7.98±0.44^{b,c,d,e,f}	9.39±0.51^{c}
Acrostichum aureum	10.97±0.63^{g,h}	14.59±0.54^{g,h}	18.90±0.54^{f,g}
Ficus religiosa	10.61±0.12^{f,g}	10.53±0.41^{d,e,f,g}	18.33±1.12^{f,g}
Lagerstroemia macrocarpa	10.94±0.54^{f,g}	12.59±0.03^{f,g,h}	20.07±0.88^{h}
Alstonia scholaris	9.61±0.52^{e,f}	14.25±0.38^{g,h}	20.57±1.62^{g,h}
Anthurium andraeanum	12.55±4.69^{g,h}	12.56±0.21^{f,g,h}	12.41±0.46^{d}
Plerocarpus Indicus	13.88±2.52^{h}	16.01±2.81^{h}	17.82±4.35^{f,g}
Dracaena sanderiana	11.00±4.68^{f,g}	16.02±1.62^{g,h}	19.00±2.8^{f,g}

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 (a-h), symbol classify group of data following the column.

4.2.2 The relationship between benzene adsorption and quantity of wax

One factor that might affect benzene adsorption efficiency by dried leaf material is the quantity of wax. To investigate this, crude wax was extracted from the samples, and the relationship between quantity of wax and benzene removal efficiency was analyzed. The results demonstrate that in general material of higher wax content removes benzene to a greater amount than material of lower wax content. To confirm the relationship between quantity of wax and benzene adsorption by leaf material, the correlation coefficient was analyzed, and a high R-square value, equal to 0.6512 was found for the logarithmic curve (Fig 4.5). The result could be discussed in the term of thickness of wax because although high wax quantity materials could uptake benzene well, higher quantity of wax than 0.25 g g^{-1} absorbent, benzene had been uptake stably (Topp, *et al*., 1986). Benzene transportation might be limited in how deep it can diffuse through the wax layer, so although high quantity of wax has higher thickness, benzene could not transport deeply to accumulate on the underside. In addition, some plant leaf material with low quantity of wax took up high benzene. In the other hand, some leaf materials have high wax quantity, but low benzene uptake had been found. These results suggest that not only quantity of wax but also wax composition might be a factor in benzene adsorption efficiency.

Table 4.3 Benzene adsorption efficiency by each plant leaf materials and their wax weight.

Plants species	Wax weight (mg g^{-1} of absorbent)	Benzene adsorption efficiency (μmole g^{-1} of adsorbent) at Day 3
Homalomena rubescens	$0.01±0.00^{a}$	$1.10±0.28^{a}$
Citrus hystrix	$0.06±0.01^{b,c}$	$9.19±2.63^{b,c}$
Musa paradisiaca	$0.06±0.02^{b,c}$	$15.05±1.14^{e}$
Mangifera indica	$0.05±0.00^{b,c}$	$6.79±3.05^{b,c}$
Catura metet	$0.01±0.00^{a}$	$3.58±0.04^{a}$
Lagerstroemia inermis	$0.05±0.00^{b,c}$	$9.45±0.88^{c}$
Cananga odorata	$0.04±0.01^{b}$	$6.36±0.24^{b}$
Cassia siamea	$0.16±0.04^{d}$	$7.66±0.17^{b,c}$
Bougain villea	$0.05±0.00^{b,c}$	$7.06±1.17^{b,c}$
Litchi chinensis	$0.21±0.02^{e}$	$23.46±2.10^{h}$
Coccinia grandis	$0.05±0.00^{b,c}$	$6.64±0.33^{b,c}$
Dieffenbachia picta	$0.15±0.01^{d}$	$19.37±0.25^{f,g}$
Attacus atlas	$0.10±0.00^{d}$	$17.08±0.21^{e,f}$
Polyalthia longifolia	$0.07±0.01^{c}$	$9.39±0.51^{c}$
Acrostichum aureum	$0.10±0.00^{d}$	$18.90±0.54^{f,g}$
Ficus religiosa	$0.10±0.00^{d}$	$18.33±1.12^{f,g}$
Lagerstroemia macrocarpa	$0.18±0.03^{e}$	$20.48±0.88^{h}$
Alstonia scholaris	$0.11±0.01^{d}$	$20.57±1.62^{g,h}$
Anthurium andraeanum	$0.14±0.02^{d}$	$12.41±0.46^{d}$
Plerocarpus Indicus	$0.10±0.02^{d}$	$17.82±4.35^{f,g}$
Dracaena sanderiana	$0.25±0.00^{e}$	$19.00±2.8^{f,g}$

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 (a-h), symbol classify group of data following the column.

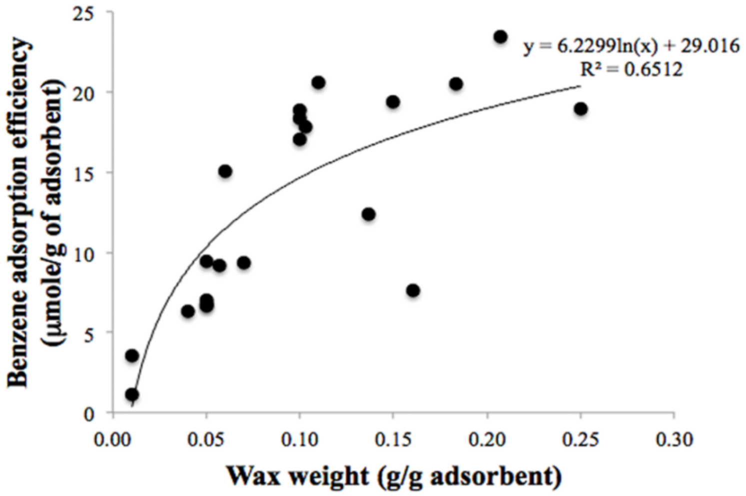

Figure 4.5 Relationship between benzene adsorption efficiency and wax quantity.

A comparison between benzene adsorption efficiency of various plant leaf materials grouped by wax weight is shown in Fig 4.6. The increasing of quantity of wax was considered as one factor affecting benzene adsorption, but there are still significant differences between plant materials with similar wax content. The result confirmed that the composition of wax could clearly be a factor affecting benzene adsorption by plant leaf materials.

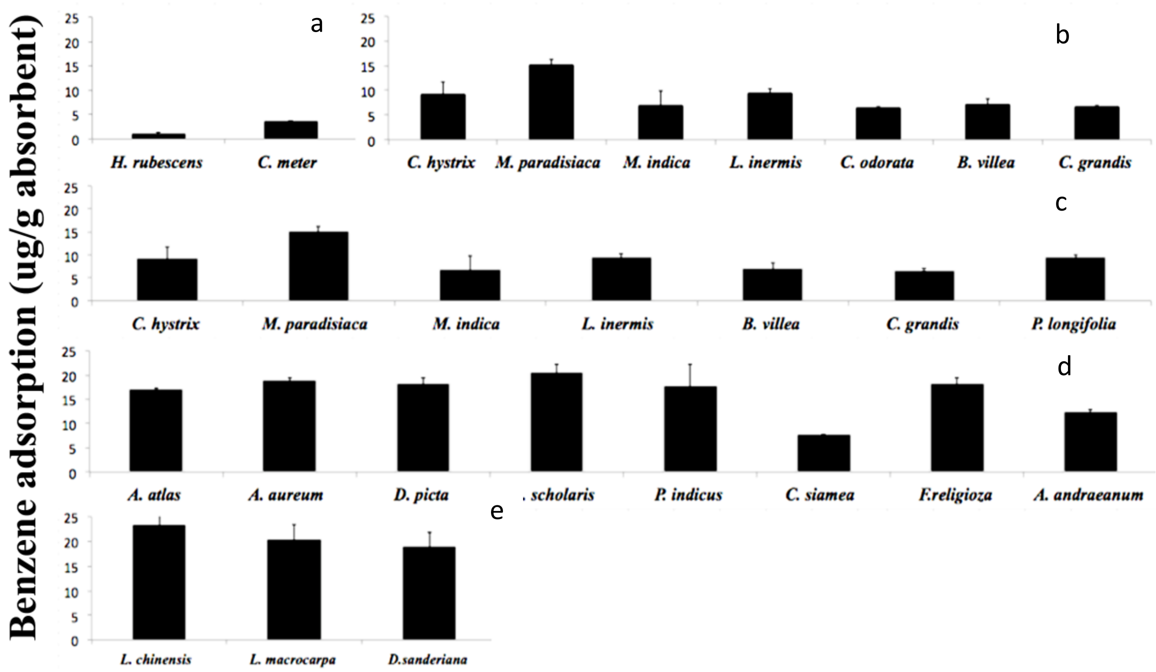

Figure 4.6 Comparison of benzene adsorption efficiency of plant leaf materials grouped by non-significantly difference in wax quantity by Duncan multiple range test: a) 0.01 -0.03 mg g^{-1} of absorbent b) 0.04-0.06 mg g^{-1} of absorbent c) 0.05-0.07 mg g^{-1} of absorbent d) 0.1-0.16 mg g^{-1} of absorbent and e) 0.18-0.25 mg g^{-1} of absorbent (average and SD in each dot and error bar, respectively).

4.2.3 The relationship between benzene adsorption and composition of wax in each plant species

Fatty acids have been commonly known as a main composition of plant wax. The wax of selected plant leaf material was measured and analyzed by GC-MS (Fig 4.7). Material from *M. paradisiaca* leaves was chosen for analysis, as previous results showed that it has significantly higher benzene removal efficiency than other materials with similar wax content.

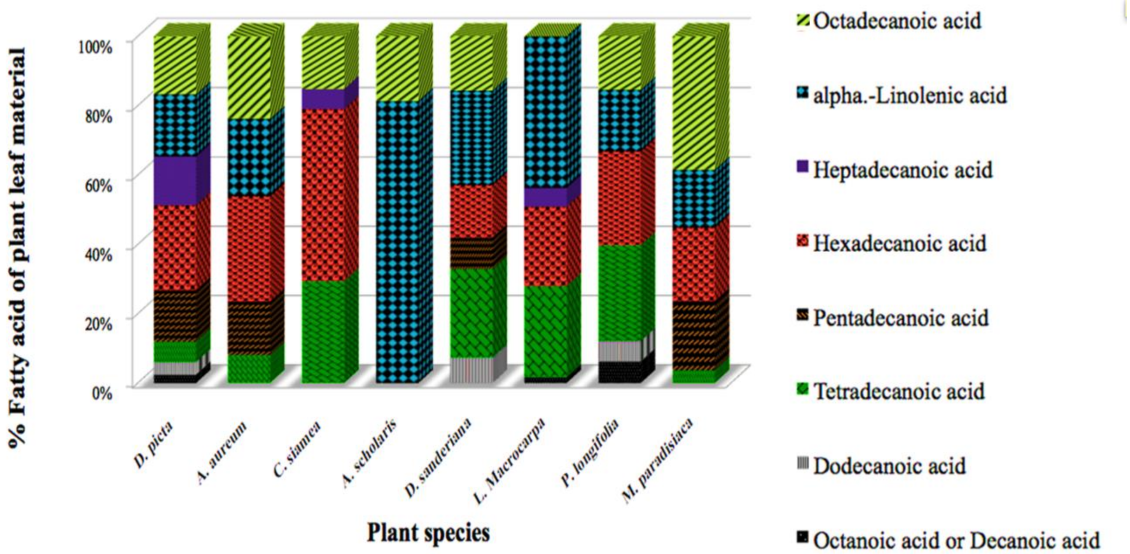

Figure 4.7 Percentage of fatty acid composition in wax of each plant leaf materials.

Plant leaf material from *P. longifolia* and *M. paradisiaca* are in the same grouping by wax weight, but *M. paradisiaca* leaf material was found to be more efficient in benzene removal (Fig 4.6). From the result, fatty acid composition was observed, and octadecanoic acid was found 38.5% of whole fatty acid composition in *M. paradisiaca* leaf material (Fig 4.7), and low among of octadecanoic acid had been found in *P. longifolia* leaf material.

Material from *C. siamea* was of interest because this material has significantly lower benzene removal efficiency (Fig 4.8b) compared with other materials with a similar wax weight. The fatty acid composition of *C. siamea* and three other plant materials of similar wax weight but higher benzene removal efficiency are shown in Fig 4.8a. The result suggested that alpha-linoleic acid may be the main factor for benzene removal, so material from *C. siamea*, which this fatty acid has not been found, have low benzene removal efficiency.

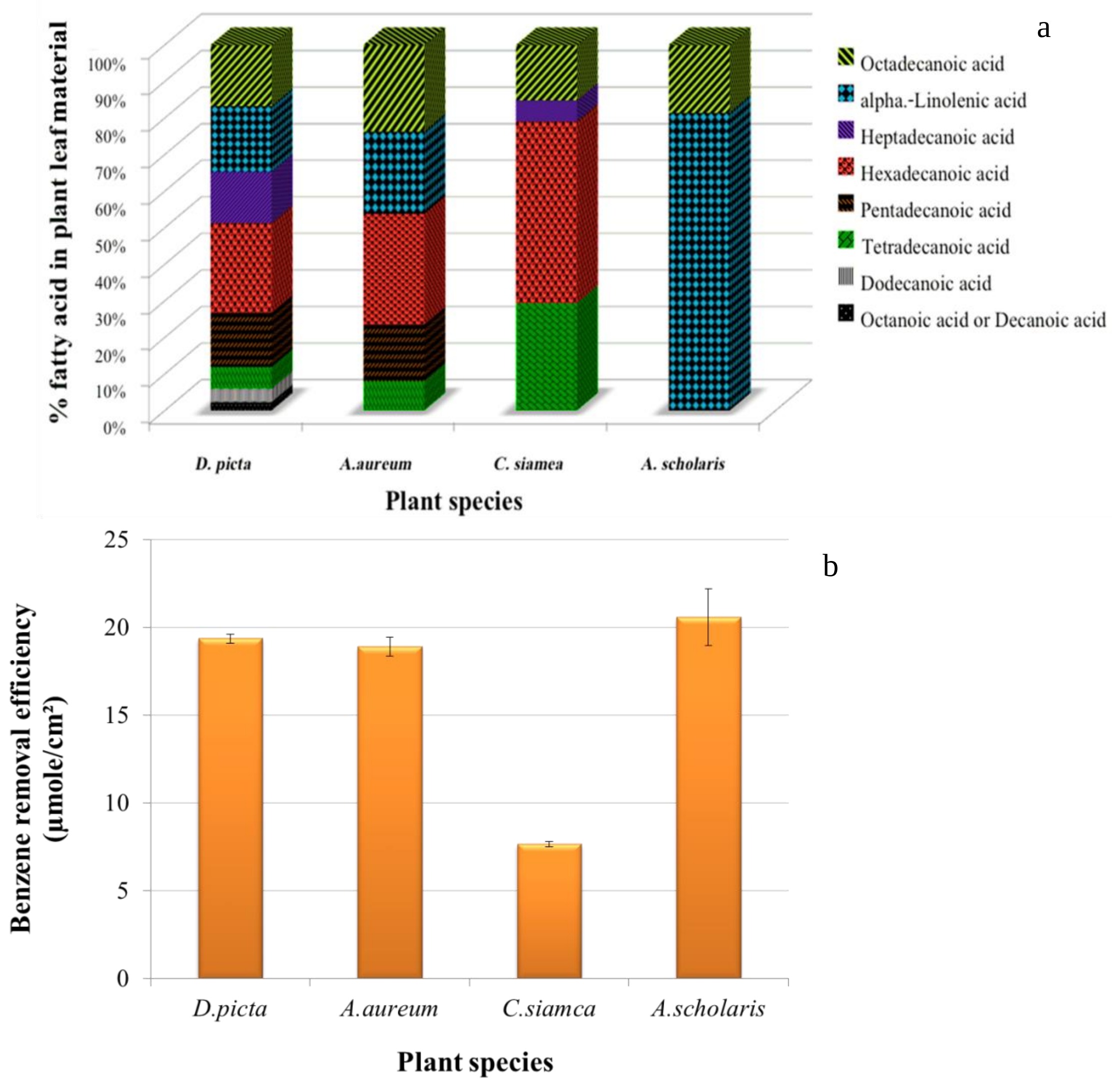

Figure 4.8 Percentage of fatty acid composition in wax of plant leaf materials that contain wax in the range of 0.10-0.16 mg g^{-1} of adsorbent (a) and benzene removal efficiency of these 4 plant leaf materials (b).

Alpha-linoleic acid was found in all species investigated except *C. siamea*. The increasing number of carbon on fatty acid molecules can decrease the polar fatty acids and might increase the solubility in benzene (Hoerr and Balston, 1944), so alpha-linoleic acid and octadecanoic acid might enhance benzene adsorption in plant leaf materials. A positive linear relationship between alpha-linolenic acid, but not octadecanoic acid, and benzene adsorption efficiency was identified (Fig 4.9).

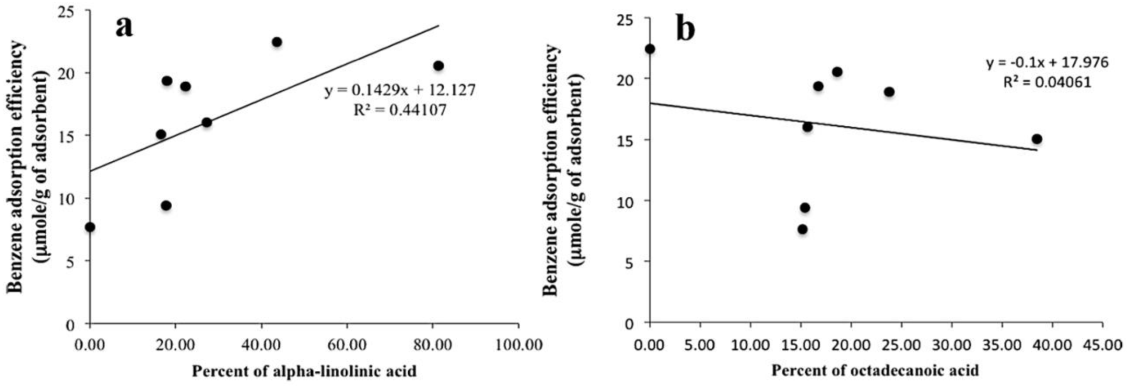

Figure 4.9 Linear regression of benzene adsorption efficiency and percentage of alpha-linoleic acid (a) and octadecanoic acid (b).

Fig 4.9 found that positive linear regression between benzene adsorption and percentage of alpha-linoleic acid had been present with 0.4410 of R-square, but linear regression between benzene adsorption and percentage of octadecanoic acid was not appeared. The result suggested that high quantity of alpha-linoleic acid might enhance benzene adsorption efficiency. Similar result had been found in xylene adsorption by pure alpha linoleic acid experiment. Not only fatty acid but also group of alkane was analyzed in Fig 4.10.

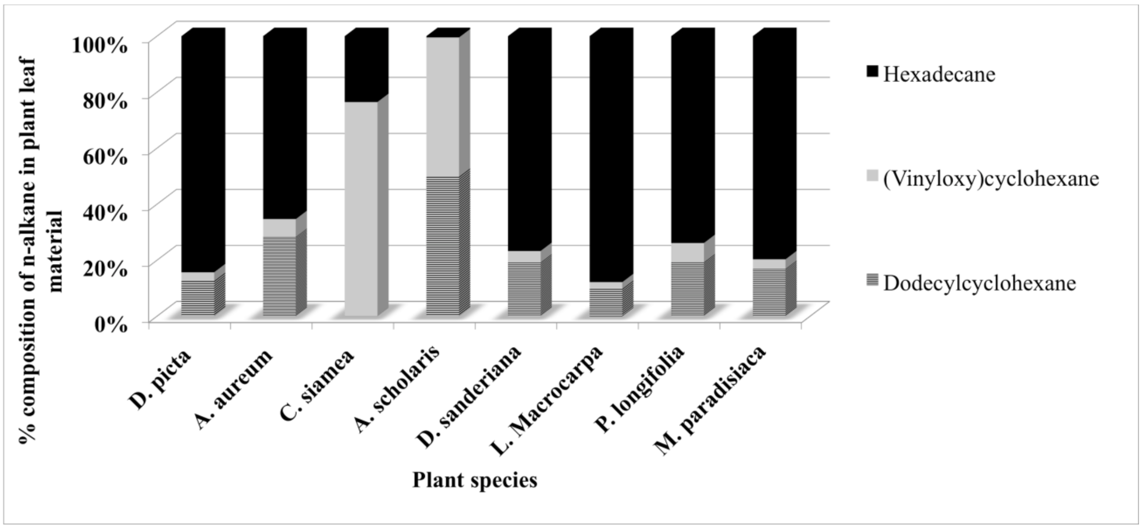

Figure 4.10 Percentage hexane composition in wax of each plant leaf materials.

High dodecyl cyclohexane in *A. scholaris* leaf material, which is a high benzene adsorption material, was found to be higher than other plant leaf materials. This result implied that dodecyl cyclohexane might also involve in benzene adsorption

4.2.4 Benzene adsorption by leaf material in a continuous system

For the application, the plant leaf materials of *D. picta, A. aureum, F.religiosa, L. macrocarpa, A. scholaris,* and *D.sanderiana* were ground and immobilized on glass beads. These materials were used as adsorbents in a continuous system with an air retention time of 3-5 min. Initial benzene of 55 ppm was controlled and continuous feed into the system. 80% benzene removal efficiency was found in *D. picta, A. aureum, A.*

scholaris, and *D.sanderiana*, and 60% benzene removal efficiency was found in *F. religiosa, L. macrocarpa* (Fig 4.11). Most of plant materials had a limit capacity within 120 h. However *A. aureum* and *A. scholaris* leaf materials were occurred as the highest benzene removal capacity that had a limit capacity within 132 h.

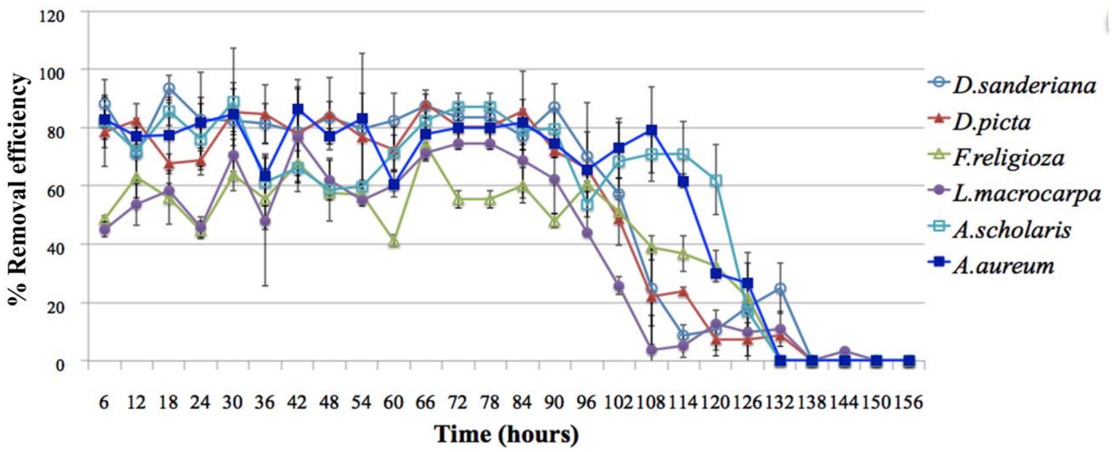

Figure 4.11 Benzene removal efficiency (%) in a continuous system of 6 plants biomaterials.

4.2.5 Benzene adsorption mechanisms

Capacity of each plant leaf material was calculated in the unit of umole/g absorbent and shown in Table 4.4. Benzene adsorption materials were desorbed by using hexane as an eluent. High quantity benzene desorption was found about 99% in selected materials.

Table 4.4 Benzene adsorption capacities of 6 plant biomaterials and percentage of benzene desorption in these selected materials.

	D.sanderiana	*D.picta*	*F.religioza*	*L.macrocarpa*	*A.aureum*	*A.scholaris*
Total porosity (cc g^{-1})	0.0703	0.0717	0.0461	0.1385	0.0694	0.2382
Micro pore volume (cc g^{-1})	0.0137	0.0181	0.0084	0.0176	0.0124	0.0377
Capacity (u mole/g)	20.37	20.32	16.08	20.77	21.15	21.50
%Desorption by hexane	>99.5%	>99.5%	>99.5%	>99.5%	>99.5%	83.11

Physical adsorption might be involved for benzene adsorption mechanism in wax of these materials because hexane can desorb benzene easily from most of plant leaf materials. However only 83.11% benzene desorption was occurred in *A.scholaris* leaf material because high porosity was found clearly in biomaterial from this plant leaf. Physical sorption mechanism was confirmed by FT-IR result. Functional groups on adsorbent surface were observed (Fig 4.12). In addition, although a few studies reported benzene adsorption mechanism by plant leaf materials or other natural materials, benzene adsorption by activated carbon presented that physical adsorption is the main benzene adsorption mechanism (Ramirez, *et al.*, 2005; Rozwadowski and Wojsz, 1987; Stoeckli, *et al.*, 2001)

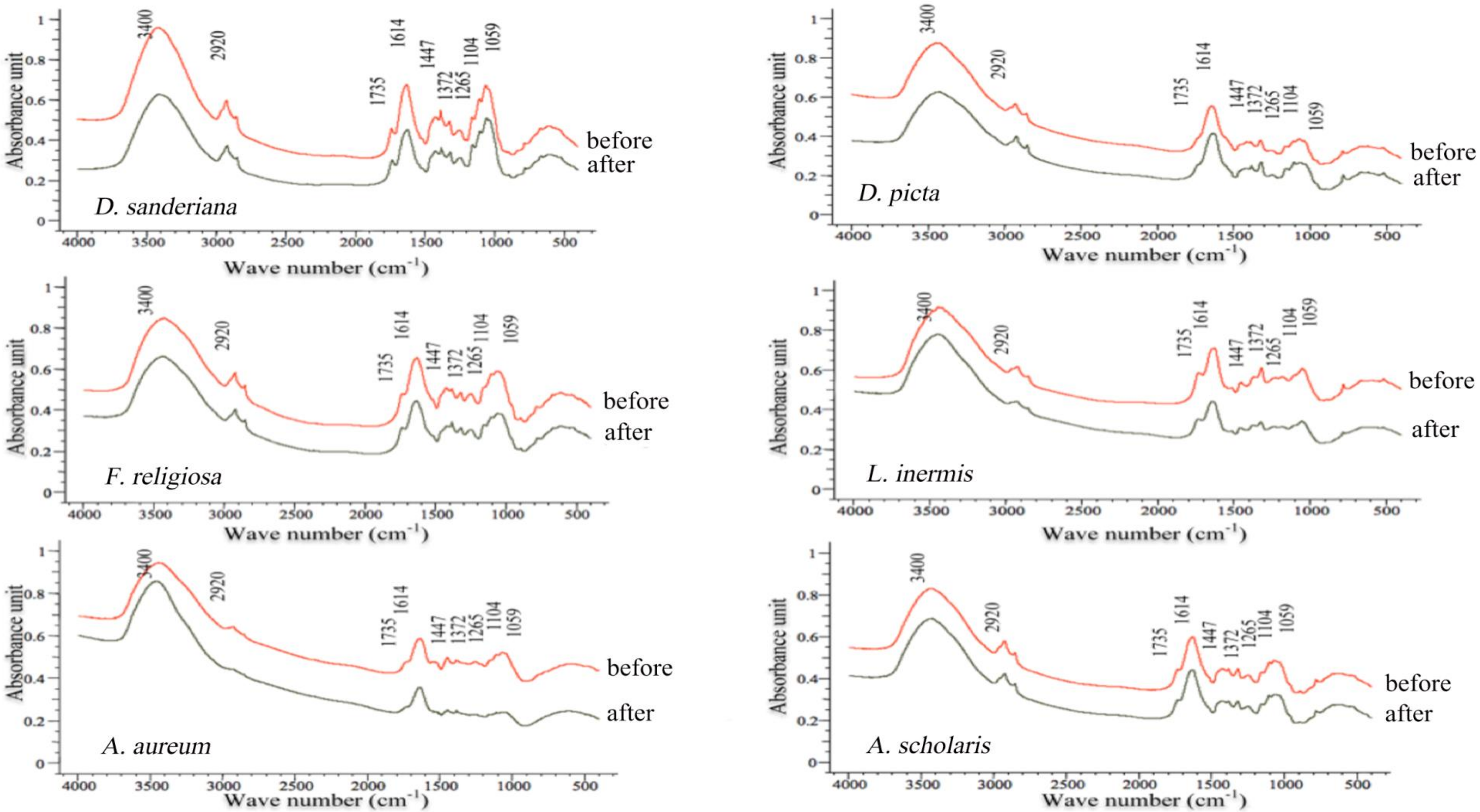

Figure 4.12 Functional groups on the surface of adsorbent before and after benzene adsorption in selected plant leaf material.

Fig 4.12 shows the same tendency of functional groups of plant leaf material surface before and after benzene treatment process, and the same pattern of functional group of every plant leaf material surfaces were found as (O-H, around 3400 cm^{-1} and C-H, at 2920 cm^{-1}). In addition, many fingerprint regions were found between 1800 and 1000 cm^{-1}. The peaks that were observed at 1,735 and 1,614 cm^{-1} showed C=O and C=C, respectively. A Methoxy (O-CH_3) group was also found at 1,447 cm^{-1}. At 1,372 and 1,265 cm^{-1}, a fingerprint region was presented as O-H deformation originating from the phenolic group, and C-OH in primary alcohol and secondary alcohol was shown around 1,059 cm^{-1} and 1,104 cm^{-1}, respectively. This result suggested that the main mechanism for benzene adsorption by plant leaf materials should be physical adsorption.

4.3 Biofilter application

4.3.1 Loading rate evaluation of biofilter application

P. putida, which could completely uptake benzene and use benzene as a carbon and energy sources, was applied in this study (Heald and Jenkins, 1996; Reardon, *et al*., 2000). This strain was commonly applied for benzene removal in many biofilter system (Harwood, 1989) In this experiment that glass bead immobilized with plant leaf materials had been used to be packing bead in biofilter system. Enriched medium was feed to be the rich of nutrient support for the growth of microorganisms every day. 55 ppm of benzene concentration was feed continuously into the system. The flow rate was varies to evaluate suitable loading rate for the system. Benzene removal efficiency in 4 phases showed the difference of flow rate affected benzene removal (Fig 4.13). The result show that phase 1 and 2 of the experiment, the system that contain plant leaf material cassava-bead immobilized with *P. putida* could adapt to remove benzene completely, but in high flow rate in phase 3 and 4, low benzene removal was occurred. In cassava-bead immobilized with *P. putida*, benzene removal was lower than plant leaf materials-bead immobilized with *P. putida* in every phase. In other treatment such as *A. aureum* cassava-bead without *P. putida*, *A. scholaris* cassava-bead without *P. putida* and glass bead immobilized with *P. putida* that were feed by enriched medium, very low benzene removal was found. The result suggested that benzene can slowly solute in enriched medium, so enriched medium can decrease benzene adsorption efficiency. The relation between benzene loading rate (g m^{-3} h^{-1}) and elimination capacity (g m^{-3} h^{-1}) in phase 1 and 2 was shown in Fig 4.14. Low R-square was found in control system such as *A. aureum* cassava-bead without *P. putida*, *A. scholaris* cassava-bead without *P. putida* and glass bead immobilized with *P. putida* that were feed by enriched medium. In treatment system, cassava-bead immobilized with *P. putida*, *A. aureum* cassava-bead immobilized with *P. putida* and *A. scholaris* cassava-bead immobilized with *P. putida* were found that have R-square about 0.96, 0.92, and 0.91, respectively. *A. aureum* cassava-bead immobilized with *P. putida* and *A. scholaris* cassava-bead immobilized with *P. putida* shown as highest benzene removal efficiency and was more effective than cassava-bead immobilized with *P. putida*. Suitable loading rate of biofilter system using plant material as a packing bean was around 10 g m^{-3} h^{-1}.

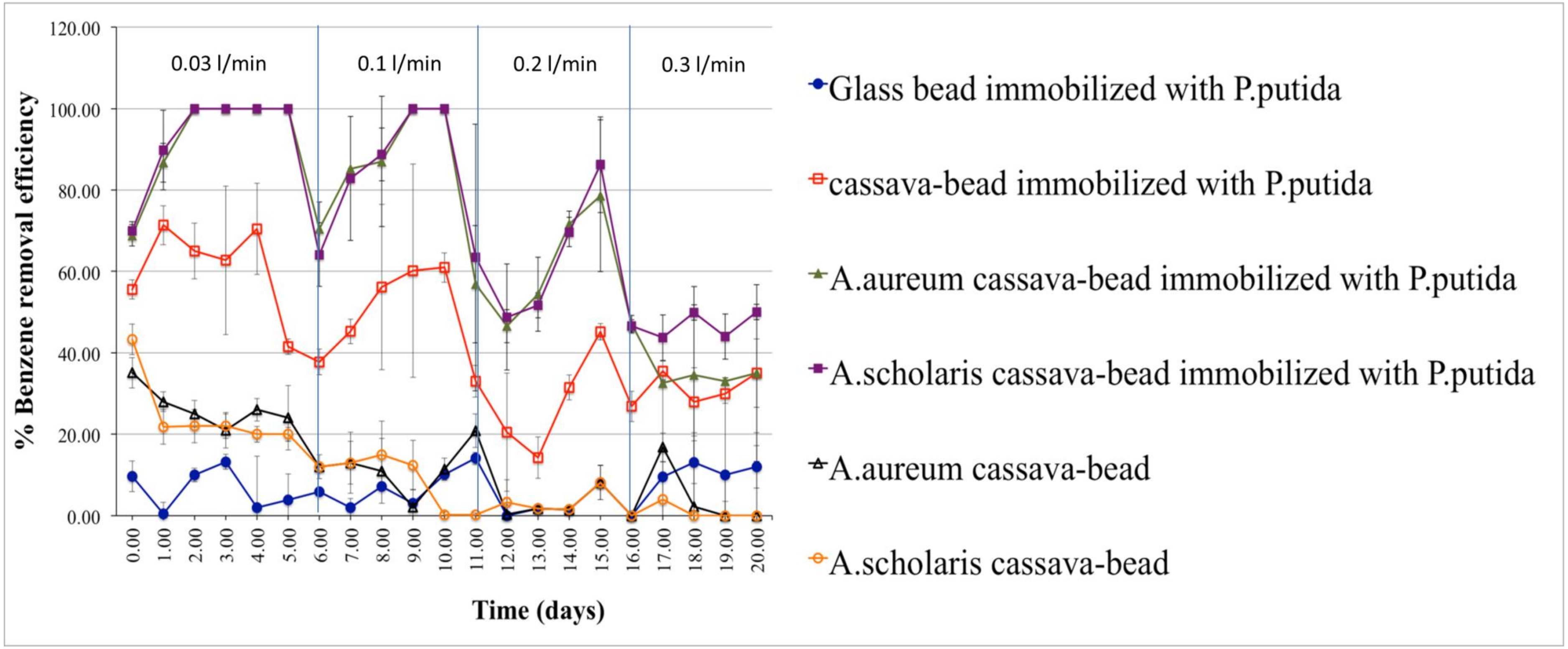

Fig 4.13 Benzene removal efficiency in phase 1-4 of each bio filters system: Phase 1, 2, 3, 4 refer to 0.03, 0.1, 0.2, and 0.3 L min^{-1} of flow rate, respectively.

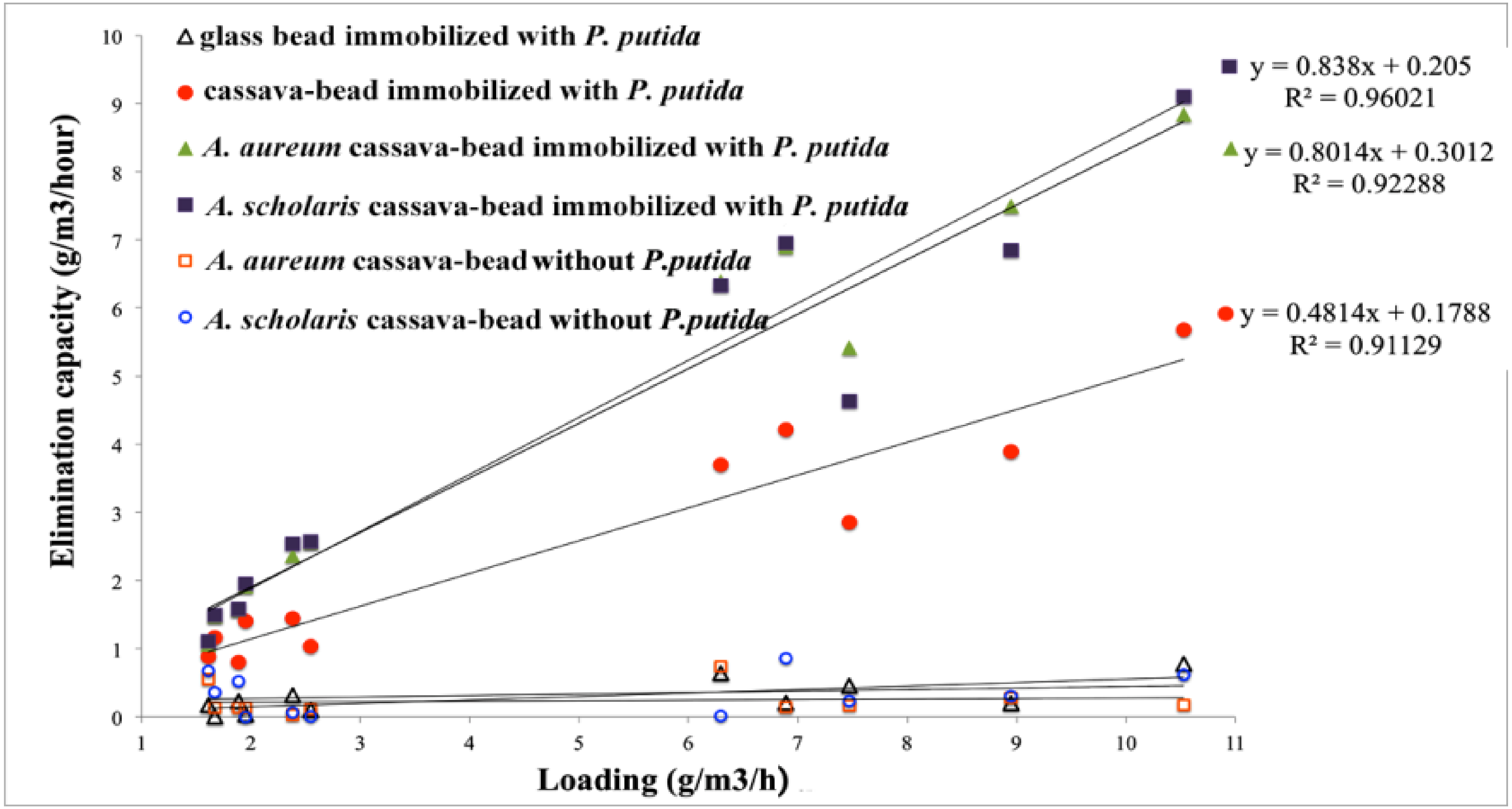

Figure 4.14 The relation between benzene loading rate (g m^{-3} h^{-1}) and elimination capacity (g m^{-3} h^{-1}) in phase 1, 2 of each bio filter.

4.3.2 Suitable nutrient evaluation of biofilter application

Although organic packing materials such as plant leaves might be the carbon and energy source for microorganisms (Devinny, *et al*., 1999), enrichment nutrient support was still required in the nutrient-poor medium (Leson and winter, 1991). From loading rate evaluation, 0.1 L min^{-1} of airflow with initial concentration of benzene 55 ppm was found that is suitable rate for benzene removal. This flow rate was applied in varies nutrient experiment to find the best nutrient. The result showed that enriched medium was a suitable nutrient because the systems that were feed by enriched medium have clearly high benzene removal efficiency if compared with other treatments that were feed by minimum medium and sterile water. This result suggested that *P.putida* could difficultly use plant leaf materials as a carbon source and grow slowly in minimum medium and sterile water feed system. The same result has been found in many studies (Barona, *et al*., 2004; Clark, *et al*., 2004; Weckhuysen, *et al*., 1993; 1994). Not only benzene removal efficiency but also slope of the relation between loading rate and elimination capacity was clearly shown that enriched medium was the best nutrient in the experiment. High R-square value in every treatment and linear equation was shown in Fig 4.16.

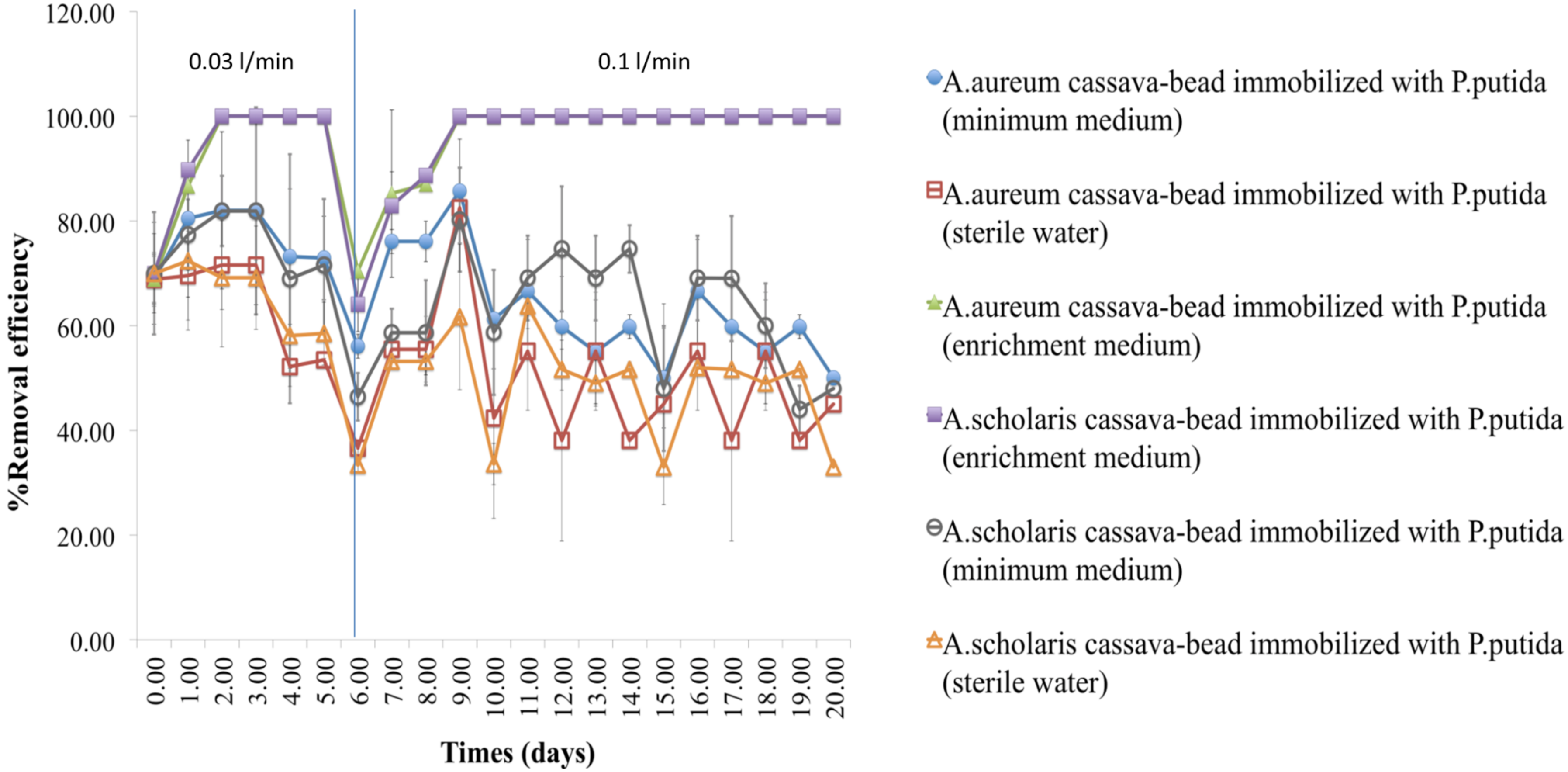

Figure 4.15 Benzene removal efficiency by leaf materials cassava-bead immobilized by *P. putida* at different nutrients.

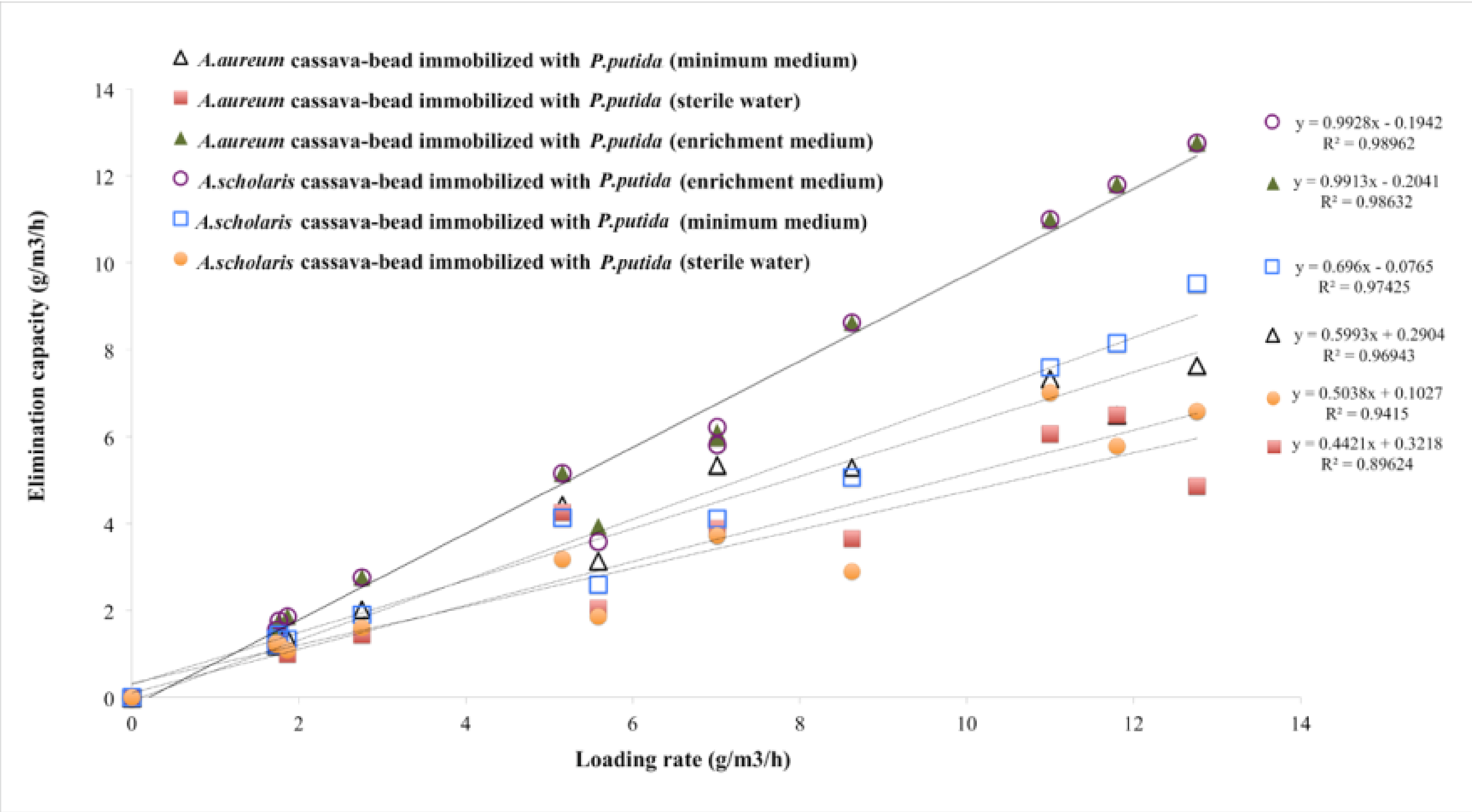

Figure 4.16 The relation between benzene loading rate (g/m^3/h) and elimination capacity (g/m^3/h) in phase 1, 2 of benzene removal efficiency by leave materials cassava-bead immobilized with *P. putida* in different of supported nutrient.