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Transformation towards
Smart, Resilient & Sustainable
Communities

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CONTENT

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Urban Community Garden: Drivers and Motivations

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Community garden has gained much popularity in urban areas owing to its potential that it may bring to the environment, economy, and social development. Greening the cities with urban garden and farming activities is one of the means that help cities to improve urban resilience, as well as improve the food security of the urban community. Such effort directly supports the interlinked principles of New Urban Agenda and addresses the Sustainable Development Goal 11 (Sustainable Cities and Communities), 13 (Climate Action), and 15 (Life of Land). Traditionally, gardens have always played a significant role in achieving a sustainable community. Furthermore, community gardening has the potential to achieve resilience at different levels: individual, social group as well as the natural environment. Today, the awareness of the expected benefits of community garden continue to increase. Previous studies highlighted that the majority of the urban community has initiated community gardens as a mean to overcome food shortage, stress relief, and boost sense of community. This paper examines the drivers and motivations on the involvement in the community garden in an urban setting. The drivers and motivations are evaluated based on a questionnaire survey and guided interview with selected participants of Sungai Bunus Community Garden, Kuala Lumpur. Findings of the study indicated that through active involvement in gardening activities increases sense of community, enhances gardening skills, improve food security and most important, motivate to start own garden for self-consumption. Also, majority of the respondents associated the benefits of the activity to satisfy their personal benefits related to gardening activities i.e. to explore new ideas on gardening (88 %), to find solutions (84 %), resources (80 %) and reinforcement of existing knowledge (72 %). Indirectly, the community garden has successfully unified the urban community within the vicinity, inculcate healthy lifestyle through gardening, contribute to carbon reduction in Kuala Lumpur and support the Sustainable Development Goals. Hence, such community garden needs to be advocated to provide further impact and collectively strengthen the sustainability and improve the people's awareness of the human-nature relationship.

1. Introduction

Community garden has gained much popularity in urban areas owing to its potential that it may bring to the environment, economy, and social development. Greening the cities with urban garden and farming activities is one of the means that help cities to improve urban resilience, as well as improve the food security of the urban community. Such effort directly supports the interlinked principles of New Urban Agenda and addresses the Sustainable Development Goal 11 (Sustainable Cities and Communities), 13 (Climate Action), and 15 (Life of Land). Community resilience is referred to the ability of communities to survive, adapt to, and recover from loss and disruptions that may affect their daily lives (Nursey-Bray et al., 2014). Traditionally, gardens have always played a significant role in community resilience. Rural communities, in particular, have relied on compound and backyard gardens for food shortages during the economic and political crisis. Rezai et al. (2016) pointed out that urban agriculture has gained popularity in Malaysia due to its potential to provide urban dwellers access to

fresh produce at low cost. Today, the awareness of the expected benefits of a community garden continues to increase. Previous studies have highlighted that the majority of the urban community initiated community gardens as a mean to overcome food shortage, stress relief, and boost sense of community. This paper examines the drivers and motivations of the community garden participation in an urban setting with specific focus on Sungai Bunus Community Garden, Kuala Lumpur as part of the effort to rejuvenate the abandoned/unused land along the riverbank of Sungai Bunus.

2. Literature review

2.1. Understanding urban community garden

Community gardens are distinguished from a private garden in terms of its ownership, authority and access, where it reflects the sense of a public garden (Ferris et al., 2001). Community garden as defined by Glover from Beilin and Hunter (2011) states that it is a piece of land where people from a community able to produce food or grow flowers for personal use. It has collective benefits thus the community share the resources. These gardens are collectively operated by a group of people or a community from various setting such as- neighbourhood, institutions, hospitals, prisons, faith community, organizations etc. and contribute to individuals from all age, ethnicity, and socioeconomic status (Draper and Freedman, 2010). The concept of community garden became widespread during the two world wars in order to overcome war-time food shortage in Europe, UK and America (Egli et al., 2016). These gardens were an important initiative taken by the communities in order to supply of rations, food and necessary nutrients. Today, community gardens not only serve the purpose as an alternative food supply but also provide the opportunity for income generation and outdoor activities as well. Indirectly, they create interaction among communities and sense of belonging to the place (Agustina and Beilin, 2012).

2.2. Types of community garden

Allotment gardens are the first variation of urban garden where individual gardeners rent plots owned by local municipality which have well-defined contract and aim for reducing poverty. Later other types of community gardens were popularized which shifted the emphasis from food production and supply to bringing communities together, educating the community, relieving stress, strengthening sense of community and resilience. Community gardens include neighbourhood gardens, institutional gardens, mixed and specialized production gardens etc. (Trendov, 2018). Ferris et al. (2001) identified eight categories of community gardens while conducting research on community gardens in San Francisco and considered these typology comprise most types of gardens worldwide. Among the category of community gardens are as follows:

i. Leisure gardens- These are the most common types of community gardens initiated in a neighbourhood for recreation and leisure purpose.
ii. Child and school gardens- School gardens are built and maintained by the students which are important platform for educational activity (part of science curriculum) as well as food production.
iii. Entrepreneurial gardens- In disadvantaged neighbourhood community gardens are built to alleviate poverty and social exclusion by providing opportunity to generate income for the participants.
iv. Crime diversion gardens/Work and training gardens- These community gardens are used to divert young people from negative activities in addition to encourage training and income opportunity.
v. Healing and therapy gardens/Quiet gardens- These gardens are part of treatment or healing, managed by hospital community that offers community care, rehabilitation program and memorials.
vi. Neighbourhood pocket parks- A civic park created voluntarily by neighbourhood community in a reclaimed unused or under used land which is open for residents and public as well.
vii. Ecological restoration gardens/parks- The community parks that are established from the social objective of restoring a place to its natural form to control the ecological balance. These gardens involve voluntary approach as well as scientific expertise.
viii. Demonstration gardens- Some of the community gardens are part of research on sustainable agriculture or organic food, which also serve the purpose of teaching residents about composting and water conservation.

Along this line, the Sungai Bunus Community Garden falls under three categories listed above; (i) neighbourhood pocket park, (ii) ecological restoration garden and (iii) demonstration garden.

2.3. Benefits of community garden

Urban community garden has many benefits not only confined to food security, environment and economy, but also in societal wellbeing by positively influencing individual emotional experience and social community

integration (Egli et al., 2016). Improvement to food security is one of the most cited benefit of community gardens. According to Corrigan (2011) community, gardening and producing food within neighbourhood can overcome unequal food distribution problems and ensure nutritious food to everyone. Community gardens establish economic stability through producing foods and creating provision for selling production in local markets and encouraging community supported agriculture and food processing microenterprises (Corrigan, 2011). On health related benefits, urban community farming promotes fresh fruits and vegetables consumption among participants as well as community members that increase positive dietary habit (McVey et al., 2018). Moreover, gardening encourages outdoor physical activity among youth and adults which lead to improved physical fitness (Draper and Freedman, 2010). Urban community gardens are often an alternative for public parks for socialisation, where community members can enjoy nature and enrich personal perspectives (Draper and Freedman, 2010). Community gardens is also often act as public space that require collaboration and negotiation (He and Zhu, 2018).

With regards to community sustainability and resilience, community gardens are often created by group of people in network to leverage power by creating strong connection (Ghose and Pettygrove, 2014). These gardens enhance collective efficiency of participants by encouraging to share resources inside and outside the community network which bring together people from different social background and strengthen sense of community (Draper and Freedman, 2010). Community gardening provide opportunity to youth for participatory learning and enhance academic and social skills. Additionally engaging young people in gardening activities result in lower crime rate and maintain favourable neighbourhood environment (Draper and Freedman, 2010). In terms of social and cultural aspects, studies in US show that gardeners experience significant connection with their gardens that indicate social attachment with the place and community. They appreciate the experience of gardening and take pride in their neighbourhood (Petrovic et al., 2019). Community gardens reflect the participants' culture through the produced plants, activities, events held, design and helps preserve cultural diversity (Draper and Freedman, 2010). Gardening activities require interaction and cohesion which help strengthen urban community resilience (Shimpo et al., 2019). By strengthening community resilience urban gardens help accomplish SDG 11 of making cities and communities sustainable. Overall, community gardens have the potential to establish social empowerment and improve social cohesion (McVey et al., 2018).

Often, urban community gardens are managed by spontaneous participation of the members of the community where people from different background come together and gardening activity serve in diverse manner for each individual. For some people, gardens provide economic benefit and supply healthy food, on the other hand, it also boosts sense of ownership and pride and relieves stress (Egli et al., 2016). In terms of governance, in Europe and US, these agricultural zone in urban areas are legitimized by government that indicates these community gardens are maintained by both bottom-up and top-down approach (He and Zhu, 2018). Also, community gardens contribute to restore ecological balance by bringing back biodiversity; birds and insect species in city centre, flood mitigation through infiltration and greens that cool the air in high density areas (Middle et al., 2014). Furthermore, community gardening have the potential to achieve sustainability at different levels of individual, social group and natural environment (Okvat and Zautra, 2011). Gardening activities in unused lands rejuvenate the place as well as benefit the society. Also, urban community gardens play important role in community recovery after disasters or extreme conditions by creating opportunities to collaborate and building networks within community members.

3. Methodology

This paper assesses the drivers and motivations on the involvement in the community garden in an urban setting. The drivers and motivations are determined based on a questionnaire survey and in-depth interview with selected participants of Sungai Bunus Community Garden, Kuala Lumpur. The potentials of this community garden are evaluated based on a questionnaire survey and guided interviews with selected participants of Sungai Bunus Community Garden, Kuala Lumpur. Twenty-five participants of the community garden responded to the questionnaire survey which were distributed after their gardening sessions and eight participants were interviewed. The questionnaire survey gathered information on their perceptions towards community garden, the motivation of their involvement and the perceived change of before and after their involvement in gardening activities.

4. Findings and discussion

Sungai Bunus Community Garden, initiated in 2017 aimed to rejuvenate the abandoned Sungai Bunus riverbank and to improve the water quality of Sungai Bunus River, a 9-kilometre stretch river that flows in Kuala Lumpur. Through partnerships with various organisations and corporate agencies located at the vicinity of the site, the garden was progressively developed by these committed volunteers. Among the main active partners of Sungai

Bunus community garden are Universiti Teknologi Malaysia, Expressionz, Bernama and Ecoknights. The community garden progresses through the weekly gardening activities. Since the location of Sungai Bunus Community garden is not within close proximity to residential neighbourhood, therefore, each partner take turn to organise and conduct monthly event to ensure continuous effort and sustainability of the garden. The weekly slot is also valued as a short retreat for working people and students to make time for some gardening and recreational activities as well as an opportunity to socialise with others. The community garden is also being recognised by Kuala Lumpur City Hall as a showcase to present the idea of community garden within an urban setting.

Figure 1: Sungai Bunus Community Garden, Kuala Lumpur Photo: Author, 2018

The focus of the study is to gather the participants' perceptions on their involvement in the Sungai Bunus community garden. Similar to other established community gardens (Shimpo et al., 2019), the participation in the gardening activities at Sungai Bunus Community Garden are mostly on voluntary basis. Findings from the survey revealed that the motivation of participation in the community garden is related to the three aspects of environmental stewardship, opportunities for social engagement, as well as recreational activities. Based on the survey, it is revealed that motivation to set up own garden is the top motivator for their involvement with mean score (M) of 4.24, followed by greater confidence in gardening activities (M = 4.04) and opportunities for social interaction to improve social support (M = 3.96). Nevertheless, prior studies also revealed that one of the reasons for an individual to volunteer for gardening activities is because of his/ her great interest in nature and leisure activities. A study conducted by Torres et al. (2018) revealed the first motivations of regular volunteering in gardening activities are environmental purposes, social interaction and for self-satisfactions. For environmental purposes motivations, the main reason for the establishment of Sungai Bunus community garden is to rejuvenate the environment of the area. With its location situated at the riverbank of Sungai Bunus, the community garden has the potential to gradually improve the water quality of the river and enhance the community's awareness of the human-nature relationship.

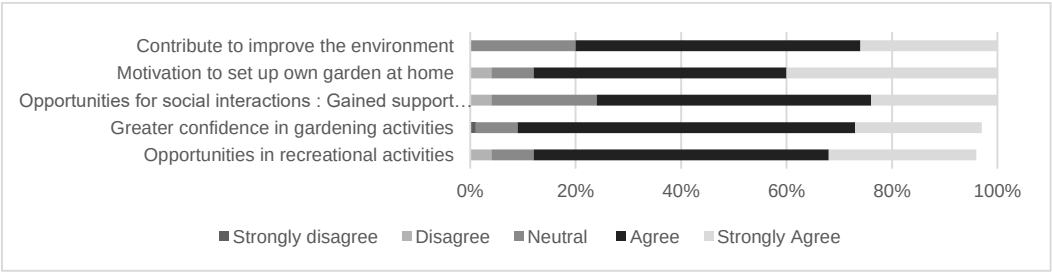

Figure 2: Motivation of participation

Figure 3 illustrates the perceived benefits of participation in community garden. Since the nature of the activities are on voluntary basis, those involved are participants that have strong interest in gardening. The involvement is also motivated by the fact that they are targeting to gain knowledge and improve their skills in gardening. This finding corresponds to previous studies that when it comes to aspects of volunteering, self-motivation is essential. Ryan et al. (2001) explained that the act of volunteering is much related to personal benefits and emotional motivation (Ryan et al., 2001). The result reported in Figure 3, shows similar pattern. Majority of the respondents of the study associate the benefits of the activity to satisfy their personal benefits related to gardening activities i.e. to explore new ideas on gardening (88 %), to find solutions (84 %), resources (80 %) and reinforcement of existing knowledge (72 %).

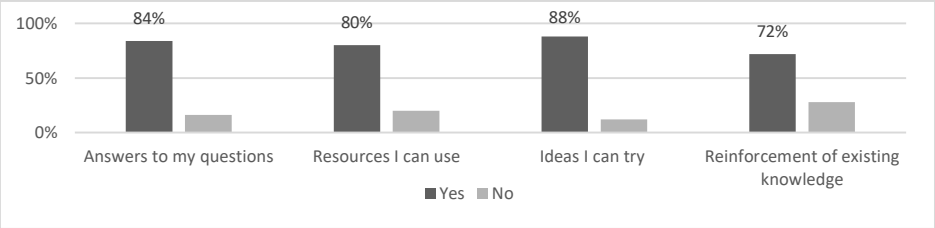

Figure 3: Benefits of participation in Community Garden

During the interview sessions, most of the interviewees indicated that they improved their knowledge and skills in gardening after few gardening sessions with Sungai Bunus Community Garden. More than 70% of the participants have reported that it gave them positive impact on various aspects of gardening. Their knowledge on types of plants, planting method, composting process and the use of organic fertilizer have improved. With reference to Table 1, on the scale from 1 to 4, improved on knowledge of composting was reported as the best progression (before M = 1.91, after M = 3.48). This has resulted to improved quantity and quality of harvests that contributes to higher self-satisfaction among the participants. It has also become the motivator to attract more volunteers to be involved in developing and supporting the community garden that translates to stronger sense of attachment. Similar to the study conducted by Veen et al. (2015), the sense of attachment developed towards the community garden is a positive indication of growing community development. Knowledge and skill exchange during the gardening activities are well acknowledged by the participants.

Table 1: Impact of participation in Community Garden (Before and after)

	Before	After
Type of plants	1.96	3.44
Knowledge on Planting method	2.32	3.48
Knowledge of Composting	1.91	3.48
Knowledge on fertilizer (organic)	2.20	3.40

Based on the interviews with selected participants, there are some concerns raised on the issue of funding the community garden. As the garden is on voluntary terms, financial resources are limited. Current funding is also based on contributions by the partner organisations/ agencies and support from Kuala Lumpur City Hall, through its Local Agenda 21 Unit. The sustainability of the community garden is still questionable as the funding is only on short term basis. Some participants have expressed that the garden need to produce more harvest (to be sold) in order to sustain the community garden in the long run.

5. Conclusion

This study has highlighted the factors that motivate the involvement of the community in an urban setting. Sungai Bunus Community Garden is a unique one because of its location and nature of participation. Participants of the community garden are among working populations with mutual interest in gardening activities that aimed at the same cause. Based on voluntary basis, participants are committed at sustaining the garden through the weekly gardening activities. With the support and recognition from Local Agenda 21 Unit, Kuala Lumpur City Hall, participants felt appreciated and motivated to further progress in the development of the community garden. The most obvious finding emerged from this study is that participation in the community garden brings a lot of benefit to the personal and social well-being as well as environmental preservation. Findings of the study indicate that such community garden needs to be further advocated. Indirectly, the community garden has successfully unified the urban community within the vicinity, inculcate healthy lifestyle through gardening, contribute to carbon reduction in Kuala Lumpur and support the Sustainable Development Goals. Also, there is a need to research the effectiveness of community gardens as a means to promote environmental changes, social, health, and economic benefits. However, the information presented in this study is only limited to the community garden of Sungai Bunus. Findings may vary if applied in a different category of community garden i.e. community garden within residential neighbourhood where the participants would be the local residents within the neighbourhood.

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Effects of Technological Parameters of Enzyme Treatment on The Protein Extraction Yield from Defatted Peanut Meal

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In the process of producing protein concentrate and protein isolate from plants, protein extraction is a decisive process affecting the product recovery performance. In this study, defatted peanut meal was used as raw material for protein extraction. Viscozyme L with cellulase activity was used to treat defatted peanut meal to support the protein extraction process. Effects of enzyme concentration, treatment time, initial pH of peanut mixture and water and enzyme-treatment temperature on protein extraction efficiency were examined. The results showed that the enzyme treatment process improved the extraction yield of peanut protein. With cellulase concentration of 30 U/g, 120 min treatment time, initial suspension pH of 4.5 and enzyme-treatment temperature of 45 °C, protein extraction yield reached 74.3 %; this value increased by 17.2 % compared to the control sample without enzyme treatment. In addition, the use of enzyme did not alter the protein composition of extract compared to the control sample.

1. Introduction

Peanut is an important oil plant in Vietnam and many other countries in the world. Defatted peanut meal, a by-product of edible peanut oil production, is a cheap and rich source of protein. In Vietnam, defatted peanut meal is usually used to produce animal feed or soy sauce. The protein content of defatted peanut meal is around 47 – 55 %, and peanut protein contains various essential amino acids (USDA-NAL., 2005). In developed countries, defatted peanut meal is used to product protein concentrate and protein isolate. Peanut protein extraction product is a good source of protein both for human consumption and for food industry as potential technical substances (Yu et al., 2007).

In the production of protein concentrate and isolate, extraction is a critical process due to its great impact on the product yield and quality. Protein is located inside the plant cells and protected by cell walls. Therefore, the destruction of cell walls will increase the protein extraction yield. Alkali is often used as the conventional solvent in peanut protein extraction. The chemical composition of cell walls is 90 % polysaccharide, of which cellulose is the main component (de Vries and Visser, 2001). Therefore, the usage of enzyme product for cell wall hydrolysis will considerably improve the protein extraction yield (Rosenthal et al., 2001). However, there are only a few published researches about using cellulase to assist protein extraction process.

In this study, cellulase was used to assist the protein extraction process from defatted peanut meal. The objective of this study was to investigate the effect of technological parameter of enzyme treatment, such as cellulase concentration, time, pH and temperature on extraction yield.

2. Material and Methods

2.1 Defatted peanut meal

Peanut variety of Arachis hypogaea VD1 was used in the present work. The peanut was provided by the Research Institute for Oil and Oil Plants (Ho Chi Minh City, Vietnam).

Defatted peanut meal processing: the peanut was soaked in 0.5 % NaOH solution for 5 min to remove the silk sheath, followed by drying at 55 °C to a moisture content of 7 %, then pulverised and passed through a 355 µm sieve. The peanut meal was defatted by the Soxhlet method (AOAC 960.39, 2000), and the total lipid content

in the meal was less than 2 %. Finally, the obtained product was crushed and then passed through a 355 µm sieve. The defatted peanut meal was kept at 4 °C for use in all experiments. The defatted peanut meal contains 9 % moisture, 36 % crude protein and 2 % crude lipid.

2.2 Cellulase

Viscoenzyme L preparation (liquid) is a mixture of cellulase and hemicellulase, provided by Novoenzymes (Denmark). This product is produced from *Aspergillus aculeatus* mold.
Viscoenzyme L has the optimum pH of 4.0 to 6.5, and optimum temperature of 40 °C to 55 °C. The enzyme activity is 102 ± 0.5 U/mL, in which 1 U is the amount of enzyme necessary to hydrolyse carboxymethyl cellulose into glucose with the reaction speed of 1 µmol glucose/min).

2.3 Experiment design

Based on the results of the study about protein extraction from plants with enzyme treatment (Rosset et al., 2014), the protein extraction from defatted peanut meal was proceeded as followed: Mixed the peanut meal with distilled water in 250 mL beaker with material/solvent ratio of 1/20 (w/v), then used HCl 2 N solution to adjust pH of the mixture to the chosen values. The amount of defatted peanut meal in each beaker was 5 g per 100 mL distilled water. Next, the mixture was heated in a thermostatic tank to the suitable temperatures and the enzyme was added with the chosen amount. The enzyme treatment was performed following the procedure in Table 1. When the enzyme treatment ended, the pH was adjusted to 9.0 with NaOH 2N. Finally, the mixture was centrifuged at 3000 rpm in 20 minutes to collect the supernatant, which was crude protein extract. The control samples were proceeded similarly to experiment samples but without adding enzyme.

Table 1: Experiment design for investigating the effect of technological parameters in enzyme treatment to protein extraction yield from defatted peanut meal

	Changing variables	Fixed variables
1	Enzyme concentration: 0; 10; 20; 30; 40 (U/g material dry weight)	• pH: 4.5 • Temperatures: 45 °C • Processing time: 60 min
2	Enzyme treatment time: 0; 30; 60; 90; 120; 150 (minutes)	• Enzyme concentration: from results of experiment 1 • pH: 4.5 • Temperatures: 45 °C
3	pH of suspension samples: 4.0; 4.5; 5.0; 5.5; 6.0.	• Enzyme concentration: from results of experiment 1 • Enzyme treatment time: from results of experiment 2 • Temperatures: 45 °C
4	Enzyme treatment temperatures: 40; 45; 50; 55; 60 (°C)	• Enzyme concentration: from results of experiment 1 • Enzyme treatment time: from results of experiment 2 • pH of suspension samples: from results of experiment 3

2.4 Analytical, calculation and data analysis methods

Crude protein content was determined by Kjeldahl method (AOAC 984.13, 2000), the total nitrogen content is multiplied with 5.47 to calculate the crude protein content.
The particle size distribution of the material particles at the end of the protein extraction was determined by laser scattering method on the Horiba device, model LA 920 (Japan) according to the procedure proposed by Hong et al. (2002).
The protein profile in the extract was analysed by electrophoresis on sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE) according to the procedure in Laemmli (1970).
Protein yield was calculated by the following Eq (1):

$$H = \frac{C_p V}{m_p} 100\% \qquad (1)$$

where Cp = protein concentration in the extract (g/L); V = extract volume (L); mp = protein content in the defatted peanut meal (g) used in the protein extraction.

Each experiment was carried out in triplicate, and the results were presented as mean ± standard deviations. One-way analysis of variance was performed with the Statgraphics plus software (version 3.2). The experimental results were compared by Multiple range tests with p = 0.05

3. Results and Discussion

3.1 Effect of enzyme concentration on protein extraction yield

Results from Figure 1a showed that control samples without enzyme addition had the lowest protein extraction yield (57.7 ± 0.4 %). When the enzyme concentration increased from 0 to 30 U/g, the yield also increased 17 %. However, there was no statistically significant difference in extraction yield when the enzyme concentration increased from 30 to 40 U/g. As explained in the introduction section, protein is located inside the plant cells. Moreover, protein is often connected with other molecular like polysaccharide (de Vries and Visser, 2001). The cellulase in Viscoenzyme preparation hydrolysed the cellulose that constructed the plant cell walls, and as a result increased the protein extraction yield. The cellulase and hemicellulase also hydrolysed the connection between protein and cellulose or hemicellulose, which helped releasing the protein molecular and making the protein more soluble. Jung et al. (2006) used IndiAge Super L preparation which had cellulase activity and pH 7.0 to extract soybean protein. With the amount of enzyme preparation 5 % compared to material, the protein extracted increase 9.2 % compared to control samples. However, Rosenthal et al. (2001) found that enzyme treatment of soybean meal with different enzymes (cellulase, hemicellulase and pectinase) reduced the protein extraction yield compared to control samples. This was caused by the pH, which was adjusted at 4 - 5 for both enzyme treatment and protein extraction; this pH range was optimal for enzyme activity but could increase protein denaturation. In our study, the enzyme treatment was done at pH 4.5 but the pH of the suspension peanut meal was adjusted to 9.0 when extracting protein. This pH adjustment between acidic pH for enzyme treatment and alkalic pH for protein extraction was necessary when using enzyme preparation with acidic pH to assist the peanut protein extraction.

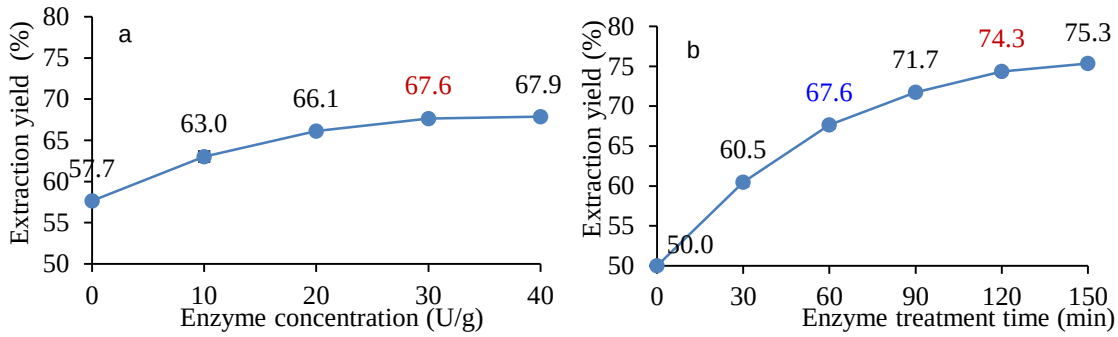


Figure 1: (a) Effect of cellulase concentration on protein extraction yield; (b) Effect of enzyme treatment time on peanut protein extraction yield

From the results of this experiment, the enzyme concentration of 30 U/g was chosen to investigate the effects of other technological parameters of subsequent experiments.

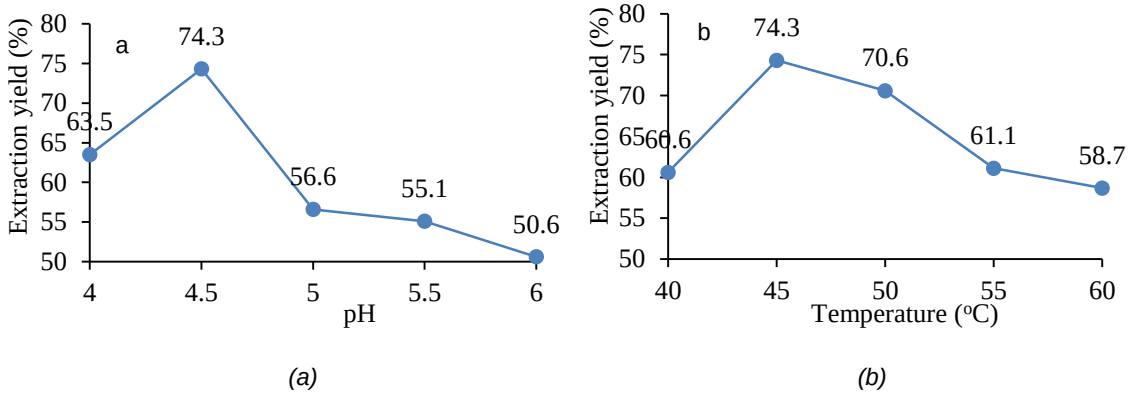
3.2 Effect of enzyme treatment time on protein extraction yield

The results from Figure 1b showed that protein extraction yield was peaked at 74.3 ± 0.9 % when enzyme treatment time reached 120 minutes. If enzyme treatment time increased further from 120 minutes to 150 minutes, the corresponding protein extraction yield had no statistically significant difference ($p > 0.05$). As a result, 120 minutes was the suitable time for enzyme treatment of peanut meal with Viscoenzyme L preparation. Guan and Yao (2008) also used Viscoenzyme L preparation to assist protein extraction of defatted rice bran and found that suitable treatment time is 60 minutes. The enzyme treatment time is different between materials because of the amount and distinct structure of cellulose in each material.

3.3 Effect of pH on protein extraction yield

From the results shown in Figure 2a, the protein extraction yield reached maximum value at 74.3 ± 0.9 % at pH 4.5. When the pH increased or decreased from 4.5, the protein extraction yields always reduced. These results were in accordance with the theory of enzyme catalytic activity. Every enzyme has an optimal pH for a particular substrate; at any non-optimal pH, the catalytic activity of the enzyme is reduced.

The results from our study was similar with the conclusion from another study that also used Viscoenzyme L to assist protein extraction from defatted soybean meal; the extraction yield was peaked at pH 4.6 (Rosset et al., 2014). The pH value of 4.5 was chosen to investigate the effect of other technological parameter in remain experiments.



(a) *(b)*

Figure 2: (a) Effect of pH on peanut protein extraction yield; (b) Effect of temperature on protein extraction yield

3.4 Effect of temperatures on protein extraction yield

The results from Figure 2b shows that the protein extraction yield was peaked at 74.3 ± 0.8 % when temperature was 45 °C. At other temperatures apart from 45 °C, the extraction yields always reduced. This could be explained that the Viscoenzyme L preparation hydrolysed cellulose more efficiently at the optimally catalytic temperature (45 °C), and thus improved the protein release from plant cells into solvent. Moreover, Yu et al. (2007) concluded that enzyme treatment at high temperatures could denature protein and reduce the solubility of protein in solvent.

In conclusion, the temperature suitable for enzyme treatment of defatted peanut suspension with Viscozyme L preparation to assist protein extraction was 45 °C.

The solid phase at the end of the protein extraction process was photographed with electronic microscope (Figure 3). Both the samples with enzyme treatment and the control samples without enzyme treatment had starch granules on the surface. The granules in the samples without enzyme treatment (Figure 3a) had intact structure, the cells were only damaged lightly. On the opposite, in the samples with enzyme treatment (Figure 3b), the cells were not intact, and the cell walls were destroyed.

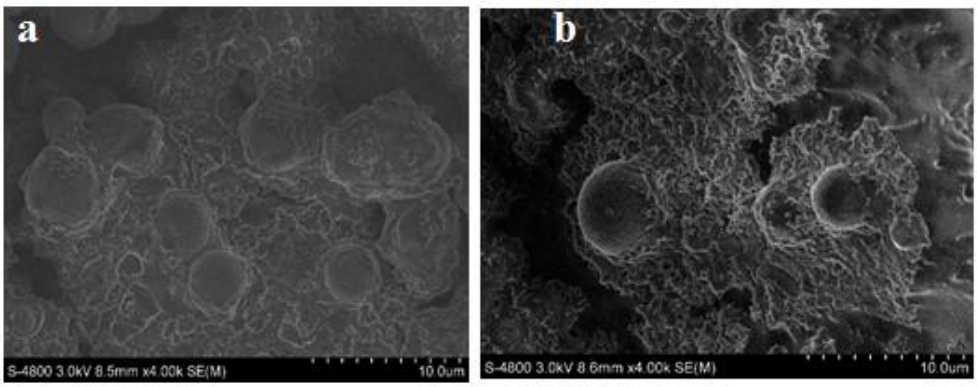


Figure 3: Photos of material particles surface at the end of protein extraction with electronic microscope: (a) Samples without enzyme treatment; (b) Samples with enzyme treatment

Figure 4 presents the particle size distribution at the end of the protein extraction process. The samples with enzyme treatment had the largest particle segment of approximately 700 µm; while the samples without enzyme treatment had the largest particle segment of 900 µm. The mean size of sample with and without enzyme treatment was 104 µm and 137 µm respectively. These results showed that enzyme treatment effectively reduced particle size and thus increased the contact surface between particles and solvent, which resulted in

increase in protein extraction yield. In our knowledge, there are not many published studies about the particle size reduction when using enzyme preparation to assist protein extraction from plant cells.

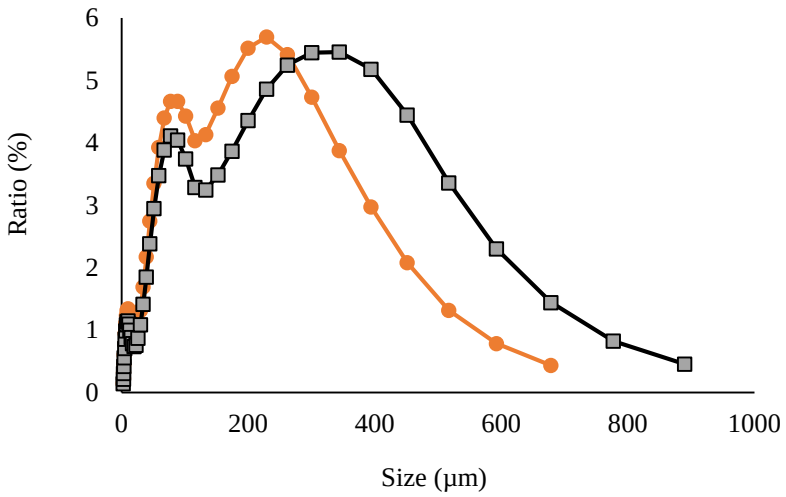


Figure 4: Particle size distribution of material at the end of protein extraction: (■) Samples without enzyme treatment; (●) Samples with enzyme treatment

The protein profile of the protein extract was presented in Figure 5. The protein segment of samples with and without enzyme treatment were similar. Peanut variety VD1 in this study had protein segments with molecular weight ranges from 10 to 70 KDa. Shefcheck et al. (2006) also reported similar results with peanut variety Arachis hypogaea (Shefcheck et al., 2006). However, some previous studies discovered protein with molecular weight higher than 70 KDa on other peanut varieties but their physicochemical properties still not published (Javaid et al., 2004). Almost all the peanut protein with molecular weight higher than 70 KDa is hetero-multimeric proteins (Shefcheck et al., 2006).

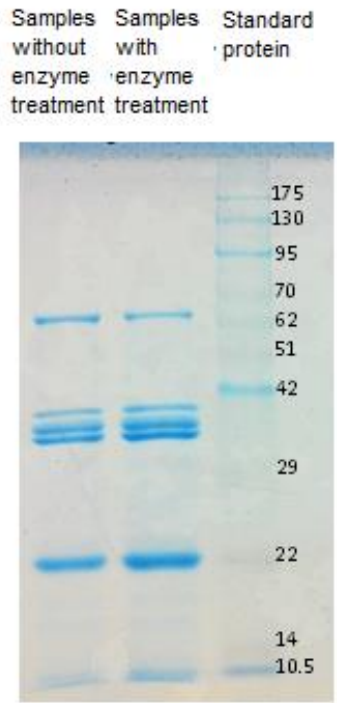


Figure 5: Electrophoresis on polyacrylamide gel of peanut protein extract

4. Conclusion

Treatment of defatted peanut meal with cellulase enzyme had considerably increased the protein extraction yield but had not changed the protein profile of protein extract compared to conventional protein extraction methods. The usage of cellulase preparation was an effective method to improve the protein extraction yield from defatted peanut meal.

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Rural Web as A Tool to Project Trajectories For Green Economy

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Malaysia has set a target by looking at green growth as an important game changer in the Eleventh Malaysia Plan and there has been initiatives and directions made in efforts of transitioning towards a green economy. Rural development plays a strong role in achieving this target as it would involve primary production sectors of agriculture and forestry. A shift towards green economy in rural areas is vital as it would contribute to a resilient, low-carbon, resource-efficient, and socially inclusive environment for the rural community. Thus, there is a need to understand the transitional process towards green economy in order to project greener development trajectories for rural areas in Malaysia. Rural web is a tool to highlight the differing responses to the squeeze on rural economies that would enable the stakeholders to reflect on current and future development trajectories. It is a comprehensive theoretical framework composed by the interrelations and mutuality that exist between actors, resources, activities, sectors and places within rural areas. This paper presents the application of the rural web dimensions in analysing the rural dynamics and development trajectories of Kampung Sedili Kechil. This paper also shares the potential for a green economy for Kampung Sedili Kechil. The study area was found to be moving along a sustainable endogenous development pathway which valorises its natural resources for economic purposes. It was also found that the initiatives done in Kampung Sedili Kechil were fulfilling green characteristics.

1. Introduction

According to the Eleventh Malaysia Plan, Malaysia has set a target and an intention to shift from the conventional ways to greener trajectories by looking at green economy as an important element in ensuring sustainability of the nation's natural resources, minimising pollution, and strengthening the security of energy, food and water supply (Ministry of Economic Affairs Malaysia, 2018). Many countries all over the world have started embarking on green economy and green growth plans and strategies. This global green initiative resulted from the need to focus on the link between the economy and environment as issues of poverty, environmental degradation, inequality and climate change became a significant global concern. New consensus on sustainable development were then brought by global leaders through the signing of Sustainable Development Goals (SDGs) and Paris Agreement specifically to address these concerns and consequently, green economy emerges as an economic planning that would best reflect its transformational visions (Green Economy Coalition, 2012). In order to pursue the national target of green growth, rural development can be seen as an important area of importance. Rural development is a process of social, economic, environmental and cultural transformation that targets the well-being of the people living in the rural areas (Ministry of Rural Development Malaysia, 2019).

2. Research background

Green economy can be defined as "An economy that results in improved human well-being and reduced inequalities over the long term, while not exposing future generations to significant environmental risks and ecological scarcities" (UNEP, 2015). Basically, green economy is an economy that encourages economic opportunities which are not in any way could interrupt the sustainability of the environment and social well-being

apart from also providing new-forms of socio-economic opportunities that promotes environmental objectives (ENRD, 2017a). The sustainability of any region is highly dependent on its policies and performance of any country could only be improved by its key strategies (Fan et al., 2019). Thus, the greening of any economy starts with making green initiatives for the region.

2.1 Transitioning to a green rural economy

The transition to green rural economy is about improving people's lives and livelihoods in rural areas, balancing natural resource use with maintaining incomes, and trading the risks of making a change with the opportunities that change will bring. There are many practical examples of activities and initiatives which embrace the principles of the green economy in rural areas. Taking an example from Sweden, 'Suderbyn Permaculture Ecovillage' is an initiative that serves as a model for rethinking rural development in terms of living in an environmentally friendly manner, patterns of consumption, ethical financing for developments and interactions with wider society. The decisions for the project was taken on the basis of a communal consensus approach in order to ensure participatory governance. Entrepreneurial activities of the ecovillage also have a focus on social entrepreneurship and green businesses, instead of financial profit. The initiative has managed to plant Sweden's largest permaculture forest food garden which is a space-efficient perennial garden of edible plants in order to provide the locals with food, avoid waste and ensuring food is purchased in bulk from local farmers and wholesalers (ENRD, 2017b). Among many others, the goals necessary for rural areas to move to a green economy is through the transition towards a low-carbon economy and increasing practices of sustainable farming and forestry. Promoting the right initiatives for rural areas can be about strengthening existing good practice or more fundamental changes to the way the rural economies operate thus it is important to take note on the key features and characteristics that would best support the transition to a green economy (see Table 1).

Table 1: Initiatives that supports transition to green rural economy (ENRD, 2017a)

Key Features	Characteristics
Respond to demand	Demand-driven, responds to gaps in the market, social concerns or economic opportunities
Target environmental objectives	Clear, tangible and economically sustainable environmental objectives
Balance economic and social objectives	Takes systematic approach, including the economic and social benefits that can be achieved
Innovate	Involves new products, new services, new technologies, new business models and/or smart adaptations or combinations of old ones
Involve multiple actors	Engages and motivates key people from all relevant spheres (public, private and community) from production to consumption
Lever in public and private support	Explores a variety of funding sources and seek to avoid dependency on grants
Evolve	Projects grow, evolve and adapt to changing environments and their own successes and failures
Enable future learning	Environmental, economic and social objectives are clearly specified and monitored, enabling results-based learning for the future
Communicate results	Communicates their successes and achievements, to encourage others
Change mindsets	Demonstrates that there need not be any conflict between economic, environmental and social objectives.

2.2 Rural Web

Instead of being a result of direct policy interventions, processes of rural development are being shaped by the creative patterns called the rural web. Rural web is a tool to study the different responses that has arisen through the squeeze of economy in rural areas. The role of rural web is to enhance and mobilise different territorial capitals towards a sustainable rural development. The rural web can be used as an explanatory scheme, a concept and also a tool for exploring, analysing and interpreting the dynamics that defines rural territories, supporting the idea that every territory has its own dynamics (Milone & Ventura, 2010). As the rural web tool highlights the different responses to the squeeze of economy in rural areas, it suggests that every region is responding differently through its own intertwined institutions of society and economy (Paddock & Marsden, 2015). Defined as a collection of individuals, resources, activities and processes that interacts with each other within a territory, the rural web consists of six theoretical dimensions that seeks to improve the quality of life in rural areas (See Table 2). The interrelationships between these six dimensions will create synergies when they

reinforce each other mutually (Pakeltienė, 2015). Through rural web analysis, the acknowledging of interplay between all six dimensions will help to generate knowledge on how to achieve a set of strategies for a greener rural development. Therefore, any positive interrelations between the six dimensions would indicate a greater coherence with the logic of a green economy while the absence of interrelation points out the missing links that hinders the transition towards a greener rural economy.

Table 2: Dimensions of sustainable development, rural web and its characteristics

Dimensions of Sustainable Development	Dimensions of Rural Web	Characteristics of Rural Web Dimension
Economic	Endogeneity	The degree to which rural economies are: i. built upon local resources ii. organised according to local models of resource combination iii. strengthened through the distribution and reinvestment of produced wealth within the local/regional constellation
	Novelty	New insights, practices, artefacts and/or combinations (of resources, technological procedures, bodies of knowledge, etc.) that carry the promise that specific constellations function better.
	Market Governance	Institutional capacities to control and strengthen existing markets and/or to construct new ones
Social	Social Capital	The norms and networks that enable people to act collectively or, more specifically, the ability of individuals, groups, organisations or institutions to engage in networks, cooperate and employ social relations for common purpose and benefit
	New Institutional Arrangements	New institutional constellations that solve coordination problems and support cooperation among rural actors
Environmental	Sustainability	The existence of the social and ecological conditions necessary to support human life at a certain level of wellbeing through future generations

3. Methodology

The collection of primary data for case study region was done through a questionnaire based survey and in depth interviews towards 90 respondents of Kampung Sedili Kechil which consists of the local villagers, head of the village, and local business owners. The data collection was done to draw the respondent's demographic profile, structural characteristics and key elements of the study area in order to identify the rural web and its dynamics. Open coding were used for the gathered data and SWOT analysis was conducted taking into account of seven territorial capitals The territorial capitals of the study area were analysed with the purpose of finding out the evolutional dynamics triggered by the local initiatives in the territory The description of the territorial capitals and its evolution would reflect the formation of the rural web in ways that it emerges, thus producing effects that would provide a progressive structuring of the territory's web. The findings were found through qualitative analysis in order to use subjective judgements for an exploratory research mainly through in depth interviews, due to the unquantifiable information collected. The case study area is a village called Sedili Kechil, located in Tanjung Sedili which is a coastal region in Kota Tinggi District, Johor. Located by the bank of the Sedili Kechil river, Sedili Kechil is about 40 km from the city. Despite being located at a coastline, the economic activities of the local population are diverse. Sources of income for local population in Sedili Kechil ranges from fishing and small enterprises to cultivation of palm oil and development of tourism products.

4. Findings

4.1 Territorial Capitals of Sedili Kechil

According to the Bryden's theory, the rural regions have an economic development that depends on the way a mixture of its immobile resources which are tangible and less tangible interact with each other locally (Terluin, 2003). These resources are also conceptualized as territorial capitals, consisting of environmental, economic, human, cultural, social, institutional and symbolic capitals (Milone & Ventura, 2010). The environmental capital is local assets that are purely natural without the creation of human action, also consisting of the resulting product of the interaction between nature and man (Giaime, 2011). The environmental capital of Sedili Kechil is the beauty of its natural scenery, the location of the village, which is situated along the coast, its mangrove forest

ecosystem with its distinctive elements of biodiversity, river ecosystem and marine ecosystem. Sedili Kechil is a territory with high environmental quality and biodiversity. The agricultural sector of Sedili Kechil is mainly on the involvement of the local community in palm oil cultivation activities, as well as other crops such as fruits and vegetables. Due to the location of Sedili Kechil being at the coastal region of Johor, fishing activities are one of the major incomes for the local community and they rely heavily on marine produce. The presence of local attractions such as Sedili Wetlands, homestays and handcrafting classes also helped generate income for the local economic performance. Thus, the presence of many tourism products, marine and agricultural produce makes up the economic capital of Sedili Kechil. On the other hand, cultural capital of a territory can best be desribed as the historic legacy and the existence of an artistic heritage and folk traditions (Sassi, 2008). As for the cultural capital, Sedili Kechil is known for its unique and distinctive identity. This is translated into its features and designs of its traditional homes, which can be seen through the designs for homestays businesses that has taken the advantage of its local identity by cooperating the homestays with the unique concepts of traditional Malay houses through its architecture and creative carvings. As for social and human capital of Sedili Kechil, they can strongly be seen through its high levels of volunteering among its local communities in various activities which proves their strong sense of place and local identity. Institutional capacity is best described as the existence of strong roles in local institutions that produces great interaction among the local organization as well as the development of mutual awareness by the local organization in participating in a development project (Ferrario and Coulson, 2007). The institutional capital of Sedili Kechil has high levels of local planning which can be seen through the effective cooperation of its local actors, Village Community Management Council (MPKK) and the involved organizations in managing local economic activities namely Fishermen Association.

4.2 Rural Web Application on Sedili Kechil

Based on the analysis done using the rural web model, it is possible to identify a pattern of domain interrelations representing the rural transition of Sedili Kechil that moves along a sustainable endogenous rural development trajectory. This is based on the initiatives done by actors which are largely concentrated on the valorisation of local resources. This can be seen through the valorisation of its local environmental assets on Sedili Wetlands. The high environmental values of Sedili Wetlands were valorised in a way that it would improve the economy through ecotourism, and at the same time spread awareness of the need to take good care of the local's valuable identity. Many other initiatives in Sedili Kechil were also started by valorising its local resources, such as the centre of breeding and hatchery for horseshoe crab. Horseshoe crab is known to be one of the marine resources that strives in the environmental condition in Sedili Kechil, thus the act of breeding and providing the supply of horseshoe crabs for consumers has managed to raise the local economy by making great use of the local resources. Local resources of Sedili Kechil are not limited to only the marine animals as the fish scales and shells were also used as resources for a local handicraft business. Another important component of endogeneity is the sense of place that brings a strong local identity. The strong identity of the local community in Sedili Kechil has managed to provide unique ideas to the local enterprises mainly in the tourism sector. One of the homestay businesses, Homestay Uchu has a distinct theme where the homestay was created to match the local's traditional Malay concept. The local identity was being taken into account as a unique quality in order to promote the homestay business and this has contributed to the strength of endogeneity as a primary domain in Sedili Kechil. The domain interactions between endogeneity as a primary domain, novelty, and social capital as a lubricant which enhances and cemented these positive interrelations creates a trajectory that focuses on further valorisation of local resources, thus making the endogeneity as both the initiator and also the outcome. The webs unfolding in Sedili Kechil also causes the sustainability domain to be the outcome alongside endogeneity. This endogenous rural development process is deemed sustainable as it brings about the preservation and further valorisation of the territory's environmental capital, the rediscovery of cultural capital, a mediatic role of social capital and a new vibrant economic potential. Based on the analysis of the local initiatives through rural web dimensions, a web configuration of Sedili Kechil was identified where the most important domain which contributes to its local economy is the endogeneity domain. The domains with medium importance turned out to be market governance, novelty and social capital. As for the domains which were of least importance, it was found to be the new institutional arrangements and sustainability. According to the theory of rural web model, the web's density plays a huge role in determining the strength of a rural territory in raising its competitiveness and community well-being. The denser the web configuration, the higher the strength. This form of density is achieved through the mutual interactions between all domains. It was discovered through this study that the mutual relationships between domains of the Sedili Kechil's web existed except for the interaction between the domain of novelty and institutional arrangements (See Figure 1). Thus, Sedili Kechil's web can be described as a dense web, pointing out the information that the territory is capable of raising its competitiveness through its unique economic initiatives.

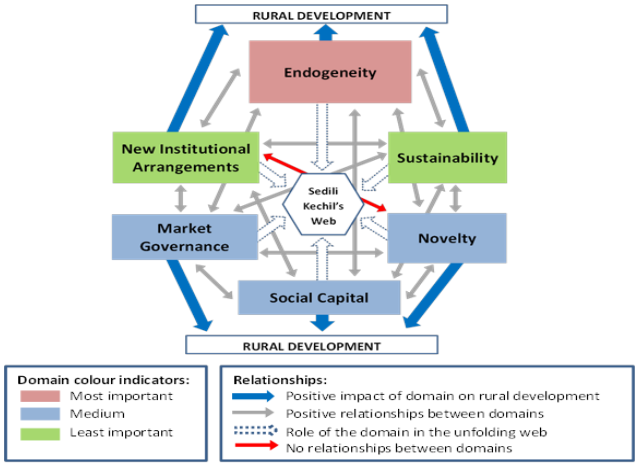

Figure 1: Sedili Kechil's rural web configuration

4.3 Potential of Green Economy in Sedili Kechil

The initiatives done in Sedili Kechil towards raising its competitiveness reflects the characteristics of how green initiatives would appear, thus raising the potential of a green economy in the territory. This was achieved in Sedili Kechil through its responds to gaps in the market, social concerns and economic opportunities. The sustainable use of environmental capital such as ecotourism in Sedili Kechil also targets environmental objectives, besides also balancing its economic and social objectives for the village through creation of income and motivating the collective efforts of its community. Multiple actors were also involved in the economic activities in Sedili Kechil where an example of this would be the Sedili Wetlands which was initiated by the state's regional development agency and enriched by the local community. The achievement of Sedili Kechil's initiatives in relation to the characteristics of a green rural initiatives is scored with positive (+), negative (-) and not related (0) as a means to assess its green potential. The initiatives in Sedili Kecil were found to be mostly positive towards fulfilling a green characteristic. However, the locally owned palm oil weighing and collection centre did not fulfil green characteristics and was assessed as negative since it failed to target environmental objectives and it did not change mind sets (See Table 3). Such initiative did not encourage the locals to address the environmental impacts caused by the planting of palm oil trees in the village. It was found that the ease of access to a weighing and collection centre possesses higher effect towards environmental degradation compared to the economic value it initially plans to contribute.

Table 3: Matrix of initiatives strengthening Sedili Kechil's web and green characteristics

Key Characteristics of a Green Rural Initiative	Sedili Kechil's initiatives				
	Sedili Wetlands	Centre of Breeding and Hatchery for Horseshoe Crab	Locally Owned Palm Oil Weighing and Collection Centre	Malay Traditional Concept Homestay	Marine Handicraft Workshops
Respond to demand	+	+	+	+	+
Target environmental objectives	+	+	_	0	+
Balance economic and social objectives	+	+	+	+	+
Innovate	+	+	0	+	+
Involve multiple actors	+	+	+	0	+
Lever in public and private support	+	+	+	+	+
Evolve	+	+	0	+	+
Enable future learning	+	+	0	+	+
Communicate results	+	+	0	0	+
Change mindsets	+	+	-	+	+

5. Conclusion

It is known that the concept of rural web has been well accepted in rural planning as a comprehensive framework that is able to synthesize the extensive body of theoretical and empirical work of rural development. In addition to that, the rural web model also can be used in many different studies in order to better plan for the well-being of rural community. One of its many valuable contributions to rural planning is it represents new ways of directing the pathways for new paradigm of rural development, which coincides with the principles of a green economy. By using the concept of rural web as a tool to move towards a green rural economy, the provision of green economic strategies for rural areas in Malaysia could be proactively planned out and concrete recommendations highly relevant for policy analysis can be derived.

Acknowledgments

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Inactivation of *A.* hydrophila in sterilized striped-catfish pond water at different phage-to-host ratios

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Disease outbreaks and antibiotic resistance crisis are considered as major challenges to confront the sustainable development of striped catfish farming in the Mekong Delta - Vietnam (MKDVN). One of the most common disease types is hemorrhagic septicemia caused by *Aeromonas hydrophila* bacteria with a high fatality rate. It recently shows an inadequate control due to antibiotics resistance of A. hydrophila in stripped catfish. This has led to the following alarming results of output reduction, export loss, community health and environment risk. Phage therapy is the therapeutic use of phages (that are virus infecting only bacteria) to prevent and treat pathogenic bacterial infections. Aim of this study was to investigate effect of different phage-to-host ratios (or multiplicity of infection – MOI) of about 0.5, 3.0, and 20.0 on inactivation of A. hydrophila in sterilized striped-catfish pond water. It resulted in different concentrations of the host bacteria and phages.

1. Introduction

In Vietnam, fisheries is a key national economic sector that produces 4–5% of the national Gross Domestic Product (GDP). Vietnamese fishery products are exported to more than 140 countries and territories in five continents earning over US$7.9 billion in 2016. The Mekong Delta in Vietnam (MKDVN) has traditionally been the major region of aquaculture produce accounting for over 52% of national aquaculture crop, and 65% of aquaculture export. Striped catfish (Pangasianodon hypohthalmus) is the native freshwater catfish of the MKDVN. By 2016, striped catfish is the highest item of fish-produce exports in Vietnam accounting for 23%. The striped catfish farming covers an area of close to 6,000 hectares and harvests over 1.2 million tones. However, many problems confront sustainable development of the striped catfish farming in the MKDVN, in which disease outbreaks and antibiotic resistance crisis are considered as major challenges. In 2015, over 730ha (12% of the total farming surface devoted to striped catfish) in the four provinces of An Giang, Dong Thap, Hau Giang and Vinh Long were bacterial infected. One of the most common types is hemorrhagic septicemia (32%) caused by Aeromonas hydrophila bacteria with a high fatality rate (Dang & Nguyen, 2010). Antibiotics are commonly used as a measure of prevention and treatment of the bacterial infection on farms. However, it recently shows an inadequate control due to antibiotics resistance of A. hydrophila in stripped catfish. Quach et al. (2014) has shown that antibiotic resistance ratio of A. hydrophila isolates in ill striped catfish in the MKDVN was 100% for certain kinds of antibiotics, i.e., ampicillin, amoxicillin, cefalexin, trimethoprim/sunfamethoxazol, was 93% for tetracyclin and 63% for florfenicol. This has led to the following alarming results of output reduction, export loss, community health and environment risk. Due to these adverse impacts, there is an urgent need to come up with more effective solutions as a replacement of antibiotics in fisheries industry in the region.

Bacteriophages (or phages) are virus infecting only bacteria, hence they are non-toxic for human, animal and plant cells. In addition, phages own a high host specificity meaning they are not harmful for useful bacteria in environment as well as in human and animal digestion systems. Phage therapy is the therapeutic use of phages to prevent and treat pathogenic bacterial infections. The therapy became available since 1930s, but the method has only gained serious attention in the aqua industry in the last thirty years, especially in the face of wide spreading of antibiotic resistances in bacteria. Phage therapy has shown its efficacy in treatment of bacterial diseases in fish and shellfish in several cases, such as Edwardsiella tarda causing edwardsiellosis in Loach

(Wu et al., 1981; Wu & Chao, 1982), Lactococcus spp. causing lactococcosis in Yellowtail (Nakai et al., 1999), Aeromonas salmonicida causing Furnunculosis in brook trout, rainbow trout, Atlantic salmon (Imbeault et al., 2006), A. hydrophila causing tail and fin rot in Loach (Wu et al., 1981; Jun et al., 2013). Phage therapy against various kinds of fish and shellfish pathogens have been reviewed by Richards (2014). However, until now, to the best of my knowledge, there is no research of phage therapy to Aeromonas hydrophila infection in striped catfish.

One of the most parameters that needs to be considered in phage production and also in phage therapy is molar ratio of phage-to-bacteria. It is also called as multiplicity of infection (or MOI). Tran et al. (2018) showed a high activation of A. hydrophila by phage at MOI of 50. However, a lower MOI value than 1 will have some advantages. First, it will avoid damaging host cells before replication of newly generated phages (Asami et al., 1997). Second, it will lower the production cost when a small volume (low concentration) of inoculum phage is required. To clarify this point, aim of this study was to investigate effect of different MOI values of about 0.1, 1.0, and 10.0 on inactivation of A. hydrophila in sterilized striped-catfish pond water.

2. Materials and methods

2.1 Preparation of bacterial stock

The host bacterial strain A. hydrophila CA.1.2T was provided by Prof. Dang Thi Hoang Oanh (Can Tho University, Vietnam). This strain was previously isolated from ill striped catfish in the MKDVN. The bacterial strain was recovered from glycerol stock preserved at -80 ^{o}C by culturing on TSA and then incubated at 28 ^{o}C for 24 h. Pure bacterial strain was cultured in TSB medium at 30 ^{o}C for 24 h. Bacterial cells was harvested by centrifugation at 4,000 rpm for 3 minutes and washed 2 times with 0.85% NaCl solution after discarded medium. Bacteria density was determined by culturing on TSA and count number of colonies after 24 hours. The concentration of A. hydrophila CA.1.2T was about 10^9 – 10^{10} CFU/mL.

2.2 Phage production

Phage TG25P was used in this study. Phage stock was prepared in SM buffer [100 mM NaCl, 10 mM MgSO4, 0.01% gelatin, and 50 mM Tris-HCl (pH 7.5)] at concentration of 10^9 – 10^{10} PFU/mL. Protocol of phage stock preparation was shown elsewhere (Hoang et al., 2014). Next, phage-containing product was obtained from fermentation of host bacteria in culture mainly containing molasses and yeast extract (not shown details in this study).

2.3 Challenge test in pond water

Inactivation of *A. hydrophila* CA.1.2T cells in a striped catfish pond water sample by TG25P phage was examined. The bacterial culture was shaken at 30 ^{o}C, 120 rpm in TSB until its OD_{600} of 0.1 (~10^7 CFU mL^{-1}) was achieved. The culture was centrifuged at 10,000 xg, 4 ^{o}C, 5 min to obtain a pellet. The pellet was suspended in the same volume of sterilized pond water. The centrifugation and suspension were repeated to discard residuals of TSB. The final pellet was suspended and serially diluted in sterilized pond water to obtain a bacterial concentration of ~10^5 CFU mL^{-1}. The solution was divided into six aliquots, each of 70 mL in 250-ml Erlenmeyer flask. The first three aliquots were mixed with TG25P phage-containing product at a multiplicity of infection (MOI) of 0.5, 3.0, 20; and the other three aliquots were added by respectively volumes of product containing denatured phages (no lytic activity of phages). In addition, three 70-mL aliquots of sterilized pond water were prepared in 250-ml Erlenmeyer flasks and added by respective volumes of TG25P phage-containing product as above (no host bacteria contained in these three flasks). All the mixtures were incubated at 30 ^{o}C. Sampling was performed at different intervals. In case of the mixture of host bacteria and phage, each sample was divided into two aliquots. One aliquot was serially diluted and spread onto Trypticase Soy Agar (TSA) to estimate bacterial concentration. To the other aliquot, the phage titer was estimated by serial dilution and the double agar-layer method, as described elsewhere (Hoang et al., 2014). In case of no phage addition, the sample was serially diluted and spread onto TSA to estimate bacterial concentration. In case of no host bacteria addition, the phage titer was estimated as described above. The experiment was conducted in triplicate.

3. Results

3.1. Phage-containing product

Phage production in a standard condition was shown earlier (Tran et al., 2018). In this study, a phage-containing product was obtained from a fermentation of host bacteria in culture mainly containing molasses and yeast extract. The product has phage concentration of about 10^9 PFU/mL (Figure 1).

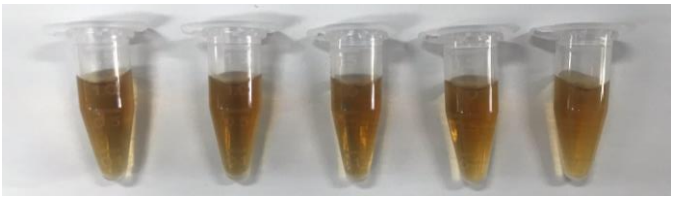

Figure 1. Phage-containing products

3.2. Inactivation of *A. hydrophila* in pond water by phage at different MOIs

Fig. 2 shows a time course of host cells during the experiment. At MOI = 0.5, the first 2 h of incubation, almost no change of host bacterial count was seen for both experiment (with or without phages). The host bacterial count in the challenge with TG25P decreased (about 1 log) in the next 5 h of incubation in the run with phages. However, after 7 h, in the run without phages, an increase of bacterial count appears and maintains at about log 5-6. In the negative control (host cells without phages), a similar increase also maintains for 48 h. Compare to the phage concentration shown in Fig. 3 at MOI = 0.5, an increase of approximately 1 log appears in the run with phage mixture. In the run without phages, phage concentration maintains stable for 48 h indicating a high stability of phage activity in sterilized striped catfish pond water.

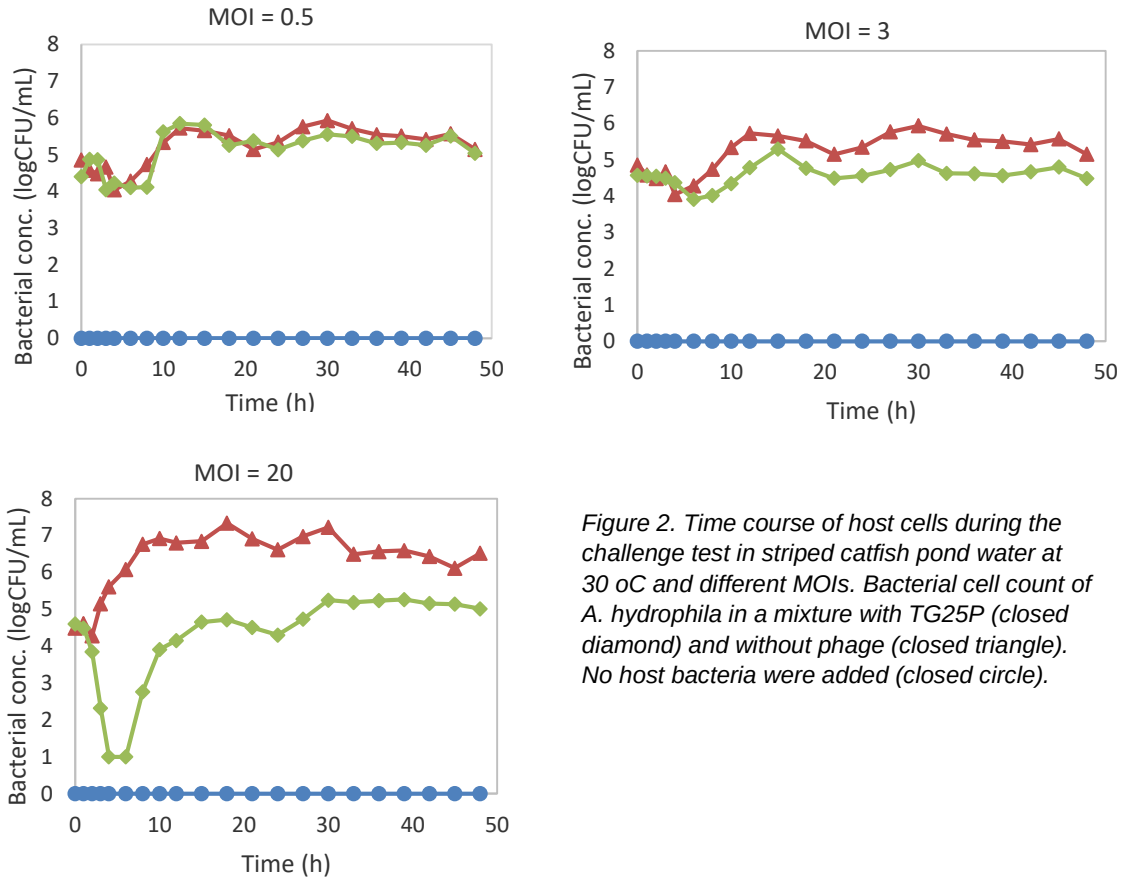

Figure 2. Time course of host cells during the challenge test in striped catfish pond water at 30 oC and different MOIs. Bacterial cell count of A. hydrophila in a mixture with TG25P (closed diamond) and without phage (closed triangle). No host bacteria were added (closed circle).

In the run with MOI = 3.0, a different of approximately 1 log between runs with or without phages appeared after 6 h (Fig. 2) respectively to about 1.5 log change of phage concentration (Fig. 3). In the run with MOI = 20, a sudden increase (about 3.5 log) of bacterial count appears at the run with phages. The bacterial count starts to increase after 6h and reaches stable at about 15 h. In the control run (without phages), bacterial count sharply increases (3 log) and reaches stable at 10 h. Together with lysing host cells, phage particles were also newly generated. Phage titer increased during the first 2 h and then keep stable (Fig. 3). In the control (phage without

host cells), phage titer was almost stable during 48 h, indicating the stability of TG25P in striped catfish pond water.

Different from Tran et al. (2018) when phage stock in SM buffer was used in the test, in this study, phage-containing product produced in a culture involving molasses and yeast extract. A rich-nutrient condition much supported growth of host bacteria. A higher increase appeared when a higher volume of phage-containing product used.

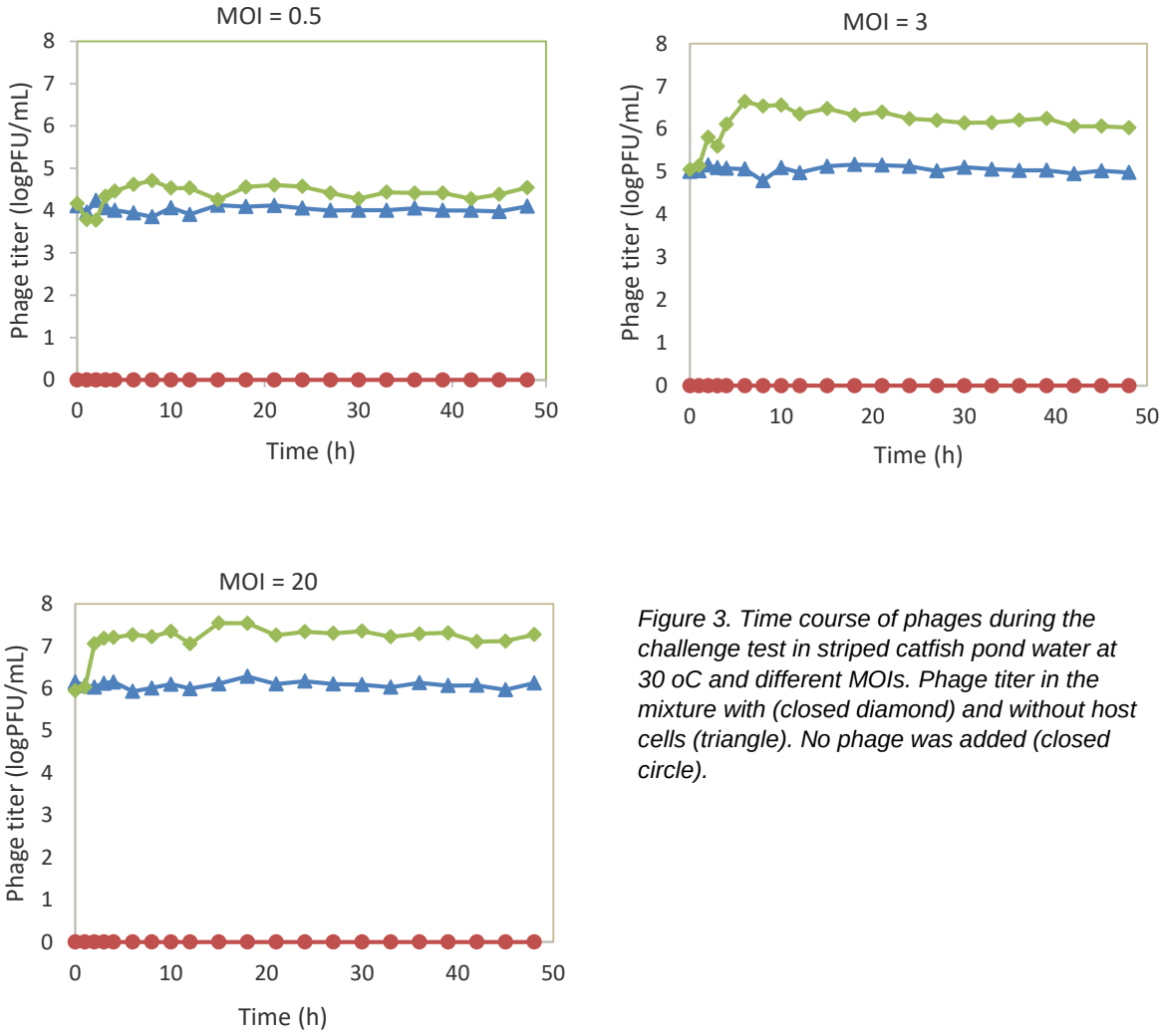

Figure 3. Time course of phages during the challenge test in striped catfish pond water at 30 oC and different MOIs. Phage titer in the mixture with (closed diamond) and without host cells (triangle). No phage was added (closed circle).

4. Discussion

In terms of production cost of striped catfish, antibiotics are the third highest item of expense (2.5%). Nevertheless, it has been estimated that even 1% fall in expenditure on antibiotics (of the total 2.5%) would alone generate a saving of approximate USD 13Millions to the fisheries industry in Vietnam (Nguyen & Vo, 2014). The low-price phage product is expected to reduce antibiotic expenditure. This is due to: (1) phages replicate extremely fast, and only a small dosage is needed at the beginning; (2) phages can be produced from simple fermentation process with low-cost materials (Richard, 2014). Diseases such as hemorrhagic septicemia can lead to significant and widespread damages. In 2014 and in the four MKDVN provinces of An Giang, Dong Thap, Vinh Long and Hau Giang alone, 730 hectares causing damages of approximate USD 170Million (Nguyen & Vo, 2014). As such the technologies identified in the study are expected to assist in the search for treatments to lower the high fatality rate, financial losses and risks for the fisheries industry, not only in Vietnam but also in other countries in the Mekong Sub-region.

The phage therapy has been developed and applied only in some developed countries. However, phage solution is still produced by expensive materials and stored at laboratory condition. In order to apply the phage therapy on striped catfish farms, the kind of 'science' data need to be developed to 'engineering' data. In other words, products containing respective phages need to be produced from low-cost materials. In this study, a low-cost phage product was investigated its ability to inactivate the host bacteria. This is the first study on usage of this kind of phage product on in vitro trial.

MOI is one of the most parameters that needs to be considered in phage production and also in phage therapy is molar ratio of phage-to-bacteria. In this study, three different MOI values were examined their effect on inactivation of host cells. A higher MOI value was more effective than a lower MOI value in terms of inactivation of host cells in the first hours. However, after 10h, bacterial counts in phage-host cases were almost the same, while bacterial count in host-only cases was higher when a higher MOI value used. The study also indicated growth of phage resistant bacterial strains to TG25P phage. The regular emergence of phage-resistant bacteria is one of the major challenges of phage therapy (Labrie et al., 2010; Oliveira et al., 2012; Mateus et al., 2014). An effective way to tackle the problem is to apply a phage cocktail (a mixture of different phages showing different types of host bacterial receptors) to inactivate phage-resistant bacteria. The previous studies interestingly indicate that when the host bacteria are resistant to phage infection, the bacterial receptors change resulting in less toxic properties (Fillippov et al., 2011; León & Bastías, 2015). This may have meanings in control of bacterial diseases on fish farms using phage therapy. In the future studies, an in vivo trial of inactivation of host bacteria by phages will be conducted.

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Assessment on the Reality of the Development of Land Fund in the Area of Phu Vang District, Thua Thien Hue Province

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Phu Vang is a coastal and lagoon district of Thua Thien Hue province with great potential for marine economic development. Recently, urbanisation had taken place strongly in the district, accompanied by an increase in demand for the land fund. This led to a need for an assessment on the reality of development of land fund in the area of Phu Vang district. In this study, the questionnaire and interview surveys were conducted with random 90 households and 12 administrative staffs, combined with document review between January and June in 2017 in area of research. Then statistical methods were used for description, analysis, comparison, and assessment. From 2014 to 2016, 67 projects with total areas of 61.88 ha of land were implemented for land fund development (LFD). It emerges that LFD was affected considerably by policies to attract investment, price of real estate, state budget and comprehensive planning as well as had significantly positive impacts on infrastructure.

1. Introduction and literature review

Worldwide, studies on land use have played an essential role in social development planning and has substantially contributed to nature conservation. Many studies mentioned about the predictions on land use change (LUC) basing on the models. Notably, a study on simulation of LUC in the urban areas in Hong Kong offered positive alternatives for future urban renewal based on different policy directions. This study has significantly contributed to the making-decision process of land use planning (LUP) (Zheng et al., 2015).
Moreover, researchers made many enhancements to the existing LUC models. A researcher enhanced the existing land use and land cover change (LULC) models by combining a Conversion of land use and its effects at small regional extent (CLUE-S) model with a Markov model in Beijing. The results indicated good consistency between predicted results and actual land use situations according to a Kappa statistic (Han et al., 2015).
Apart from the prediction of LUC, a plethora of studies reported the effects of LUC to the environment and society. A study analysed econometric-based projections of future land use change to capture changes around 1260 protected areas, from 2001 to 2051, under different land use policies and crop prices in America. This study showed that urban expansion was the major threat in diminishing conservation value and likely to continue to threaten protected areas (Martinuzzi et al., 2015).
A study examined consequences and impacts of land acquisition in China. The results showed that land acquisition resulted in increasing social tension and injustice that may pose a potential threat to stability and sustainable development (Ding, 2007). Another article studied the land acquisition for infrastructure, industries and various services and the process of displacing people from their normal activities. The results showed that despite necessity of land acquisition for industrialization, there was a lack of proper compensation and rehabilitation programme that determined success of land acquisition (Sarkar, 2007). In Chisumbanje, Zimbabwe, a study assessed the local livelihood implications of land acquisitions for biofuel development. The findings of study showed that displaced households argued the costs incurred from biofuel development outweighed benefits derived (Thondhlana, 2015). In Vietnam, a survey of 430 officials, organisations, and people

was conducted to assess present status of LFD in Yen Bai city, Vietnam. Basing on these results, implications in policy, financial, and planning were recommended in order to promote LFD work (Ho et al., 2016).
In general, the above studies mentioned, researched and evaluated the LUC, LUP and LFD other topics that were related to LFD in a host of areas. Despite a variety of above-mentioning studies, it would be a big challenge if people want to apply approaches in many parts of the world to a specific country (Zevenbergen et al., 2015). Therefore, this led to a need for intensive study in a specific area for LFD. There was no specific study on LFD in Phu Vang district. In addition, a clean land fund for various projects has gradually become urgent with strong urbanization in the researched area in recent years. Therefore, this study was conducted on the status of LFD in Phu Vang district with the aim to examine the reality of LFD; impacts of LFD on socio-economic development; factors that effected LFD as well as propose the solutions on improving the LFD in the area of Phu Vang.

2. Methodology of the study

There were two main stages in conducting this study. They were a collection of data and analyses of data. In terms of collecting data, this research used a questionnaire, interview, and document review. To be specific, data collection was conducted on the reality of LFD in the researched area. Moreover, 12 officers who worked in the government offices were interviewed in order to assess the effect of some factors which affected the task of LFD. Additionally, 90 organisations, individuals and households crucially that were compensated for land clearance were interviewed to assess the effect of the task of the LFD to the economic and social development in the area of Phu Vang district (Ho et al., 2016).
After that, the data were analysed by using descriptive statistics.

- Using the methodology on analysis in the descriptive statistics to total up the particularities of the objects following the group of objects, the average weight and the frequency of appearance by Excel software.
- Using Likert measure (Likert, 1932) to assess the groups of factors which affected the LFD by five levels: very high, high, medium, low and very low which correspond to 5, 4, 3, 2 and 1, respectively. The range of value corresponding to the level of impact on LFD was identified as follows: very large: ≥4.20; large: <4.20 and ≥3.4; average: from <3.4 and ≥2.6; small: <2.6 and ≥1.8; very small: <1.80.
- The method for calculating the range of value corresponds to the level of impact on LFD (Likert, 1932):

$$Range\ of\ value = \frac{Maximum - Minimum}{n} = \frac{5-1}{5} = 0.8 \quad (1)$$

- The magnitude of impact was calculated as a mean of weights on the Likert scale.

3. Result of research and discussion

3.1. The result of the development of land fund in the researched area

The results of LFD between 2014 and 2016 in Phu Vang district with a variety of purposes of creating land fund were illustrated in Table 1. Overall, 67 projects were developed with an auction of 61.88 ha of land.
In addition to this, the area of land used for LFD mainly came from agricultural land, with a total area of 42.69 ha compared with 19.19 ha of other lands. This was mainly because recovery of agricultural land had a low cost and simpler procedure compared with that of other sources of land.

Table 1: The result of the development of land fund in the period from 2014 to 2016

Purpose of creating a land fund	Number of projects	Total area(ha)	Agricultural Land (ha)	Other (ha)
Residential land	30	20.98	16.53	4.45
Other purposes	37	40.9	26.16	14.74
- Land for construction of non-business facilities	5	1.86	1.55	0.31
- Land for non-agricultural production and business	6	15.93	15.9	0.03
- Land used for public purposes	25	15.83	6.81	9.02
- Land for national defense or security purposes	1	7.28	1.9	5.38
Total	**67**	**61.88**	**42.69**	**19.19**

[Source: List of projects implementing compensation, support and resettlement in Phu Vang district]

LFD for residential land and serving the public accounted for the largest number of projects and total area. A total of 30 projects were developed, and 20.98 ha of land was recovered to build the new residential areas in the period between 2014 and 2016. This showed the tendency of quite strong urbanization in Phu Vang district over the years. Moreover, an auction of residential land had a tremendously important role in the state budget. A large amount of

annual state budget came from the auction of land. This was the reason why there were usually projects of LFD for residential area annually.

Besides, there were 25 projects with the recovery of 15.83 ha of land for public purposes in the same period. The vast majority of these projects were the extension of provincial roads and national highways as well as the development of an electric grid. They contributed positively to Phu Vang district's infrastructure and the aim of 100 % indigenous people using the national electricity network in 2020. Therefore, the quality of life of local people would be increased enormously.

Although there were only six projects for non-agricultural production and business, these projects used 15.93 ha of land mainly converting from agricultural land (15.9 ha). Production and business performed a fundamental role in the socio-economic development of each locality. Consequently, the ground clearance for production and business, namely trading petroleum, textile and mineral exploitation not only yielded revenue for the state budget but also combatted unemployment in the local region.

During the study period, there was only one project of land recovery for national defence and security. It was the construction of a training centre of police of Thua Thien Hue Province with a total area of 7.28 ha of land. Most of the area was converted from unused land (5.25 ha). This enhanced the efficiency of land use (most of the area was converted from unused land) in the local area as well as strengthened security for Phu Vang district. As a result, social order and peace of mind of homegrown people would be boosted.

3.2. Assessment of the effect of the factors on the development of the land fund.

3.2.1. Group of policy factors

In this group, two factors had a considerable influence on the LFD: land policy and policies to attract investment. To be specific, the policies to attract investment had an average value of 4.33 (Figure 1a). Likewise, Ho et al. (2016) also proved the significantly important role of these policies for LFD by the value of 4.4. According to Likert scale, this value represents the significant influence on the LFD. As a result, the policy of attracting investment was a key factor determining the effectiveness of LFD. Developing the policy of attracting investment through various forms of reasonable support for capital, tax and land use fees for investment projects should be a primary duty to create the most favourable conditions for LFD.

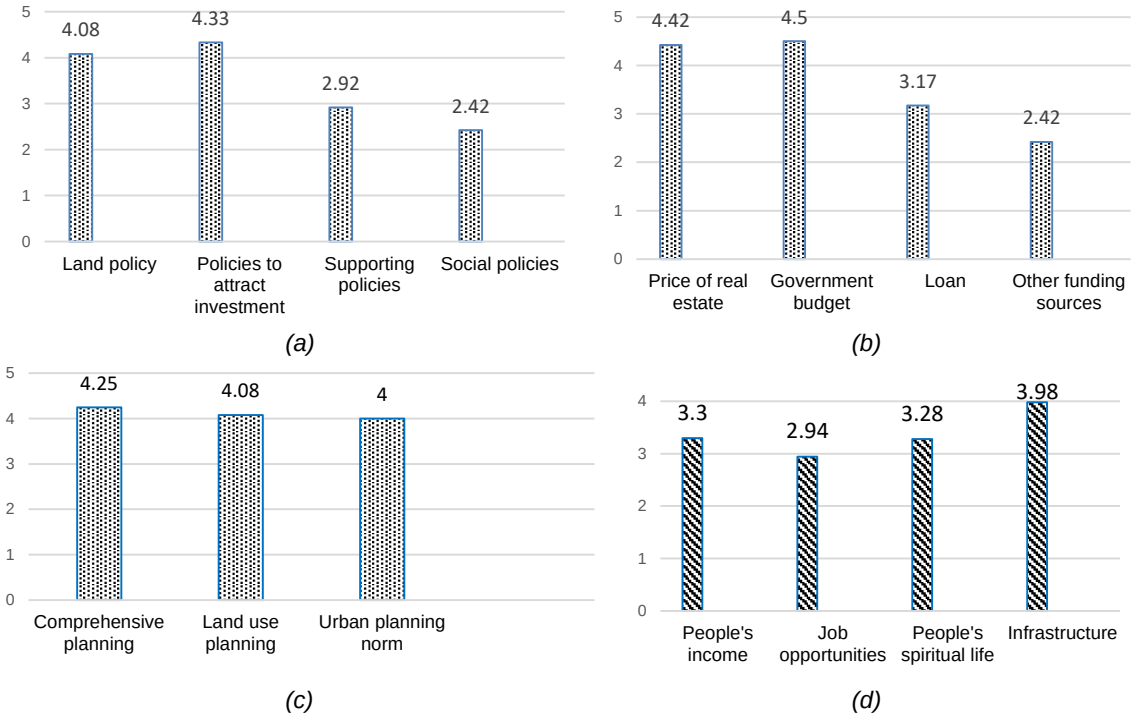

Figure 1: (a) The influence of the policy factors group on the work of land fund development; (b) Financial factors on the work of land fund development; (c) Planning factors on the work of land fund development; and (d) Impact of land fund development on socio-economic development

Figure 1a shows that land policy had an average value of 4.08. This value demonstrated the quite utmost importance of land policies to LFD. For this reason, the land policy should be received proper attention,

remarkably land policy related to the conversion of land use purposes in order to maximise the potential of the land resource in the localities and ensure the sustainable development on three aspects of the economy, society and environment in the local area.
In terms of supporting policies and other social policies, the average value was 2.92 and 2.42, respectively. This represented the low and moderate impacts of these policies on LFD. In order to enhance the efficiency of LFD, supportive policies and other social policies should be introduced after having coordination between the industries: Natural resources and environment management; Agriculture; Construction and Trade as well as consulting with specialists.

3.2.2. Group of financial factors

In Figure 1b, the two most prominent elements were the price of real estate and the state budget. Specifically, the price of real estate had a value of 4.42, and the state budget had a value of 4.5. These results reflected an exceedingly important role of the price of real estate and the government budget on LFD. This was the reason for the great interest in the land price and the state budget of specialists as well as leaders during the process of implementing LFD. Whereas, Ho et al. (2016) did not mention the government budget criteria, which is one of the most influencing factors of LFD in Yen Bai city. Nevertheless, groups of researchers revealed the price of real estate as the most important factor to LFD by the value of 4.57.
Regarding the government budget, disbursement is a vitally important work. The LFD centre of Phu Vang district needs to propose new and specific models to the disbursement of compensation, supports and resettlement in the localities so that projects will not be delayed because of slow disbursement, causing loss of resources for not only society but also local individuals.
To develop land fund, study, evaluating and constructing governmental land prices which are suitable to the actual conditions in the locality will help the compensation and ground clearance work to be carried out on schedule. Additionally, the LFD centre should coordinate with the natural resources and environment division to propose compensation and support measures by the actual value of the property of the people but still obeying the law. This is a critical, sensitive issue due to the interests of many parties.
The funding borrowing from financial institutions had a value of 3.17. This indicated an average level of influence of this factor on the LFD. Typically, the loan is only considered if there will be any problems in disbursement. Hence, if the disbursement is well conducted, the value of this factor may decrease.
The value of the impact of the other funding sources was 2.42. This value illustrated the influence of other funding sources on LFD was at a small scale.

3.2.3. Group of planning factors

Figure 1c indicates that the comprehensive planning elements and LUP have a high value of impact, namely 4.24 for the comprehensive planning, 4.08 for LUP and 4.0 for the urban planning norms. The correlation between the comprehensive plan for socio-economic development and LFD was presented in the same level in Yen Bai city (Ho et al., 2016). This was a very high value, showing a critical role of the comprehensive planning on LFD. It also stated that during the implementation of LFD, there was a need for research on the comprehensive plan for LFD.
The other two factors were LUP and urban planning norms with a value of 4.08 and 4.0, respectively. This represented a major impact on the LFD of these two factors. Furthermore, these two factors were directly affected by the comprehensive plan. For this reason, these two factors played a vital role in LFD.
Under those circumstances, urban planning norms should be considered when implementing LFD, especially for localities with relatively developed infrastructure including Thuan an town and Phu Da town of Phu Vang district. This will inevitably guarantee the provisions of the law in those areas.
In terms of planning factors, to promote the efficiency of LFD, planners should pay attention to the calculation of land use efficiency when preparing LUP schemes in localities, thereby allocating appropriate land use plans and avoiding waste. Before planning, planners should consult the experts and the results of specialised scientific researches that have been published in the area for planning to achieve optimal results.

3.3. Assessment on the effect of the development of a land fund to the economic, social development of Phu Vang District, Thua Thien Hue Province.

The survey was conducted with 90 inhabitants, households and the organisations in the area of Phu Vang district on the effect of LFD to the economic and social development. The assessment was based on four factors: inhabitants' income; job opportunities; the people's spiritual life and infrastructure.
Generally, the infrastructure made the most significant changes during the study period with a very high value of 4.33. The figure for the remaining factors were approximately 3.0, reflecting the average impact of LFD on people's incomes, employment opportunities and community's spiritual life.

3.3.1. Assessing the impact of land fund development on people's incomes

The results of this study showed that 34.44 % of respondents claimed that their income increased due to the LFD. There were 5.56 % of interviewees thought that their income increased enormously as a result of LFD. A possible explanation for this would be the widening of the road created more opportunities for business and trade, bringing about considerable revenue. A large number of respondents (47.78 %) underlined that their income remained almost unchanged after LFD. These were households that were not dependent on local income sources but tend to seek employment through labour export, the salary of government and pension.

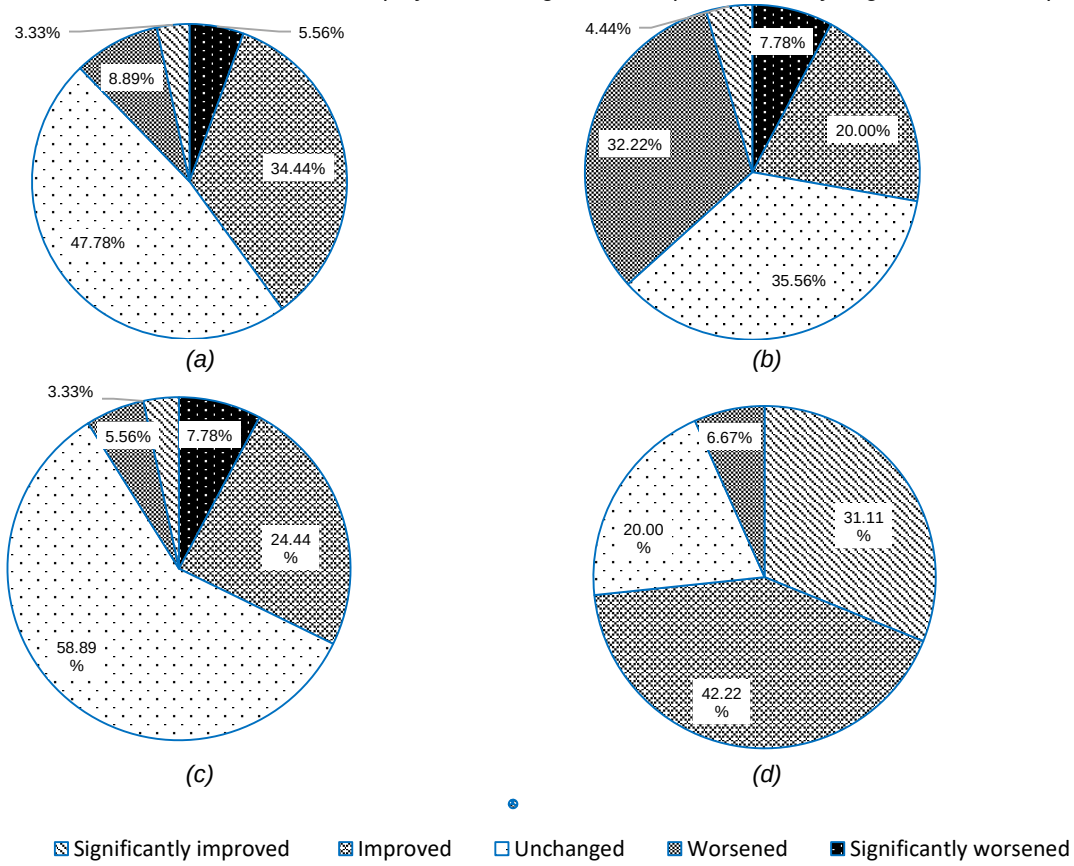

Figure 5: (a) Impact of land fund development on people's income; (b) Impact of land fund development on employment opportunities; (c) Impact of land fund development on people's spiritual life; (d) Impact of land fund development on infrastructure

3.3.2. Assessing the impact of land fund development on job opportunities

The job opportunities for employment of 29 interviewees, accounting for 32.22 %, decreased because of LFD. These interviewees were mainly from agricultural families who were not trained to change their occupations after land recovery. Eighteen respondents, accounting for 20 %, believed that their income increased after LFD. In addition to this, seven respondents stated that they found the job more easily after LFD. These people came from small home-based business or were construction workers in the localities.

The vast majority of people thought that their job opportunities remained unchanged after LFD. The main reason was that these people had done a same job for many years, so it was exceedingly difficult to change jobs that had been deeply embedded in their mind. This correlate favourable well with Ho et al. (2016) that LFD has not created job opportunities like as expectation of people.

3.3.3. Assessing the impact of land fund development on people's spiritual life

A proportion of 24.44 % of interviewees argued that LFD produced positive changes in culture and forms of entertainment. This was simply because there were projects to serve the community, for examples, projects of the construction of cultural houses and development of cultural infrastructure. These projects had ensured the basic cultural needs of people living in localities where access to recreational facilities had been limited.

Noticeably, most people thought that their spiritual life had not changed significantly when LFD was carried out. Most of the interviewed people (58.89 %) pointed out that although the facilities for cultural and community

activities had been developed the spiritual life of people would be unchanged owing to the low content of the spiritual activities.
By contrast, 56.98 % interviewees affirmed that their spiritual life has been improved due to LFD in Yen Bai city (Ho et al., 2016). This illustrated that LFD might contribute differently to people' spiritual life in distinctive areas.

3.3.4. Assessing the impact of land fund development on infrastructure

42.44 % interviewees contended that the infrastructure had positively improved. This would be the result of up to 15.83 ha of land was used for LFD projects that served the community. These were transport, irrigation and energy projects. In addition, 31.11 % of respondents noted that the infrastructure had been significantly positively changed. This was mainly because of the expansion of the existing road systems, for instances, provincial roads 10A and 49B as well as The Asian Development Bank's power grid projects. These results are scarcely distinguishable from (Ho et al., 2016) that 51.40 % interviewees supposed that infrastructure is better due to LFD.
However, there were 18 people who considered the infrastructure to be untouched. These people lived in areas which had relatively good infrastructure with Phu Thuong commune, Phu Da town as examples.
Moreover, there were six citizens (6.67 %) highlighted that the infrastructure had been deteriorated over time. This was as a result of the expansion of provincial roads and highways which affected the infrastructure of the residential areas. For example, the vehicles transporting materials had damaged inter-village and inter-commune roads with smoke, dust fall and rock fall. These factors had a negative impact on the families of those six people.

4. Conclusion

LFD served principally to the new inhabitant zones and the other public purposes. An additional finding was a vast majority of interviewees recognised that LFD created the noticeably positive changes to the infrastructure. Moreover, LFD was most seriously affected by policies to attract investment, price of real estate, state budget, as well as comprehensive planning.
In the light of this study, the findings suggested that local authorities of Phu Vang district should examine carefully the group of planning factors including comprehensive planning, land use planning and urban planning norm as well as policies to attract investment, price of real estate and disbursement of the state budget for LFD. However, it remains to be further clarified whether these findings in Phu Vang district could be applied to other areas with a different scale, namely Thua Thien Hue province, Hue city or even Vietnam.

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Health risk assessment of Glyphosate at grape farms, Tuy Phong District, Binh Thuan Province, Vietnam

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Glyphosate was recently determined as probably carcinogenic to humans due to the most heavily used pesticide in the world. Glyphosate has been much used in agricultural cultivation in Vietnam and there are no recommendations and studies related to health risks. This study aimed to (1) determine glyphosate residues in soil, groundwater, and fruit at grape farms in Tuy Phong district, and (2) assess the health risks of glyphosate residues to children, farmers in the study area. To achieve the objectives, the study conducted farmer surveys and collected samples at all 12 grape farms, then analyzed glyphosate residues by GC-MS/MS method. Health risk assessment to farmers and children was carried out by the guideline of WHO based on analyzed and surveyed results. Results show that glyphosate has no acute toxicity to users, but the accumulation of glyphosate in grapes, soil, and groundwater exceeds in 5 - 200 times higher than the European allowable limit, highest in groundwater. The pathway of glyphosate intake into the body is likely to cause a high level of cancer risk through the use of groundwater, accounting for 98.3 - 99.3 % of the total risk. Health risk of farmers is assessed as highly concerned, needed minimizing solutions.

1. Introduction

In the past decade, the use of glyphosate herbicide has become popular and now it is one of the most used herbicides in the world (US EPA, 2017). After being sprayed, glyphosate is quickly absorbed in the soil and then enters groundwater and surface water (US EPA, 2016). The increase of widespread glyphosate usage leads to genetic plant modification and human health effects (US EPA, 2017). Glyphosate residues are found in many types of samples including plant samples (EC, 2008). The maximum allowable concentration of glyphosate in US drinking water is 0.7 mg/L (Thompson et al.,1989).

Recently, public opinion is very interested in the issue of farmers abusing glyphosate herbicides in agricultural production. The amount of herbicides used by farmers increased by 20 - 30 % compared to 5 years ago, notably farmers using the concentration of herbicides increased from 1 to 2 times higher than the warning dose. The European Food Safety Agency and the International Agency for Research on Cancer have been much controversy in allowing use of glyphosate (Vogel, 2015). Glyphosate became the focus of world opinion since March 2015, when IARC under the World Health Organization announced the classification conclusion for glyphosate as a group 2A, which is high carcinogenicity (Luan, 2016).

Research has shown that glyphosate after being sprayed, remains in the air, water, food and can be absorbed to the human body through food chains. Mladinic et al. (2009) reported about experiments on lymphocytic cell lines of human and animal that showed an increase in the number of small nuclei (one of the signs of DNA destruction). Accordingly, the hypotheses about the ability to cause mutations at the telomere have set by scientists. Research in the laboratory also provides evidence of the risk of causing strange cancers in male mice such as renal tubular carcinoma, rare liver cancer and the ability to promote the development of T47D cell lines through impact on estrogen receptor. Over the years, there have been many allegations from countries about the dangers of glyphosate, typically in 2015, more than 30,000 doctors and medical professionals in the Union Federation of Health Professionals of Argentina asked for glyphosate to be banned because this chemical

causes the increase of miscarriage, birth defects, skin diseases, respiratory diseases and neurological diseases in this country (US EPA, 2015).
Glyphosate can disrupt the endocrine system and cause adverse effects in the developmental stages. In Colombia and Ecuador, the high rate of genetic modification and miscarriage in women during the season of glyphosate spraying was observed (Thompson et al., 1989). In Paraguay, women living within one kilometre radius of glyphosate spray fields faced up with risks of birth defects (Thongprakaisang et al., 2013). Recently, the special commission of EU includes 30 members was set up to evaluate the grant license process of glyphosate extension in Europe. The US EPA (2017) finalized an environmental risk assessment of glyphosate to determine whether should continue to use glyphosate.
In Vietnam, farmers have been increasing glyphosate usage by 20 - 30 % compared to 5 years ago. The dose is higher 1 - 2 times of recommended amount. The glyphosate contents in leaf samples, stems and food showed that all analytical samples had glyphosate contents exceeding the allowable standard of European regulations from 10 to 13,790 times, highest in stems and dry leaves (Nam and Tam, 2018). There are no studies related to assessing the health risks of glyphosate in agricultural production in Vietnam.
The grape growing area in Binh Thuan province was selected for this study. Grape is the main crops of Binh Thuan, with total area about 21,000 ha, total production about 400,000 tons, accounting for 73.2 % of the area and 76.9 % of national production. Glyphosate has mainly been used as weedkiller at grape farms.
A survey of 12 grape farms was conducted in Tuy Phong district, Binh Thuan province. Investigating and surveying the situation of glyphosate usage: weedkiller types, dosage, use methods, collection and handling of packages containing glyphosate after using; how to handle excess glyphosate to assess the impact on farmers' health through sociological surveys. Then, based on the data collected and the analysis of glyphosate residues in soil, groundwater and grapes, we assessed health risks according to the guidelines of the World Health Organization (WHO) (Tran, 2008). Based on risk-assessment approaches, Pinedo et al. (2017) proposed measures to manage and reduce health risk of total petroleum hydrocarbons. In this paper, from the findings of health risk assessment at grape farms, some risk management measures are taken into account.

2. Experimental

2.1 Research objects

The whole of 12 grape-growing farms in Tuy Phong district, Binh Thuan province was surveyed.
Herbicides containing glyphosate such as Grassad 480SL (Dau Trau brand) and BN-Kocan 480SL (Khung Long brand) were investigated.

2.2 Research methods

2.2.1 Sociological survey and field survey

An open and closed question system was used in accordance with the actual situation related to habits, glyphosate dosage, how to dispose of the packaging and residues of glyphosate after spraying, the amount of soil that can be contacted, the amount groundwater used and the amount of grapes consumed. The health effects before, during and after spraying on farmers were also recorded (acute effects). These are important information as input data for the risk assessment model. Interviewers were farm owners. Health risk assessment consists of three groups: two groups of children (sensitive to toxicants) and adults.

2.2.2 Sampling

Soil samples were collected at 30 cm depth following the guidance on soil quality - sampling - guidance on sampling techniques (TCVN 7538-2:2005). Groundwater sampling was carried out based on the guidance on water quality - sampling - guidance on the design of sampling programmes and sampling techniques (TCVN 6663-1:2011). Bunches of grapes were collected in zigzag shape for grape samples according to the guidance on fresh fruits – sampling method on the field (TCVN 9017 – 2011).

2.2.3 Glyphosate analysis

Glyphosate in samples were analysed by GC-MS/MS, which has high reliability, high accuracy, recovery, repeatability and reproducibility of the method according to the regulations of AOAC Official Methods of Analysis (Nam and Tam, 2016).

2.2.4. Health risk assessment

a. Health risk assessment model

To develop a health risk assessment model for the study area, the method proposed by the World Health Organization (Human health risk assessment toolkit: Chemical hazards) and the US Environmental Protection Agency (US EPA, 2016) was applied. The steps are conducted as follows:

Step 1: hazard identification

The study's risk is glyphosate, which is classified as the group 2A that may cause cancer under the World Cancer Organization (IARC). Therefore, determination of risk level is presented in Eq (1).

$$R_i = SF \times CDI_i \quad (1)$$

where i corresponds to three pathways that glyphosate can enter the human body by using groundwater, contacting with soil and eating grapes.

The total risk according to three pathways is calculated by Eq (2).

$$R_{total\ risk} = R_{soil} + R_{groundwater} + R_{grapes} \quad (2)$$

SF: slope factor $(mg/kg/day)^{-1}$, $SF_{glyphosate}$ = 0.00062 $(mg/kg.day)^{-1}$ through oral (Department of health, 2017).

Step 2: exposure scenarios
Exposure scenarios were developed by possible exposure pathways (glyphosate in soil, groundwater, and grapes to human body); the correlation between the exposed residents and the potential toxicity of glyphosate was assessed.
Step 3: exposure assessment
Applying the formula to calculate the level of exposure by the exposure pathway through gastrointestinal for different residents in the study area (Tran, 2008). Exposure pathways and related data were cited from published sources and the results of direct survey as follows:

- Random exposure with contaminated soil/dirt through gastrointestinal is presented in Eq (3).

$$CDI_s = \frac{C_s \times SIR \times CF \times FI \times ABS_s \times EF \times ED}{BW \times AT} \quad (3)$$

- Eq (4) shows gastrointestinal exposure to the use of contaminated water for cooking (using well water in the study area).

$$CDI_{dw} = \frac{C_w \times WIR \times FI \times ABS_s \times EF \times ED}{BW \times AT} \quad (4)$$

- Exposure when eating grapes through gastrointestinal (Eq 5).

$$CDI_p = \frac{CP_z \times PIR_z \times FI_z \times ABS_s \times EF \times ED}{BW \times AT} \quad (5)$$

Step 4: risk characteristics

Evaluation of the ability to harm human health through calculated values. Glyphosate is a substance belonging to the group 2A, which can cause cancer risk according to Eq (1).
CDI: Daily intake of glyphosate to the body per day (mg/kg.d). In this case, CDIs, CDIdw and CDIp were calculated in Step 3.
Eq 2 calculates the total risk from carcinogens according to three exposure pathways. Risk characteristics are summarized and summed for assessing qualitative and quantitative risk levels according to the following rating scale:

- R $<10^{-6}$: Low risk of cancer, acceptable
- 10^{-6} < R $<10^{-4}$: Average cancer risk, may or may not have a reduced decision, more research is needed
- R > 10^{-4}: High risk of cancer, mitigation measures are needed

3. Results and discussion

3.1 The status of use and glyphosate effects to farmer's health

All farmers often sprayed herbicides containing glyphosate when the grass had just developed. Farmers also added that the use of herbicides was partly based on their production experience, so 100 % of grape farms used

the exceeding dosage of manufacturer's regulations. Based on the survey results, the glyphosate herbicide dosage was chosen to add more at most 30 cc (accounting for 45 %), the lowest was from 30 to 50 cc, accounting for 11 % and the dosage was higher 50 cc, accounting for 22 % of total grape farms. This leads to increase glyphosate residues in the environment and agricultural products. The isolation time of herbicides after spraying from 2 weeks to 1 month is relatively ensured and follows the manufacturer's recommendations.
Although attended to training courses about the use of pesticides, farmers often did not record guidelines. Pesticide containers after use were designed to gather in dumping places, and then taken away for destruction. However, the fact of the survey showed that pesticide packaging was thrown indiscriminately on the farm ground.
Most farmers used underground water sources for daily activities such as eating, bathing, using for irrigation, and diluting herbicides accounting for 82 % of surveyed farms. The average amount of groundwater consumed monthly from 1,000 m3 to 2,000 m3. Herbicide washing water was directly dumped into the ground, so the groundwater source is likely to be greatly affected.
Survey results show that 92 % of surveyed farmers have never suffered from acute poisoning or skin diseases, only one farmer (8 %) had ever experienced vomiting when he smelled herbicides.
According to previous research results, glyphosate can break the endocrine system and cause adverse effects in developmental stages, the high rate of genetic modification, and miscarriage for women in Colombia and Ecuador (Luan, 2016). Danish scientists also discovered glyphosate residues in groundwater in fields sprayed with herbicide (Thonprakaisang et al., 2013). The carcinogenic mechanism of glyphosate has also been explained through immunological and neurotoxic tests (acute and subcutaneous toxicity) based on four studies in mice showing that glyphosate increases the cancer incidence (US EPA, 2016). This shows that although the use of glyphosate at grape farms in Tuy Phong district has not caused yet any cases of acute poisoning, the widespread use and frequent contact with glyphosate from the environment and food may be chronic poisoning to farmers. The next content assesses the details of glyphosate residues in the component environment and in grapes.

3.2 Health risk assessment of glyphosate in the study area

The values of C_{max} and $C_{average}$ of glyphosate residues in the environment and grapes at 12 farms in our previous study (Nam and Tam, 2018) are shown in Table 1.

Table 1: Content of glyphosate (C_{max} and $C_{average}$) in soil, water and grapes

No	Exposure pathways	C_{max}	$C_{average}$	EU standard	Vietnam standard
1	Soil (mg/kg)	1.30	0.625 ± 0.339	-	0.1
2	Groundwater (µg/L)	19.1	5.08 ± 3.81	0.1	-
3	Grapes (mg/kg)	0.14	0.041 ± 0.054	0.1	0.1

Glyphosate residues in soil exceeded 5 - 13 times higher than the allowable level of the Vietnam standard, QCVN 15: 2008/BTNMT, exceeded 10 - 191 times compared with 0.1 (µg/L) of the EU allowable standard in groundwater. Two grape samples had glyphosate residue levels higher 1.4 times compared with the EU allowable limit. This is a serious warning for policy makers and authorities in Vietnam.

3.2.1. Exposure assessment

Eq(3) – Eq(5) were applied to calculate CDI for three groups (adults, children 6 – 12 years old and 1 – 6 years old) with two cases, the greatest risk (Cmax) and average risk (Caverage) throughout life for carcinogens.
The results in Table 2 show that exposure concentrations of glyphosate through gastrointestinal from the use of contaminated groundwater and grapes were highest in the adult group and lowest in the 1 – 6 years old group. Specifically, the exposure concentration in the adult group ranges from 7.48 to 66.5 times higher than the lowest group. The reason is that adults contact directly with groundwater for crop cultivation, breath glyphosate polluted air through spraying, and eat more grape than children. Exposure concentrations by three pathways at maximum concentrations were 2.1 to 3.4 times higher than average concentrations. The main entry pathway of glyphosate into the human body is through the use of groundwater, followed by contact with contaminated soil and the use of grapes.

Table 2: Glyphosate exposure concentrations through gastrointestinal from soil, groundwater and grapes

Exposure pathway	Soil (CDI_s) (mg/kg/d)		Groundwater (CDI_{dw}) (mg/kg/d)		Grapes (CDI_p) (mg/kg/d)	
Exposure residents	C_{max}	$C_{average}$	C_{max}	$C_{average}$	C_{max}	$C_{average}$
Children 1-6 years old	3.83×10^{-4}	1.84×10^{-4}	0.0298	0.00794	1.58×10^{-5}	4.59×10^{-6}
Children 6-12 years old	1.27×10^{-4}	6.09×10^{-5}	0.0395	0.0105	1.51×10^{-4}	4.39×10^{-5}
Adults	3.59×10^{-4}	1.72×10^{-4}	0.2230	0.0594	1.05×10^{-3}	3.05×10^{-4}

3.2.2 Risk assessment

The results of risk assessment through gastrointestinal are presented in Table 3.

Table 3: The characteristics of cancer risk of glyphosate through gastrointestinal at a high risk

Residents	R (Risk) - C_{max}	R (Risk) – $C_{average}$	Comment
The characteristics of cancer risk of glyphosate through gastrointestinal from groundwater ($R_{groundwater}$)			
Children 1-6 years old	$1.85*10^{-5}$	$4.92*10^{-6}$	Cancer risk in average level, may not need to be
Children 6-12 years old	$2.45*10^{-5}$	$6.52*10^{-6}$	reduced, need more research
Adults (Farmers)	$1.38*10^{-4}$	$3.68*10^{-5}$	High risk, need to make solutions
The characteristics of cancer risk of glyphosate through gastrointestinal from three pathways ($R_{total\ risk}$)			
Children 1-6 years old	$1.87*10^{-5}$	$5.04*10^{-6}$	Cancer risk in average level, may not need to be
Children 6-12 years old	$2.47*10^{-5}$	$6.58*10^{-6}$	reduced, need more research
Adults (Farmers)	$1.39*10^{-4}$	$3.71*10^{-5}$	High risk, need to make solutions

The cancer risk of glyphosate through gastrointestinal from soil and grapes intake was low, acceptable, even in the case of maximum concentrations.

The risk of glyphosate when ingested through groundwater from two groups of children in the case of moderate concentrations is acceptable. However, in the case of maximum concentration at high risk level, there should be preventive measures.

The pathway of glyphosate into the human body is likely to cause a high level of cancer risk through by using groundwater, accounting for 98.3 - 99.3 % of the total risk. Through the results of toxicological and epidemiology studies from previous studies, it is shown that people in the study area may have renal tubular carcinoma (Anh, 2016), rare liver cancer by the quickly destroyed DNA and chromosomes on animal cells (Luan, 2016), genetic modification and high miscarriage in women (Thonprakaisang et al., 2013).

The highest health risk is identified for adults and the lowest group is 1 – 6 years old children. For adults, the cancer risk is high, needs to make solutions to minimize. Glyphosate intake to adults though other contact pathways (contaminated clothing, using bare hands when opening herbicides packing or rinsing bottles, direct contact with polluted air, etc.) may increase the current level of risk. These results provide an assessment method and the risk levels can be referenced as a comparative value when conducting different solutions.

4. Conclusion

The survey results of 12 grape farms in Tuy Phong district showed that all farms used herbicides with glyphosate. Farmers here rinsed and discharged diluted water directly into the soil and surface water. Glyphosate residues were found much higher than the allowable standard in soil, groundwater, and grapes, especially 10 – 191 times higher than the EU allowable standard for all 12 groundwater samples.

The cancer risk of glyphosate from soil and grapes for all three groups was low, acceptable, even in the case of maximum concentrations. The pathway of glyphosate into the human body is likely to cause a high level of cancer risk through by using groundwater, accounting for 98.3 - 99.3 % of the total risk to adults. Children due to less work in the grape farms, the risk is only average level, more research is needed.

Vietnamese regulatory authorities, especially the Ministry of Natural Resources and Environment, need to establish standards to control glyphosate in the environment and food, improve the quality of agricultural and food products and protect human health. Groundwater for agriculture in here needs treating in proper methods and controlling water quality before usage.

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Synthesis Nano- Xonotlite from Rice Husk Ash

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Nano- Xonotlite, ($Ca_6Si_6O_{17}(OH)_2$) material was prepared from rice husk ash, as the source for SiO_2 and CaO using sol-gel method. Rice husk ash after pre-treatment and CaO powder was mixed together in 100ml distilled water with vatious molar ratio of rice husk ash/CaO. The mixed solution was place in the autoclave and heated at 200°C for 10 hours. From the XRD results, molar ratio Ca / Si = 0.7 exhibited that xonotlite is the major phase. The xonotlite crystals have a fairly uniform length from 5µm - 8µm and the thickness are 10-15 nm was obtained at this condition. In addition, some analysis method have been conducted such as TGA, FT-IR, SEM, TEM to investigate the characteristic of this material.

1. Introduction

Synthesis of calcium silicate hydrate under the hydrothermal condition has been extensively studied to manufacture some functional materials. Under different hydrothermal conditions, varieties of calcium silicate hydrate with different structures can be prepared, such as amorphous calcium silicate hydrate and crystalline calcium silicate hydrate (CCSH). Amorphous calcium silicate hydrate, for example, calcium silicate hydration gel (C-S-H gel), consists of monomer silicate anions with silanol groups (Spudulis et al., 2013). Crystalline calcium silicate hydrate, for example, tobermorite and xonotlite, is built up of layers of Ca polyhedra and condensed by wollastonite - type silicate chains on both sides. These materials are widely used in electric power, metallurgy, petroleum, chemical, building materials in the fields of furnace, piping, reactor and other insulation. The use of insulation materials in the world is being considered and growing more and more, especially for markets with large consumption demand such as USA, Japan, China, Germany, etc. The technology of producing high-temperature and non-baked materials has been known since the 1920s, when the product was an autoclave concrete. Studies have shown that the structure of high pressure and high temperature concrete materials is predominantly in the form of tobermorite. However, with this structure, the autoclave concrete material has the disadvantages of low critical temperature, high hardness, easy to crack. So researchers have found the structure of a new CCSH material is also produced under similar conditions, but in addition to the typical tobermorite structure is xonotlite mineral structure. The formation of these minerals is mainly based on the CaO/SiO_2 ratio of the material involved, the temperature and the pressure of the production process.

An important material for synthesizing CCSH is a silica source that determines the quality and cost of synthetic materials. For Vietnam, the source of silica from rice husk ash is an agricultural waste product with a huge annual reserve generated from the heating of rice husk, which will be a suitable source of materials for the insulation industry Because of abundant reserves, stable quality, low cost. However, the exploitation of this material source still has many disadvantages that need to be researched to be able to be applied to industrial production. In this study, nano- xonotlite material was prepared from rice husk ash, as the source for SiO_2 and limestone, source for CaO using sol-gel method under specific hydrothermal conditions and samples were characterized by several methods.

2. Material and Methods

2.1 Material

Rice husk was collected at Vinh Bình, An Giang province, Vietnam. Amorphous silica was synthesis from rice husk ash by precipitation method. NaOH, HCl was purchased from Sigma-Aldrich

2.2 Method

a) Synthesis silica form rice husk ash

First, a mixture of rice husk ash and 1.5 N HCl was applied in a reactor at 90°C to remove the metal oxide. The rice husk ash and diluted acid are then filtered, washed several times with distilled water and dried to prepare the silica extraction. 10g rice husk ash was treated with diluted acid with 100 ml NaOH 3N in a 3-neck flask at 90°C to extract silica from rice husk ash. Then to the gelatinization stage, during the gelatinization process, it is necessary to add 2.5- N HCl solution and mix well at room temperature to ensure that the precipitated silica particles have sufficient time to coagulate with to form a long chain silica. After the silicate gel solution is completely gelatinized, the silica gel mixture will be aged at 50°C for 20 hours for complete gelation and stable stabilization of the silica molecule. Silica has an amorphous structure obtained after the silica gel is filtered, washed several times with distilled water to dissolve the NaCl salts produced during gelatinization and drying at 120oC for 24 hours.

b) Synthesis CCSH

The nano-xonotlite crystals were prepared by hydrothermal reaction in autoclave with different molar ratio C/S equal to 0.7, 1.0, 1.5. According to the reaction between CaO and SiO2 by hydrothermal method, the water: solids ratio of the mixture is approximately 15:1. The reaction were conducted in the Teflon reactor. Put teflon reactor into the autoclave and heated at different temperatures of 10h to investigate the formation of post-reaction xonotlite minerals. After that, the autoclave was cooled to room temperature quickly. The specimens were removed from Teflon reactor and then washed with distilled water several times. The powdered xonotlite obtained after the sample was dried at 105°C for 6h.

3. Results and discussion

CCSH materials was synthesized at vary different molar ratio Ca/Si to assess the effect of this parameter on the formation of CCSH. The molar ratio of the mixture of materials used in all syntheses was provided in Table 1:

Table 1. Synthesis sample at different Ca/Si ratio.

Sample	Molar ratio Ca/Si	Temperature, time
M1	0.7	200^{O}C, 10h
M2	1.0	200^{O}C, 10h
M3	1.5	200^{O}C, 10h

Material properties were analyzed by analytical methods such as X-ray diffraction analysis (XRD), FT-IR, scanning electron microscope analysis (SEM), transmission electron microscope (TEM), thermalgravimetric analysis (TGA)

3.1 X-ray diffraction analysis (XRD)

The XRD diffraction analysis method is used to determine the composition of the crystal formations that may be present in the post-synthesis material. The main compounds are observed in Fig. 1 is xonotlite and tobermorite mineral. Sample M1 with hydrothermal in condition 200 ^{o}C, 10h at C/S molar ratio is 0.7 has the appearance of $Ca(OH)_2$ remain at angle 2θ = 34.078 ^{o}, 54.351 ^{o}. Besides that, peak $CaCO_3$ remain appears with very small intensity at angle 29.378^{o}. As can be seen in Fig. 3-4, xonotlite and tobermorite are also the main mineral but they has very low intensity, and the signal of peaks is broader then its of sample M1. The powder synthesized under C/S molar ratio at 1.0 has signal of CaCO3 at angle 2θ = 25^{o}, 27^{o}, 32.9^{o}, 47.2^{o}, 48.42 ^{o} . Furthermore, peak of Foshagite mineral appear at 27.29^{o} and signal of Afwillite at 44^{o}. Data of Fig. 3 represents for sample M3 with C/S molar ratio of 1.5, the main substance is $Ca(OH)_2$ and $CaCO_3$. But, the data has peak Xonotlite at 49.573^{o}, Tobermorite at 300 angle. Following the increasing of C/S molar ratio, intensity of peak of CCSH mineral has decreased. With XRD results of sample M1, xonotlite insulation material was synthesized at 200^{o}C with Ca/Si ratio = 0.7, water/solid ratio = 15 in 10h completely corresponding to XRD results of the sample taken in 4h by Edmundas Spudulis and colleagues (Spudulis et al., 2013). However, the results of XRD peaks were much lower than the results of XRD peak in this research, because the reaction time they conducted only lasted 4 hours compared to 8 hours of the sample M1. The fact that, the molar ratio of Ca/Si

has significant effect on the formation of CCSH. With the molar ratio of Ca/Si equal to 0.7, CCSH material as tobermorite and xonotlite are forming successfully. When increasing this ratio, xonotlite and tobermorite mineral have decreased intensity.

3.2 Thermalgravimetric Analysis

The CCSH synthesized under conditions of Ca/Si equals to 0.7 is confirmed as well by the data of thermal analysis for the sample M1, which are provided in Fig. 4. Initially, at the start of the analysis site, the temperature was about 35 °C, the sample mass was 100%. The TG curve of specimen M1 presents that there was amount of weight loss in specimen when heat up to 800 °C by 8.379% total. DTG curve presents the peak at 55.16 °C, there is presumably resulted from the loss of bound and free water, which is observed of temperature from 50-150 ^{o}C. At 431.14 ^{o}C, there is presumably resulting from decomposition of $Ca(OH)_2$. The volume of samples continued to decrease when heating from 600 to 700°C, and received a peak heat at 643.11 °C. At this temperature the dehydration of the crystal begins to occur, which is a typical process of xonotlite before being completely transformed into wollastonite. This proves that the sample being analyzed is mainly xonotlite. The presence of not only xonotlite, but also of tobermorite in the sample M1 is shown by exothermal effect of crystallization of wollastonite with its maximum at 781.58 °C (Vakifahmetoglu, 2014).

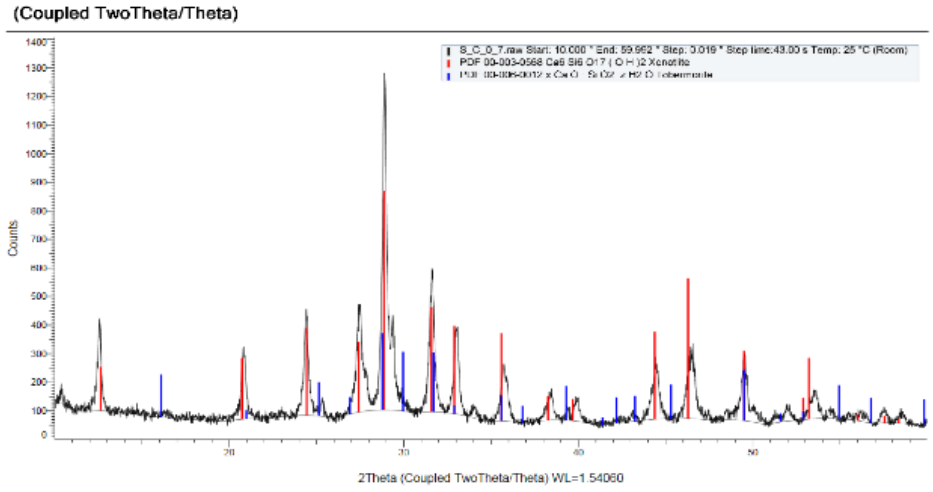

Figure 1. XRD pattern of sample M1 with molar ratio Ca/Si = 0.7

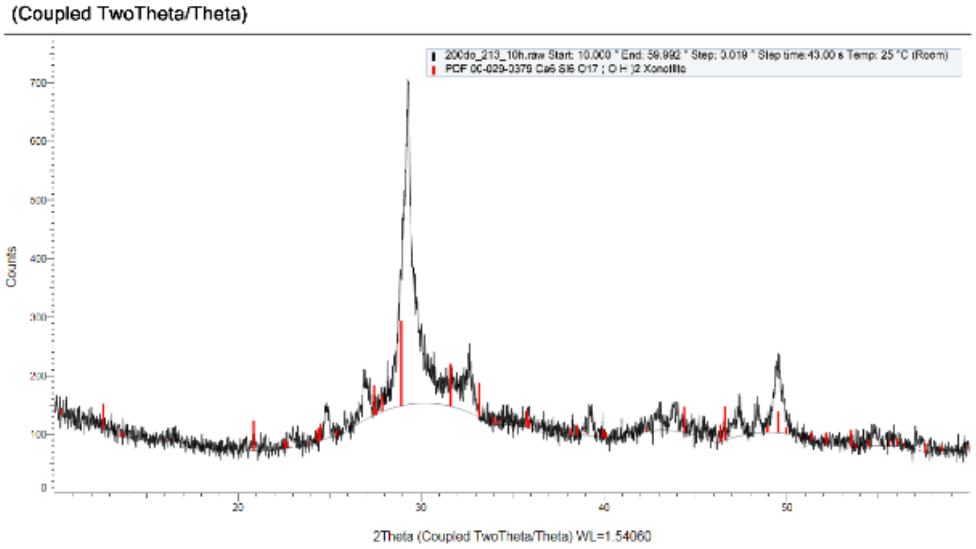

Figure 2. XRD pattern of sample M2 with molar ratio Ca/Si = 1.0

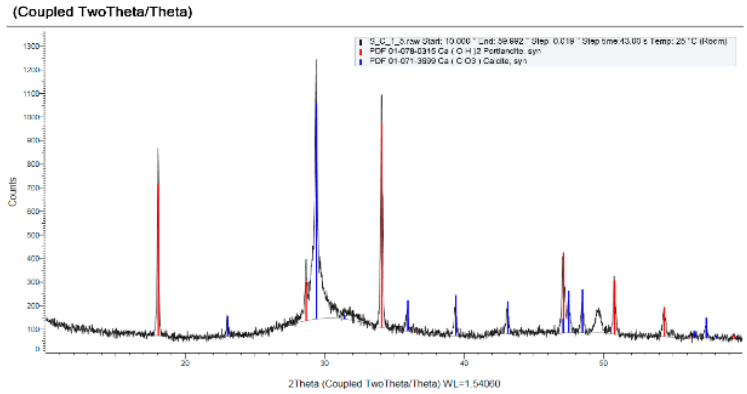

Figure 3. XRD pattern of sample M3 with molar ratio Ca/Si= 1.5

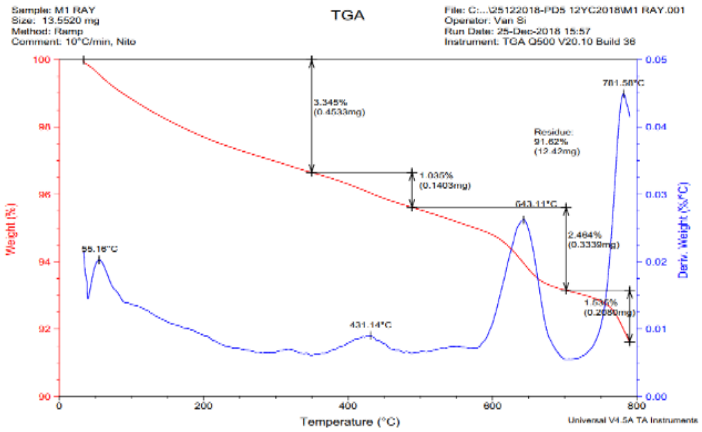

Figure 4. TGA analysis of sample M1

3.3 Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

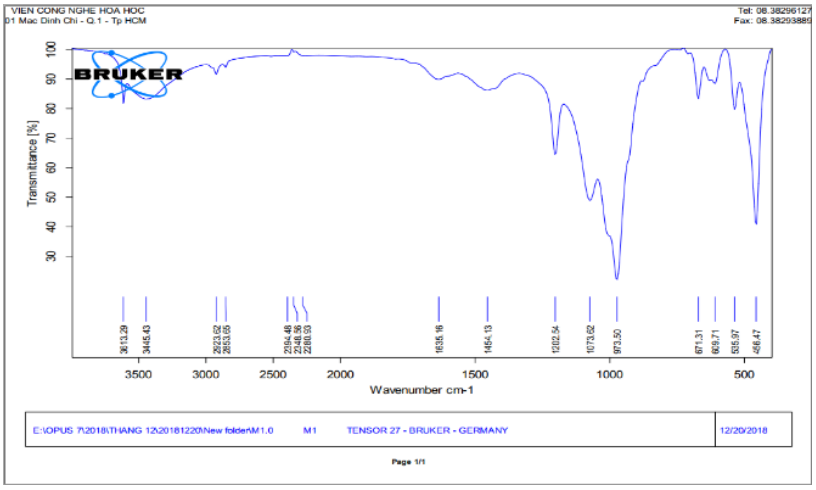

Figure 5. FT-IR analysis of sample M1

The result of infrared spectrum analysis of sample M1 is show in Figure 5. The occurrence of oscillating signals of OH- groups of adsorbed water at 3445.43 cm^{-1} and oscillation of water molecules adsorbed at the material surface at 1635.16 cm^{-1}. The results were compared with photos of infrared spectra of natural xonotlite, a sharp signal of oscillation OH- at 3613.29 cm^{-1} characteristic for OH- group adsorbed of natural xonotlite (a small amount) (Haastrup et al., 2014). The Si-O elongated oscillation bands are set at 973.5 cm^{-1}, with a shoulder at 1202.54 cm^{-1} (peak at 1202.54 cm^{-1} is the characteristic peak of the SiO_2 double chains in xonotlite (Shaw et al., 2000)). Si-O-Si bending oscillator is placed at 671.31 cm^{-1} strip. Carbonate impurities CO_3^{-2} in the natural spectrum 11 tobermorite is detected by a wide band at 1454.13 cm^{-1} (Chen et al., 2017). Infrared spectra of reactive samples in 10 hours have quite correlated results with the infrared spectrum of natural xonotlite. However, there are still indications of calcite impurities in the sample corresponding to the peaks in the tobermorite infrared spectrum in negligible amounts. Spectral results also showed that the OH group appeared on the surface of the material at the peak of 1635 cm^{-1}, but the peaks were not sharp, so it was impossible to conclude that the water-absorbing material was weak or strong.

3.4 Scanning Electron Microscopy – SEM

Samples M1 with molar ratio Ca/Si = 0.7 give the most uniform xonotlite fibers, the xonotlite fibers were clearly shaped. The phenomenon of spherical agglomeration remains but not much, this indicates tobermorite mineral exist in the sample. The fibers of crystal are long and some of them have a typical needle of the xonotlite mineral crystal. The crystals have a fairly uniform length from 5µm - 8µm.

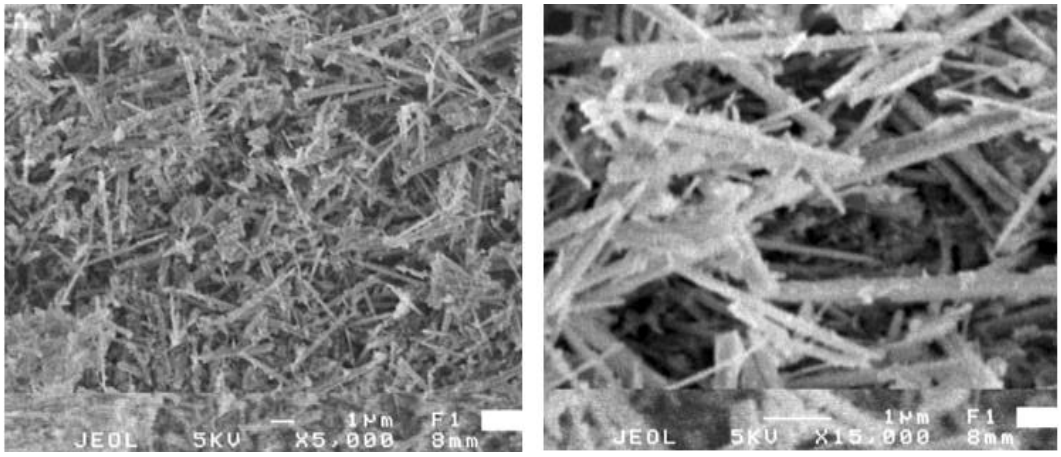

Figure 6. SEM image of calcium silicate in sample M1 with magnification of 5000 (left) and 15000 (right)

3.5 Transmission Electron Microscopic (TEM) Analysis

With the image of SEM scan, the morphology of difference types of CCSH are not clearly. The following TEM images show obviously the thickness and length of calcium silicate phases. In Fig. 7, there are two type of CCSH mineral. The thicker crystalline is presumably as tobermorite, and the needle-like rod is xonotlite mineral. Some agglomerated sphere can be CaO excessive. Following Fig. 8, the thickness of xonotlite are 10-15 nm and the thickness of tobermo`rite are 85-90 nm.

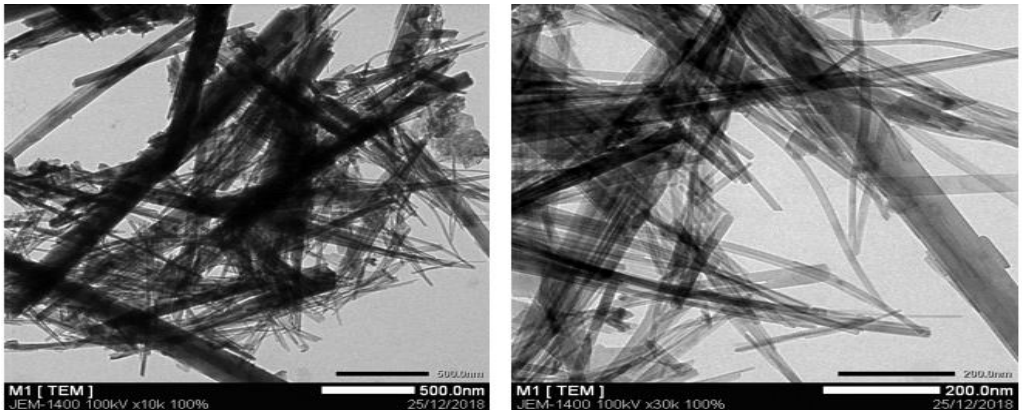

Figure 7. TEM images of calcium silicate in sample M1 with magnification of 10000 (left) and 30000 (right)

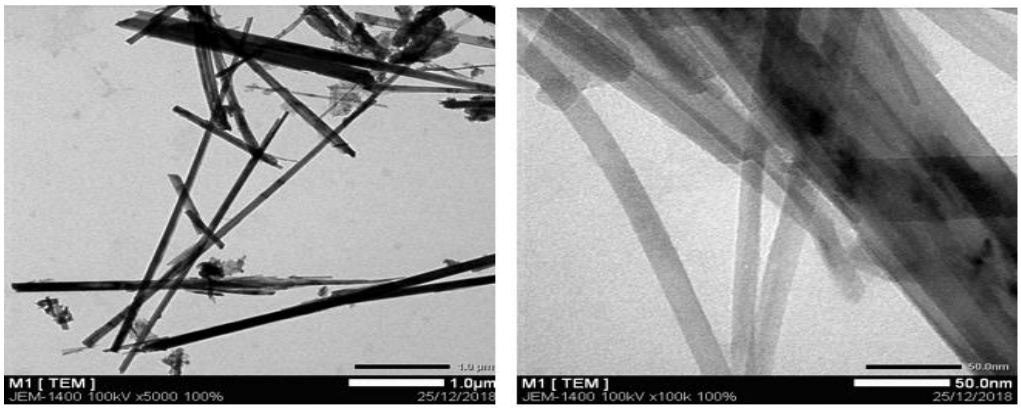

Figure 8. TEM images of calcium silicate in sample M1 with magnification of 5000 (left) and 100000 (right)

4. Conclusions

The nano xonotlite were successfully synthesized from treated rice husk ash and limestone by hydrothermal method at 200 °C with molar ratio Ca / Si = 0.7. The molar ratio of Ca/Si plays an important role in the formation of CCSH during hydrothermal reaction. When increasing this ratio, xonotlite and tobermorite mineral have decreased. In the best reaction condition (200 °C with molar ratio Ca / Si = 0.7, reaction time =10h) the thickness of xonotlite are 10-15 nm and the thickness of tobermorite are 85-90 nm.

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Technological Confidence of Higher Education Institutions (HEIs) Towards e-Learning

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This paper investigated how different individual- teachers and students, pertaining to their technological confidence in their ICT skills towards e-Learning readiness. The data were gathered from selected six Higher Educational Institutions in La Union, Philippines (n=1800) through an online survey questionnaire from August 2018 to October of 2018. Results revealed that the mean perception of teacher respondents (4.47) was significantly higher than that of the student respondents (4.21). Nevertheless, both respondents perceived that there was very high technological confidence of HEIs in the province as to their basic computer skills, internet/online skills and use of software applications. This implicated that teachers are working into a way of teaching students the easiest way through the integration of technologies and innovations towards teaching. However, the used variables could explain only little of the variance indicating that these predictors might not be the most relevant factors in the implementation of e-Learning in the province. Despite these challenges, Higher Educational Institutions in La Union are progressing beyond simple technology applications to leveraging and embedding various sources of online pedagogy, social, and collaborative Internet learning solutions for learning and educational purposes.

1. Introduction

Knowledge and expertise are key elements to success. To remain competitive and profitable, organizations thus require a workforce that is rapidly updated with new knowledge and skills, and one way of ensuring this is to use various e-learning solutions.

The concept of technology confidence can be based from the work of Bandura (1997) on the concept of self-efficiency. Thus, technology confidence refers both to the teachers' perceived likelihood of success on using ICT for educational purposes and on how far the students perceives success as being under the control of his or her teacher. According to the research of Keller (1987), which also based on the concept of Bandura (1997), confidence has different components: interest (refers to the attention factors in the environment; relevance (refers to goal-oriented activities); expectancy (refers to one's own expectation for learning).

The growth of technology addresses the gaps in teaching and learning. E-learning is the use of technology to enhance teaching and learning activities. Technology-based e-learning encompasses the use of the internet and other important technologies to produce materials for learning, teach learners, and also regulate courses in an organization (Fry, 2001).

According to Tao, et al., (2006), e-Learning as a new platform for learning, centered on electronic networks has allowed learners to receive individualized support and also to have learning schedules that is more suitable to them as well as separate from other learners. This facilitates a high interaction and collaboration level between instructors or teachers and peers than traditional environment for learning.

As information technology (IT) becomes more robust and easier to use, it increasingly permeates academic activities in higher education. The use of technology in education, commonly defined as e-learning, has become a standard component in many courses. Technology applications are not limited to the classroom. They are also replacing some classroom sessions with virtual sessions or fully replacing classroom courses with online courses. E-learning in academics which is characterized by the use of multimedia constructs will make the process of learning more interesting and enjoyable (Liaw and Huang, 2003).

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The use of educational technologies to improve services, quality, and opportunities for learning has been evolving steadily over the last three decades. E-Learning has grown remarkably in numbers of applications, diversity of technologies and variety of pedagogies. The development and integration of new instructional strategies and tools for learning have brought about fundamental changes in the way educators approach teaching and students approach learning.

In today's educational environments, teachers and students easily access to computers. To integrate technology into education, teachers' as well as students' acceptance and skills of using computers is particularly vital. Transforming the traditional educational practices through e-Learning seems very distant in many developing countries because educational institutions of higher learning are constrained by information and communication technology (ICT) infrastructure and general trends of inadequate structural support and incentives (Bhuasiri, et al., 2012).

To meet the needs of all stake holders, educational institutions endeavour to the advances in web-based learning (Lion, 2010). In 2012, the Commission on Higher Education (CHED) of the Philippine Government thru its CHED Memorandum Order No. 46, ordered the promotion and adoption of learner-centered learning. Higher Education Institutions in the Philippines were mandated to change the curriculum to Outcomes-Based Education. This is the essence of e-Learning. This initiative is promising but issues arise on the readiness of state universities and colleges in adopting such change. Thus, this paper aimed to determine the technological confidence of students and teachers' readiness towards e-Learning and test the significant difference of their responses.

2. Related Literatures

E-learning is a rich and multifaceted concept, as it is the endowment of education and training via the World Wide Web for students. Within these higher educations, virtual learning environments (VLE), learning management systems (LMS), web-based trainings (WBT) and other E-learning applications and educational technologies are accessible to all type of individuals. Agendas of schools and educational institutions have recognized e-Learning as having the prospect to transform people, knowledge, skills and performance.

The previous work of Sangra, et al. (2012) defined e-Learning as an approach to teaching and learning, representing all or part of the educational model applied, that is based on the use of electronic media and devices as tools for improving access to training, communication and interaction and that facilitates the adoption of new ways of understanding and developing learning. Moreover, Alonso, et al. (2005) stressed that e-Learning as the use of new multimedia technologies and the Internet to improve the quality of learning by facilitating access to resources and services, as well as remote exchange and collaboration can also its definition.

The utilization of e-learning, organizational learning, and knowledge management leads to the improved organizational success in the growing knowledge economy. The appropriateness of e-learning, organizational learning, and knowledge management is influential for the learning organizations to serve practitioners and researchers, increase business performance, sustain competitiveness, and fulfil expected accomplishment in the learning organizations (Kasemsap, 2016).

Ala-Mutka (2011) believed that technologies are increasingly being used in society and the economy, and this is transforming ways of working, studying, communicating, accessing information, or spending leisure time, among others. Internet and especially social technologies are used for various purposes by different groups of citizens and are also being appropriated for new social activities. Through online spaces, citizens can access resources, follow, interact, and create and share with other people globally. People from all age groups are participating in different types of online networked activities, which can support work, learning and citizenship.

Thus, the author of this paper believed that students as well as teachers' technological confidence are factors to be considered in the success towards e-Learning implementation in Higher Education Institutions in La Union.

3. Methodology

This study made use of the descriptive design method of research to describe the perception of the respondents in terms of HEIs e-learning technological confidence readiness. This design is considered appropriate in this study because it enabled the researchers to gather information that could achieve on the objectives and goals of the study through the standardized collection procedures based on highly structured research instruments and well-defined study concepts and related variables. This assessment was achieved by the use of an online survey-questionnaire. To avoid bias information obtained from the respondents, the researchers equally divided the respondents' specialization from IT and non-IT for both teachers and students. The study covered six (6) HEIs in the province of La Union, Philippines namely: Don Mariano Marcos Memorial State University (DMMMSU) - North La Union Campus, DMMMSU - Mid La Union Campus, DMMMSU - South La Union Campus, Saint Louis College, Lorma Colleges and Union Christian College from August 2018 to October 2018.

The said HEIs were chosen because of the number of students enrolled during the first semester of school year 2018-2019. Table 1 shows the distribution of respondents in the study in the different HEIs. There are two groups of respondents- Teachers and Students

Table 1: Distribution of respondents

HEI	Faculty	Student	Total
Don Mariano Marcos Memorial State University – North La Union Campus	150	150	300
Don Mariano Marcos Memorial State University – Mid La Union Campus	150	150	300
Don Mariano Marcos Memorial State University – South La Union Campus	150	150	300
Saint Louis College	150	150	300
Lorma Colleges	150	150	300
Union Christian College	150	150	300
TOTAL	900	900	1800

The scale includes 12 items distributed among three subscales. Each subscale corresponds to a five-point Likert-type response format. The responses or perceptions of the respondents were quantified using the descriptive terms and range of values in Table 2.

Table 2: Descriptive terms and range values used

Statistical Range	Mean Range	Descriptive Rating
5	4.20 – 5.00	Very Highly Confident (VHC)
4	3.40 – 4.19	Highly Confident (HC)
3	2.60 – 3.39	Moderately Confident (MC)
2	1.80 – 2.59	Slightly Confident (SC)
1	1.00 – 1.79	Not Confident (NC)

4. Results and Discussion

Respondents' knowledge of ICT tools and their perceptions of how these tools promote their learning are crucial for determining digital technology's added value in higher education settings. The study of Venkatesh and Rabah (2014) revealed that the Internet, email, and productivity tools are the most commonly used ICT tools in higher education settings.

Table 3 shows the perceived responses from the respondents on the level of technological confidence as to their basic computer skills. The teachers are very highly confident on their basic computer skills as evidence by a high mean score in all criterion. As gleaned in the table, teacher-respondents has a mean score lower than the students but still described as Very Highly Confident is the criterion "I know how to save/open documents from specific source". Higher confidence of the students from teachers can be attributed by their age gap. Jou (2012) found that respondents under the age of 30 had a more positive view than those over the age of 30. Similarly, the students rated only two criterions as very highly confident except on resolving hardware and software problems. Generally, the teachers are very highly confident whereas the students are highly confident in their basic computer skills. Not surprisingly, the study of Li and Lee (2016) found out that respondents who spent more time on the computer reported better computer skills. This association may relate to familiarity with computers and the applicability of computer facilities.

Table 3: Level of technological confidence as to basic computer skills

Criterion	Teachers		Students	
	Mean	Descriptive Rating	Mean	Descriptive Rating
I know the basic functions of computer hardware components	4.65	VHC	4.23	VHC
I know how to save/open documents from specific source	4.24	VHC	4.66	VHC
I know how to resolve common hardware or software problems	4.32	VHC	3.43	HC
Weighted Mean	4.40	VHC	4.10	HC

Table 4 reveals the result on the internet/online skills surveyed to the respondents. As seen in the table, all the criterion for internet/online skills are rated by the teacher-respondents as very highly confident. The result can be attributed to the availability of internet connection among the different HEIs. On the other hand, the student-respondents are very highly confident in their internet/online skills except in using asynchronous tools, accessing online library and familiarity with online etiquette. Accordingly, both teachers and students are very highly confident in their internet/online skills. Similarly, previous studies of Fajardo et al., (2016) and Hahnel et al., 2016 emphasized that colleges, universities, and other institutions of higher learning tries to advance online course capability in a speedily developing cyber education market. e-learning has been more and more important in institutions of higher education. The development of a range of e-Learning tools has been commencing several modifications in the academe. Internet and online skills of students, teachers and administrators are important mainly when it comes to learning delivery and support processes.

Table 4: Level of technological confidence as to internet/online skills

Criterion	Teachers		Students	
	Mean	Descriptive Rating	Mean	Descriptive Rating
I know how to navigate the web	4.24	VHC	4.66	VHC
I am familiar with online etiquette	4.71	VHC	4.01	HC
I can use web browsers confidently	4.24	VHC	4.67	VHC
I am comfortable with doing searches, setting bookmarks, and downloading files	4.79	VHC	4.49	VHC
I know how to access an online library and other resource database	4.43	VHC	3.94	HC
I know how to use asynchronous tools effectively	4.24	VHC	3.61	HC
Weighted Mean	4.44	VHC	4.23	VHC

It can be gleaned from Table 5 the level of technological confidence as to software application skills of the respondents. The teachers are very highly confident in their software application skills as revealed by their high mean ratings in all the criterion as compared with the student ratings whose response in navigating multiple opened applications is highly confident. Nevertheless, both respondents agree that they are very highly confident in their software application skills. Moreover, the study of Ouma, (2013) revealed that one of the reasons for failures in e-Learning implementation is users' poor technical and software application skills, and the possession of these skills are one of the technological aspects of e-learning. This signifies that the successful implementation of e-Learning relies on the assessment of readiness of the technological aspects in order to realize the benefits of e-Learning and reduce the barriers to e-Learning implementation.

Table 5: Level of technological confidence as to software applications

Criterion	Teachers		Students	
	Mean	Descriptive Rating	Mean	Descriptive Rating
I know what PDF files are and I can download and view them	4.82	VHC	4.44	VHC
I am comfortable with office applications (word processing, spreadsheet, etc.) and use it comfortably	4.73	VHC	4.26	VHC
I am able to have several applications opened at the same time and move between them	4.24	VHC	4.19	HC
Weighted Mean	4.60	VHC	4.29	VHC

Table 6 shows the summary of the level of technological confidence which was measured through the respondent's extent of use and capabilities in Basic Computer Skills, Internet/Online Skills and Software Application Skills. As gleaned in the table, Software Application skills got the highest mean from Teachers (4.60) and Students at 4.29 both interpreted as Very Highly Confident. The high rating signifies that the respondents are technically confident of their application skills such as downloading and viewing of PDF files from the web,

comfortable with the use of MS office applications, and can simultaneously open and move several applications at the same time. However, basic computer skills got the lowest mean of 4.40 and 4.10 from the teachers and student respondents respectively. Despite that they are the lowest, still they are interpreted as Highly Confident and Very Highly Confident. The result revealed that respondents are skilled and knowledgeable in the basic functions of computer component including saving/opening documents to/from a hard disk or other removable storage device and skilled to resolve common hardware or software problems and can access a technical support in case a problem is encountered. The level of technological confidence of result revealed that both the teachers and students were rated as Very Highly Confident in all the key area indicators implying that the six Higher Education Institutions in La Union are ready for e-Learning. The changes brought about with effective use of technology will lead to new concepts and potential to improve higher education teaching and learning process.

Table 6: Summary on the level of technological confidence

Criterion	Teachers		Students	
	Mean	Descriptive Rating	Mean	Descriptive Rating
Basic computer skills	4.40	VHC	4.10	HC
Internet/online skills	4.44	VHC	4.23	VHC
Software application skills	4.60	VHC	4.29	VHC
Weighted Mean	4.47	VHC	4.21	VHC

Table 7 presents the t-test using two sample assuming equal variances with *alpha* =.05. Result shows that the computed t-value of 4.59 is higher than the critical t-value of 1.65 (one-tail) which implies that the mean perception of teacher respondents was significantly higher than that of the student respondents (p=0.0000032) Nevertheless, both respondents perceived that there was very high technological confidence of HEIs in the province as to their basic computer skills, internet/online skills and use of software applications. Technological confidence of both teachers and the learners is one of the important factors in the success of an e-learning system because e-Learning, by definition, relies on the teacher and learner's skills and access to technology. Consequently, technological readiness can be seen as those factors that must be accomplished before e-learning can be implemented such as the available hardware and software, whereby the successful implementation of e-learning relies on a high level of ICT infrastructure readiness and users' technical skill readiness (Ouma ,2013).

Table 7: t-Test: Two sample assuming equal variances

	Teachers	Students
Mean	4.47	4.21
Variance	0.15	0.32
Observations	900	900
Pooled Variance	0.23	
Hypothesized Mean Difference	0	
Df	1798	
t Stat	4.59	
P(T<=t) one-tail	3.27	
t Critical one-tail	1.65	
P(T<=t) two-tail	6.55	
t Critical two-tail	1.97	

5. Conclusions

This study highlighted the significant relationship between teachers and students' e-Learning skills. Teachers are more likely to be more technologically confident than those of the students. To integrate technology into education, teachers' as well as students' acceptance and skills of using computers is particularly vital. So, it would definitely be fascinating to integrate e-learning as a standard device in the instruction of university students.

Understanding the perceptions of students and teachers concerning the usefulness of technology use and their proficiency and knowledge of specific types of ICT tools are both influential and critical to the success or failure of integration of ICT in higher education settings.

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Simulation System of Butterfly Valves to Mix Ethanol and Gasoline to Produce Gasohol using CFD SOLIDWORKS Software

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Production of biofuel such as gasohol and biodiesel require mixing and reacting equipment which should have high efficiency, ease of installation, low capital and operation cost. Using cavitation phenomena is one of means to achieve those. Butterfly valves which are cheap, available, wide operational range were chosen for the above purposes. The turbulent flow and cavitation after butterfly valves were investigated using SOLIDWORKS CFD software. The mixing performance of ethanol and gasoline streams to make gasohol with given concentration was the target of this simulation. The output constrains were deviation in volume fraction, head pressure and size of the system. The valve actuator's angle, distance from ethanol intake pipe, the gap between valves and valves' relative position were chosen as variables. The simulation results proposed optimized configuration of two butterfly valve for mixing gasoline and ethanol in 4" pipe system.

1. Introduction

Nowadays, a majority of energy from fossil fuel is experienced an increasing deplete due to the growing demand of these fuels. The renewable biofuels such as biodiesel and gasohol become a cure for this problem. Biodiesel production has been booming (Roslindawati et al., 2017). The enhanced oxygen content biodiesel will attain greener emission in future (Phoon et al., 2017). The gasohol has also been broadly applied for better sustainability and greener emission. Mixing ethanol and gasoline is one of key factors in producing gasohol. There are many kinds of mixer like agitator, circulation pump, static mixer and noble gas stripping, in which inline mixer shows much advantage because of simple structure, reliability of homogeneous concentration and low power consumption. Inline mixer can use static mixer pipe, venturi pipe, and orifice plate. Using orifice plate for this purpose shown good performance in both equipment and power consumption aspect (Ung et al., 2017). In this simulation research, the system of two butterfly valves was used for mixing ethanol and gasoline. The availability and low cost of butterfly valves promise high performance of this mixing system in producing gasohol due to low installation cost, low power consumption, free of space and high mixing efficiency. Firstly, construction and installation expenses depend on number of valves, length of pipes and consumed power of pump resulting in reducing the cost by using as fewer valves as possible; moreover, shortened pipes are better for lower cost of construction, operation and maintenance; costs of pump depending on the amount of required inlet hydraulic pressure. Secondly, operation expenses include the pump power and maintenance. As the lower the installation cost, the higher the operation cost because proper pumping section for better efficiency and higher required inlet pressure. Therefore, balancing the two costs is one of the goals in this research. Thirdly, the size of the equipment including pump, valves and pipes, are also essential for limited-space blending stations. As a result, the size is considered to be a criterion of design. Lastly, mixing efficiency are the most crucial criteria. The concentration of ethanol in gasohol in volume fraction must meet the specification after optimizing these previous costs to ensure the quality of gasohol.

2. Research methodology

The research was conducted in 2 phases: firstly, designing butterfly valve mixing system then secondly, fluid flow simulation in SOLIDWORKS. The valve design is broken into many parts, including flange, body, upper lever, lower lever, upper stem, lower stem, disc, rubber, opener-closer, lids, pipes, for sizing and calculating purposes, then, the parts are drawn separately with precision. The designed parts are assembled to be a complete butterfly valve by mates for joining the parts. Fluid flow simulation of the assemble: The assemble is added the flow simulation package with desired conditions. The boundary conditions contain inlets volume flow of gasoline and ethanol, and outlet pressure which is atmospheric pressure. Analysing results and calculating for storage: results are tracking for pressure and concentration of gasoline through pipes' length, then the information is operated to create figures for research. Therefore, the process is optimized by different variables and storage is designed by regulations.

The model was chosen among turbulence models such as Reynolds-averaged Navier–Stokes (RANS), Large eddy simulation (LES), Detached eddy simulations (DES) which are most popular used models though there are a few more method which are Direct numerical simulation, Coherent vortex simulation, Probability density function (PDF) methods, Vortex method, Vorticity confinement method and Linear eddy model (Lumley, 1978). Some models were tried as first then Reynolds-Averaged Navier-Stokes was used in this research.

There are many discretization methods like the finite volume method (FVM), the finite element method (FEM), the finite difference method (FDM), the boundary element method and the spectral element method are common approach used in CFD codes (Randall, 2002; Randall, 2007). The finite volume method was chosen based on simulation performance. High-resolution schemes were used because shocks or discontinuities were present in fluid flow after butterfly valves.

3. Results and discussion

3.1. Variables

There are many variables to be considered for optimizing that affect the goals: Disc's rotation angle, Ethanol inlet distance to valve, Volume fraction of ethanol, Velocity of fluid flow, Gap between two valves, Position angle of the valve to each other and to the ethanol pipe. Matrices of sequences relating to these variables and goals has been made. There are two outputs, which are the main concerns, need to be analysed: Volume fraction and pressure. Volume fraction deviation charts show the pace of case study to converge to a desired state, this is the most important value because it is the targeted efficiency of the system. Then, pressure graphs display the inlet required pressure and pressure drop through valves, this determines the installation and operation costs for pump system. In addition, sizing the system, which is mostly the length of pipe, is also essential due to the conservation in space. Starting by a normal fluid flow of velocity of 2.5 m/s for gasoline in Pipe-3 ½ of which OD is 4 in and velocity of ethanol depends on the chosen Ethanol/gasoline ratio of 20.

3.2. Results of one valve system

The advantages of a one-valve blending system are economical due to the low in installing cost and small required area for placement. However, the head pressure may be higher than that of two-valve system, moreover, the efficiency of blending may be lower. The typical pattern of flow pressure is shown in Figure 1. The inlet pipe withstands the highest pressure of over 150000 Pa for instance. The first drop is due to the charged ethanol, then, through valve, the second drop which is a vacuum pressure right after the valve is more significant due to a free of bottleneck. Figure 1b shows the pattern of volume fraction trend line by pipe length.

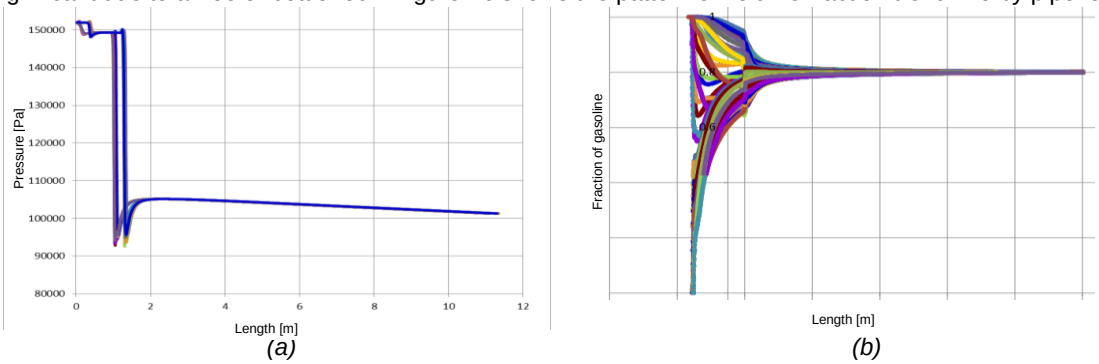
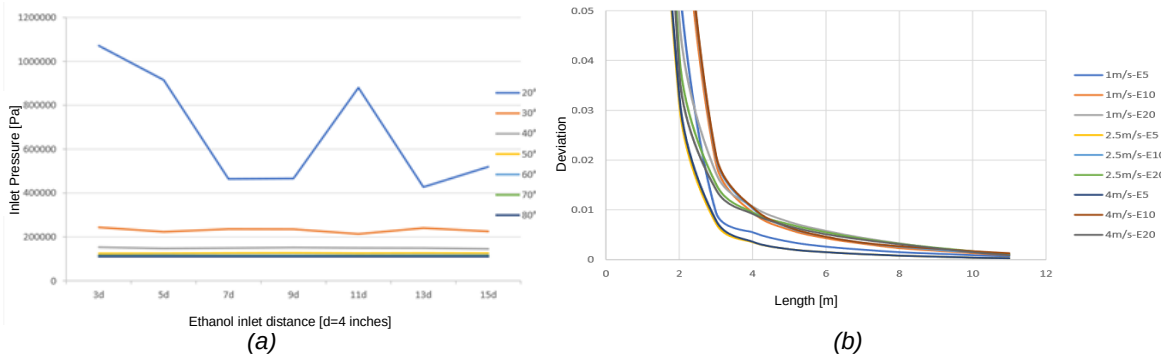


Figure 1: (a) 9d90º pressure drop by 20 trajectories; (b) Patterns of one-valve system typical volume fraction trend line

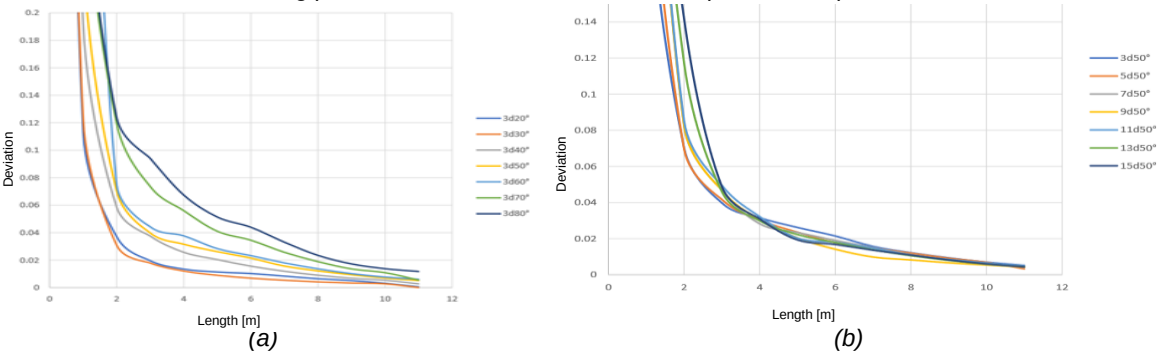


(a) *(b)*

Figure 2: (a) Effects of valve's angle and inlet distance to E number; (b) Deviation of volume fraction with various velocity and pressure

In Figure 2a, the changes of inlet pressure are manipulated by various pairs of valve's open degree and inlet gap. With 20° open, the flows are unstable, result in different value of pressure with no specific rules. However, when the valve is open to 30° and higher, flows are more stable and inlet pressure value remains approximately the same despite inlet distance. From 40° and above, the pressure value stays at roughly 150000 Pa. In summary, required inlet pressure considerably depends on open degrees, the lower the angle, the lower the inlet pressure value. However, inlet distance is not a significant variable as there is little deviation in pressure of the inlet pipe. Therefore, further studies will leave the inlet distance out of concern (fixed at 15 d), remaining only the valve angle to be considered.

Figure 2b shows that all the combination of velocity and E-ratio meets a desired deviation of 0.01 at 4 m. As shown on the line chart, the faster the flow, the lower the deviation due to the high load of particles. However, differences in ethanol content have little influence on the volume fraction. Therefore, balancing the velocity could be more efficient in the mixing process due to the restriction of inlet pressure required.



(a) *(b)*

Figure 3: (a) Deviation of volume fraction of gasoline for an inlet distance of 3d; (b) Volume fraction with actuator of 50° and different inlet distances

In Figure 3a, taking a fixed inlet distance of 3d, various cases with open degrees are simulated. Throughout 11 m of pipe length, the deviation getting closer to 0, however, different open could change the pace of the convergence. The higher of open angle results in the faster convergence to the desired volume fraction, which decreases the deviation of approximately 0. Therefore, small open such as 20° and 30° could meet a deviation of 0.02 at about 3 m long, while that of another open is dramatically higher, over 8 m for an openness of 80° to converge to 0.02 for instance. The Figure 3b shows the convergence of volume fraction through the pipe's length, researched by different inlet distances with a constant open of 50°. Overall, the trend line is similar to the previous graph, however, not much difference in each line in comparison. All the lines meet at 0.03 of deviation values at 4 m of pipe length and converged at 11 m with an approximately same value of the deviation.

3.3. Results for two valves system

In Figure 4a, the first valve actuator is controlled as a constant while that of the second one is differed. As a result, the volume fraction eventually converges to the desired value, however, there are various trends due to a variety of valve angle combinations. The deviation of a 50°x40° has the quickest convergence rate, for instance, while that of the 50°x70° is slower. Moreover, to reach a 0.002 deviation of volume fraction, the 40°

meets the desired value at 6 m, followed by 7 m, 8 m and over 9 m of 50°, 60° and 70° of the second valve angle respectively. In Figure 4b, changing the first valve open angle experience the same deal with that of the first one. A 40°x50° combo will meet a deviation of 0.002 at nearly 6 m length, following by 50°x50°, 60°x50° and 70°x50° in order of increasing in length.

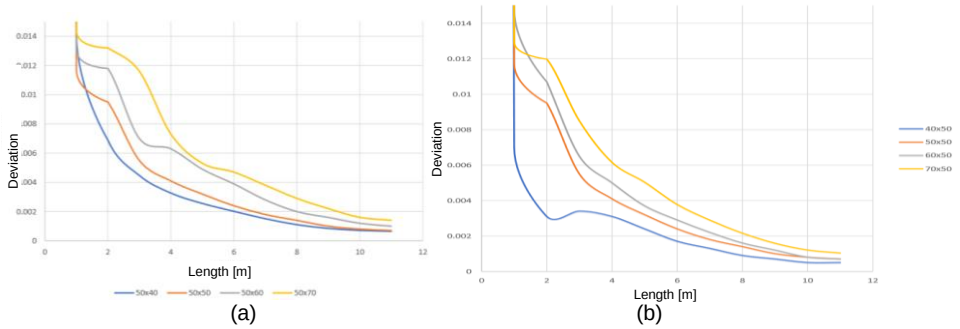


Figure 4: (a) Volume fraction deviation by changes of actuator in the second valve; (b) Volume fraction deviation with the changes of actuator in the first valve

In Figure 5a, inlet diameter is fixed at 15d, leaving the actuators of two valves are variables. As shown in the chart, inlet pressure gradually decreased when valves are slowly opened. For example, with 40°-actuator of the first valve, increasing the second valve degree will lower the pressure value, from about 190000 Pa for the 40°x40° case to over 150000 Pa for the 40°x70°. The same pattern happens when fixing the second valve actuator, for instance, from approximately 190000 Pa of 40°x40° to just over 150000 Pa of 70°x40°. Due to lower head pressure, a 50°x50° angle pair is chosen for further studies.

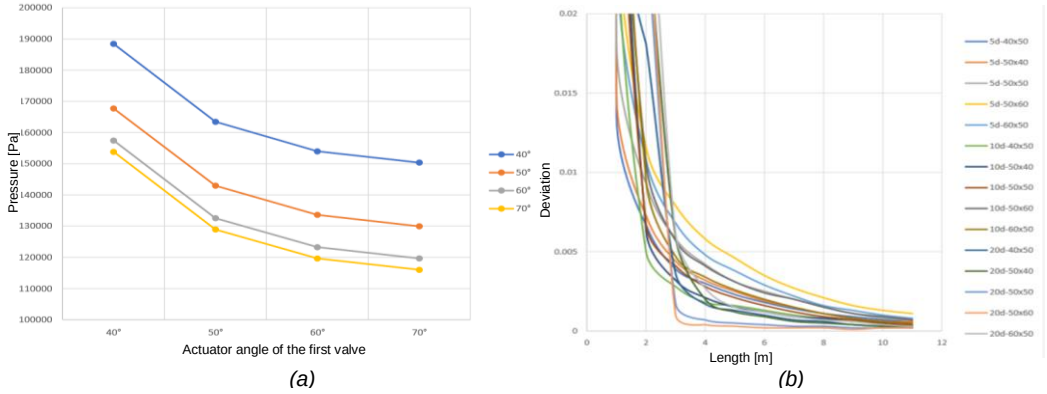
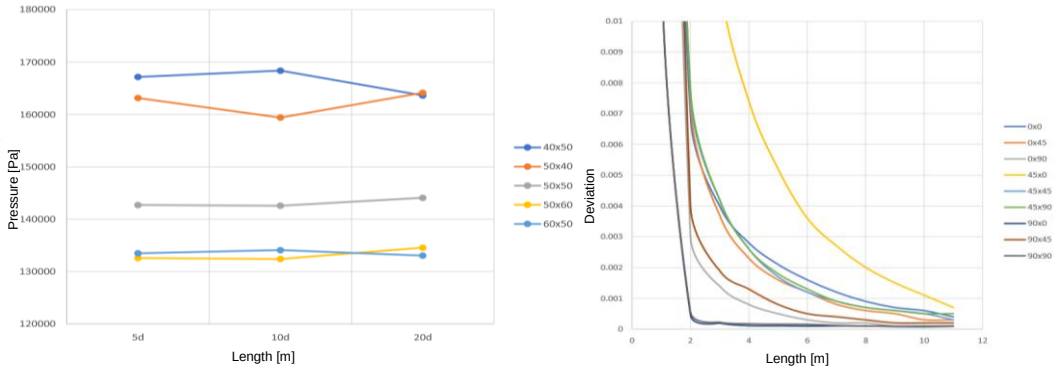


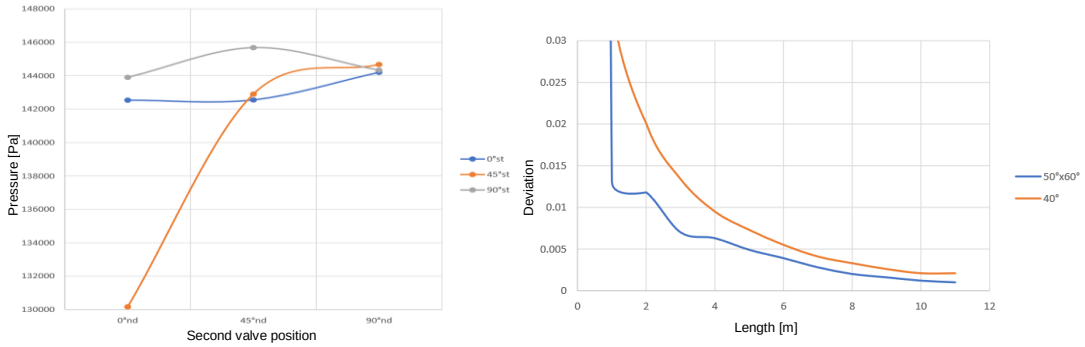
Figure 5: (a) Pressure affected by different actuators' angles of two valves; (b) Volume fraction affected by gaps and actuators' angles

Figure 5b shows that two impressive line are 20d-50x60 (first valve 50° and second valve 60°) and 20d50x50. As a result, the longer the gap could improve the efficiency for convergence, however, the system will take more space to install. Therefore, the gap of 20 d and a 50x60 valve combination is chosen for further experiments.
The Figure 6a shows that although experiencing fluctuation, there is no significant change in terms of pressure with this study. The highest pressure gap is about 170000 Pa, which belongs to the 40x50 of the gap 10d. In summary, length could affect the volume fraction, but not for pressure. Rotating valves' position can give better result in mixing efficiency. While the 90°x0° could meet a deviation of 0.001 at only above 3 m, a combination of 90°x90° could lower that value to even 1E-5. However, the 45°x0° is not as effective as the others as shown in Figure 6b. Although there are significant changes in volume fraction convergence, the pressure seems stable when valves are rotated. In Figure 7a, the biggest gap belongs to the 45°st with about 130 kPa for a 0°nd to 145 kPa when the second valve turn to 90°. The volume fraction of the 90° first valve is much better than others, while the pressure is higher, which is up to over 145 kPa, however, not too considerable. Therefore, my decision for the optimal position is 90°x0° as it has the most impressive efficiency. Figure 7b shows that the two-valve system surpass the one-valve in blending efficiency by reaching 0.005 deviation of approximately 5 m, while that of one-valve is at nearly 7 m.



(a) *(b)*

Figure 6: (a) Different gaps and valves' angle effect on fraction pressure; (b) Influence of valves positions to volume deviation

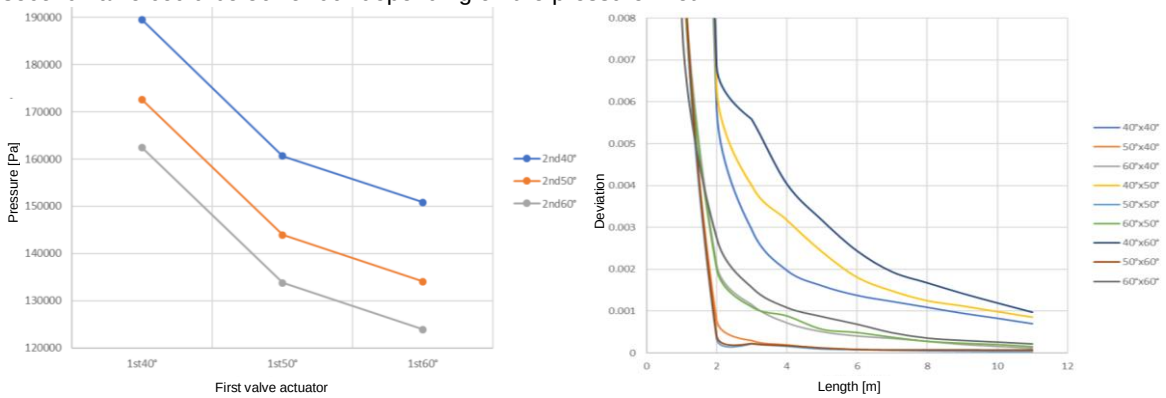


(a) *(b)*

Figure 7: (a) Valve positioning affects head pressure's value; (b) Volume fraction's comparisons of two valves 50°x60° and one valve 40°

3.4. Optimization

The pressure influenced by various actuators of two valves is as same pattern as previous results shown in Figure 8a. While in Figure 8b, various combos of actuators in both valves are conducted. Two outstanding combinations are 50°x50° and 50°x60°, which have nearly the same line trend. The two line could reach critically low deviation, followed by the 50°x40°. It seems that the first valve with 50° is an optimum variable, while the second valve could be 50° or 60° depending on the pressure inlet.

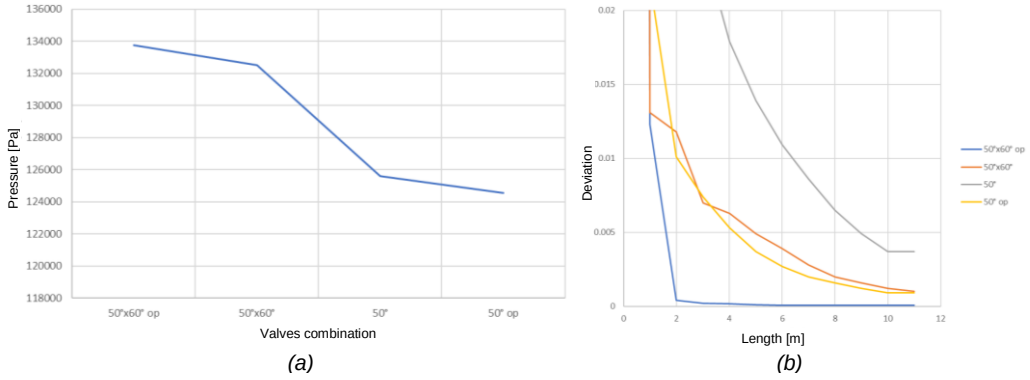


(a) *(b)*

Figure 8: (a) Pressure influenced by various actuators; (b) Volume fraction deviation by different valves of two valves angle

Figure 9a shows the comparison of pressure drop by 4 cases: the optimized 50°x60°, the normal 50°x60°, a one valve 50° and one valve optimized 50°. The pressure declines from left to right, however, not a much in

value. In terms of deviation as shown in Figure 9b, a one valve 50° is not much to be considered, while with an optimum position of valve, the one-valve could reach the deviation of a two valves combination. Finally, the ultimate combination is 50°x60° which could reach a desirable depth of deviation, 1E-5.



(a) *(b)*

Figure 9: (a) Comparison of inlet pressure between one and two valves; optimized and normal positions; (b) Comparison of volume fraction deviation between one and two valves; optimized and normal position

4. Conclusions

The ultimate goals are higher blending efficiency, lower the head pressure and reducing in length of the system. By initial study, there are six factors affecting the goals which are a distance from inlet ethanol pipe to valve, open angle of the disc, the gap between valves, E number, valve positioning and intake velocity. In the relations between inlet distance and actuator angle, pressure is critically depended on the angle of the disc, the lower the angle, the higher the pressure. Regarding volume fraction concentration, the value is still heavily influenced by the disc's angle, the lower the angle, the faster the convergence. In contrast, distance from inlet ethanol to the valve is not as effective. To conclude, actuator angle has great impact on goals, but inlet distance is not. For the influence of E number and gasoline velocity on mixing system, the results show that the higher the velocity, the higher the pressure and the same matter for E number, however, the increasing pressure is not considerable. With two-valve model, after fixing the value of the velocity, inlet distance and E number, the remaining variables are two valves position, gap and open degree. The required pressure values should be acceptable in all cases (lower than 200 kPa), therefore, the 50x50 pairs are as effective as other lower-degree pair, additionally, its pressure is just above 140 kPa, thus, it was chosen. Furthermore, at 10d gap between two valves, the deviation converges faster than that of the others. Therefore, the 10d was chosen as a fixed gap. One more reason is that the size of the system should not be too large to be restricted in placement. The final study is on valves position and there are two pairs of position are impressively optimum in volume fraction is the 90x90 and the 90x0. Regarding these two, the 90x0 has lower head pressure, so it becomes as an optimized position. Finally, the simulation shows that the 50x60 has nearly the same pattern as that of 50x50 but with a lower inlet pressure.

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Preliminary study of carbon nanotubes formation from methane over stainless steel in chemical vapor deposition system

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This work reported the formation of carbon nanotubes from methane gas, over commercial stainless steel catalyst in chemical vapor deposition system. Morphology of as-prepared carbon at various reaction temperatures was observed by SEM images. Besides, SEM-EDX analysis presented amount of carbon increases with increase of reaction temperature: very few of carbon granules were recorded at 750 ^{0}C, but ~30 at.% (atomic percent) of carbon was detected at 950 ^{0}C (the granular diameter is less than 250 nm). Interestingly, carbon nanotubes with a diameter of 20 – 40 nm and a length of 200 – 400 nm were observed at 850 ^{0}C evidencing the reaction temperature controls the carbon morphology from the initial seeds. The atomic percentages of O and Al were unchanged (in the range of 17 – 28 at.% and 13 at.%) while atomic amounts of Cr and Fe decreased with increasing reaction temperature. However, molar ratios of Cr/Fe kept constant at ~0.3 at different reaction temperatures probably associated to the growth of carbon on the Cr-Fe active site.

Keywords: *Carbon nanotubes, CVD system, methane, stainless steel catalyst*

1. Introduction

The availability of shale gas on the market has progressed potential application of methane gas (CH_4) in chemical and manufacturing sectors. Many recent studies in utilization of CH_4 in dry (Margossian et al. 2017) and steam reforming (Angeli et al. 2014) to produce clean H_2, an attractive alternative to support energy consumption without environmental impact. Besides carbon-rich sources to prepare carbon nanotubes (CNTs), like carbon monoxide, ethanol, toluene, benzene and ethylene, others of acetylene and CH_4 are commonly important carbon supplies. Chemical vapor deposition (CVD) system with an assistance of heterogeneous catalysts (CCVD) has been considered as an current standard technique to synthesized CNTs at low temperature. Hydrolysis of acetylene (Liu et al. 2004) and ethylene forward CNTs (Plata et al. 2010) over metal catalysts in a CVD system were usually carried out at about 600–950 ^{0}C and 650–850 ^{0}C, respectively. Considering on thermo-dynamic area, ΔG of decomposition of acetylene and ethylene are already negative at 200 ^{0}C while that of CH4 is favor at above 600 ^{0}C (Tessonnier and Su 2011). The stable structure of CH_4 should be concerned in fabricate CNTs in CCVD system. Catalysts for CNTs development in CVD system were reported as metal active cites, for instance, Ni, Co, Mo, Fe and alloys supported on Al_2O_3, SiO_2, MgO, etc., (Wen et al. 2007). It was reported that the crystallinity of CNTs from decomposition of C_2H_2 at 950 ^{0}C follows a sequence of Fe/SiO_2 > Ni/ SiO_2 > Co/ SiO_2 (Lee et al. 2002) while that process run well at 800 ^{0}C over Fe-Mo/MgO catalyst (Liu et al. 2004). Fe-Mo alloy supported on Al_2O_3 was also reported an excellent performance

in preparation of CNTs from CH_4 at 900 ^{0}C (Li et al. 2001). This revealed that Fe metal is an vital site to produce CNTs from hydrolysis of hydrocarbon. Moreover, catalysts for fabrication of CNTs with a CVD system were prepared as complicated processes. Many works reported the ability of CNT growth from acetylene (Camilli et al. 2011; Pakdee et al. 2017) or from plastic post-consumer wastes (Panahi et al. 2019) over stainless steel (SS) substrate in CDV at 700 – 850 ^{0}C. However, studying the growth of CNTs on SS catalyst from stable structure of methane has not been reported. This work will attempt to synthesize carbon nanotubes from CH_4 in the aid of commercial stainless steel substrate in CVD system. The preliminary experiment will study the impact of reaction temperature on morphologies of carbon formation. Scanning electron microscope (SEM) will be used to observe the formation of CNTs, as well as to analyze percentage of carbon on surface of SS substrate via SEM-EDX technique.

2. Material and Methods

2.1 Material

Pure CH_4 (99.999%) and Ar (99.999%) considered as carbon source and carrier gas, respectively were supplied from Air Liquide Vietnam.

FeCrAl alloy substrate (obtained from Goodfellow, Oakdale, PA, USA) (Oye et al. 2010) was prepared with a square of 5.0 cm x 5.0 cm, and 0.5 mm of thickness for studying the formation of carbon in a CVD system. The substrate was ultrasonicated in acetone for 15 min, then in isopropanol for 15 min, and finally blown dry with nitrogen for ready as catalyst in this study. The pre-treated FeCrAl sample was noted as stainless steel (SS) substrate.

2.2 Synthesis of CNTs in CVD system

A thermal CVD apparatus (CNCVD-200TH, ULVAC) was used to synthesize carbon nanotubes. In the CVD apparatus, only SS substrate was heated from room temperature (RT) to desired reaction temperature at a fast rate (100 ^{0}C/min). Vacuum pump was operated during heating and reaction to ensure low pressure in the reactor. Argon flow was conducted in CVD system during heating up to prepare a clean vacuum medium. At a desired reaction temperature, a mixture of 10 vol.% of methane in argon as a feeding flow was conducted to CVD system (at 1000 mL/min of Ar flow) and kept there for 60 minutes. After that, switching off reactant flow and turning on argon valve to cool reactor down to RT will be run. The SS substrate after reaction was collected and noted as C-750, C-850 and C-950 for carbon formation at reaction temperature at 750, 850 and 950 ^{0}C, respectively.

The morphology and element analysis of the synthesized carbon are examined with a field emission scanning electron microscope (FE-SEM; Hitachi S-4800, Japan)

3. Results and discussion

Surface analysis of bare SS foil was observed in the Fig. 1. The SEM images showed a fair smooth surface composed from 52 at.% of Fe, ~16 at.% of Cr and ~ 13 at.% of Al (Table 1). The presence of Fe and Cr elements on the surface of FeCrAl substrate consisting to the composition of commercial stainless steel (Huang et al. 2004). Therefore, SS substrate as catalyst was used instead of the FeCrAl alloy to observe the formation of carbon from CH_4 in Ar flow.

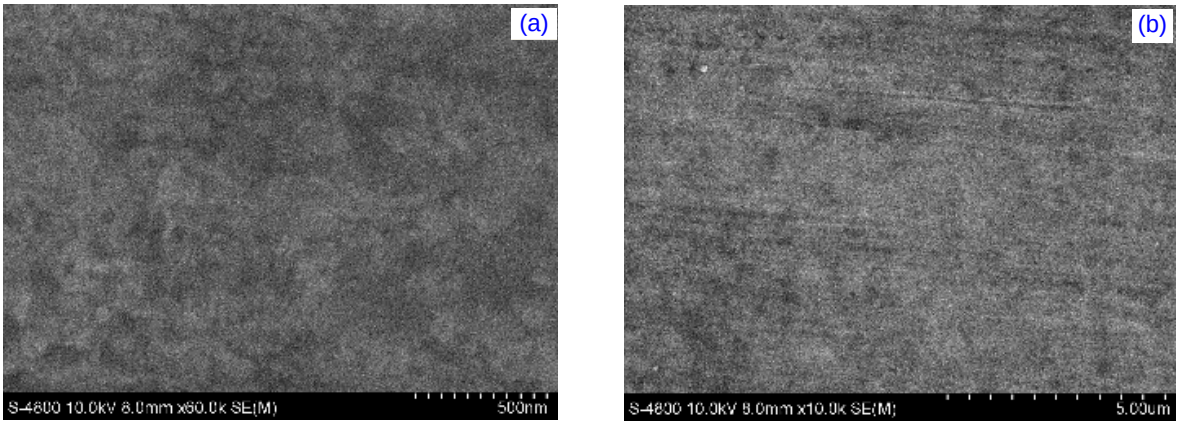


Figure 1: SEM images of stainless steel foil at high (a) and low magnifications (b)

Effect of reaction temperature on morphologies of carbon was presented in Figure 2, 3 and Figure 4. There are some carbon granules were detected at 750 ^{0}C of reaction temperature from SEM images (Figure 2a & b). The SEM image at low magnification of sample C-750 is almost similar to that of SS sample (Figure 1b and Figure 2b). Increasing reaction temperature to 850 ^{0}C resulted many carbon tubes whose 20 – 40 nm of diameter and 200 – 400 nm of length revealed the formation of carbon nanotubes (Figure 3a). The image of C-850 at low magnification showed a huge carbon with long tail (Figure 3b) suggesting the prior development of carbon along the length axis. Many un-uniform spheres of carbon that diameter is less than 250 nm was detected at 950 ^{0}C of reaction temperature indicating a preferable growth of carbon in three dimensions (Figure 4a). SEM image of C-950 at low magnification confirmed full coverage of carbon on SS substrate at 950 ^{0}C. Moreover, the lack of carbon nanotube from C-950 sample demonstrated a favorable growth of carbon along the width axis. It is proposed that long tail of carbon on C-850 sample will be only developed from initial carbon seed at suitable reaction temperature.

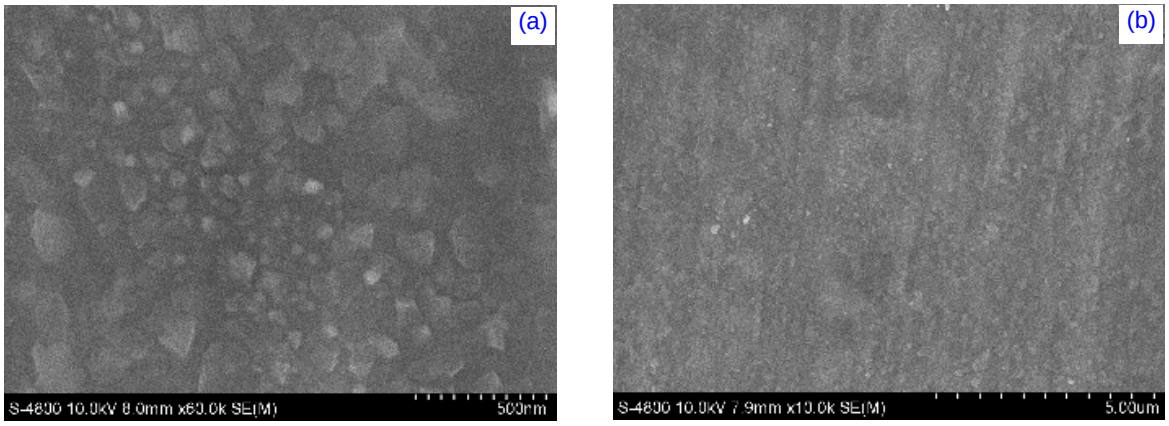


Figure 2: SEM images of C-750 at high (a) and low magnifications (b)

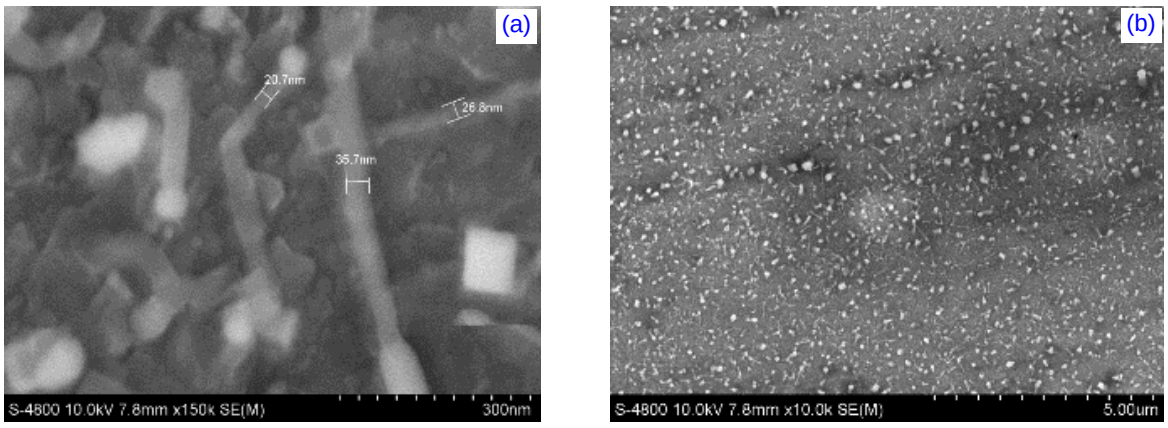


Figure 3: SEM images of C-850 at high (a) and low magnification (b)

Table 1 showed the atomic content of elements from SEM-EDX analysis. Clearly, amount of carbon increased from ~4 to ~10 at.% with increasing reaction temperature from 750 to 850 ^{0}C. There is about 30 at.% of carbon observed at 950 ^{0}C of reaction temperature (Table 1). That is consistent to full coverage of carbon spheres over SS substrate (Figure 4b).

The percentages of O and Al elements are in the similar range of ~17 – 28 at.% and 13 at.%, respectively while amounts of Cr and Fe decreased in increase of reaction temperature. Interestingly, the molar ratio of Cr/Fe kept constant value at ~0.3 before and after growth of carbon indicating that the Cr-Fe sites on SS substrate were relative to the formation of carbon from CH_4/Ar via CVD method. In comparison to C-750 sample, lengthening of carbon tail over C-850 revealed the formation CNTs on initial carbon seed was catalyzed by Cr-Fe sites. Detection of carbon spheres at 950 ^{0}C of reaction temperature demonstrated clearly the impact of reaction temperature on formation of carbon morphologies over SS catalyst.

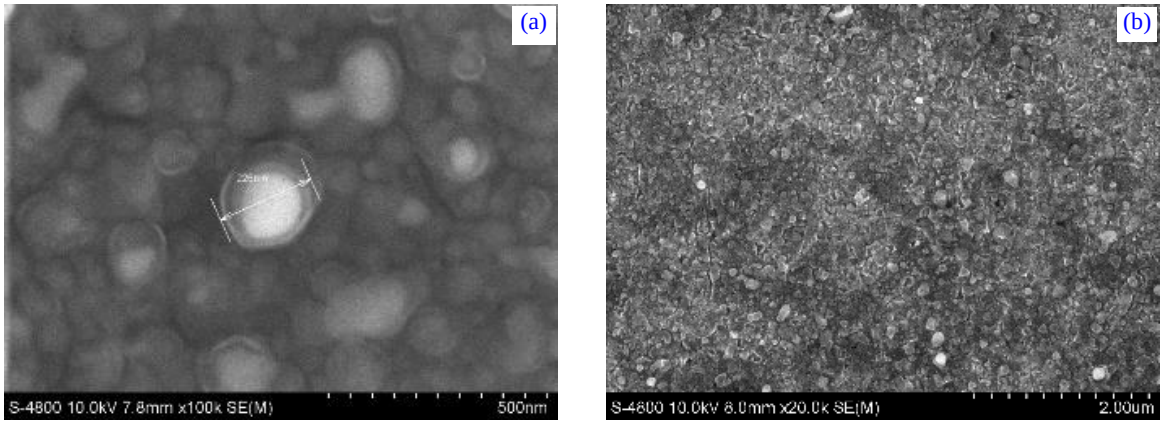
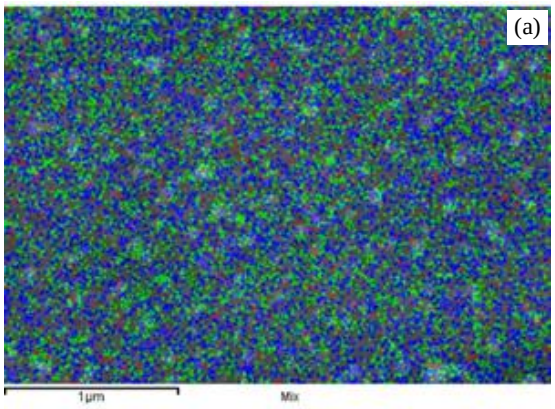
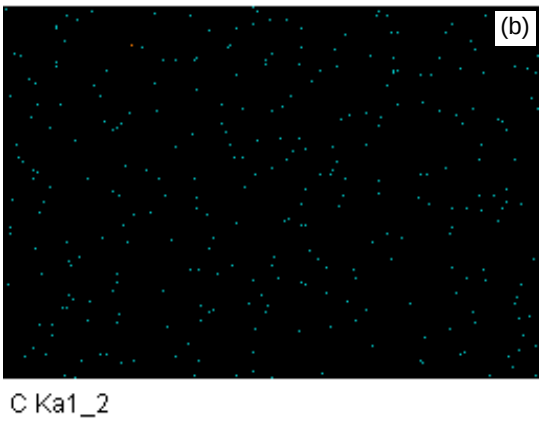
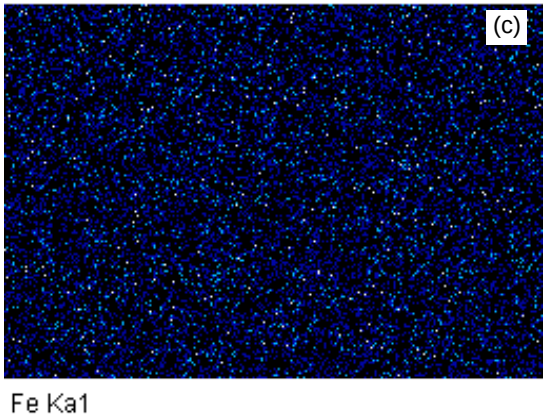


Figure 4: SEM images of C-950 at high (a) and low magnifications (b)

SEM mapping analysis in Figure 5 presented well spatial distribution of C, Fe, Al, Cr and O elements on C-850 samples catalyst suggesting good formation of carbon on SS catalyst at 850 ^{0}C of reaction temperature.
Our study showed preliminary work of fabrication of carbon nanotubes from CH_4 reactant with a CVD system. The optimal conditions in manufacture of carbon nanotubes over Fe-Cr sites are at 10 vol.% of CH_4 in Ar feeding flow (1000 mL/min of Ar flow), at 850 ^{0}C of reaction temperature in a CVD system.







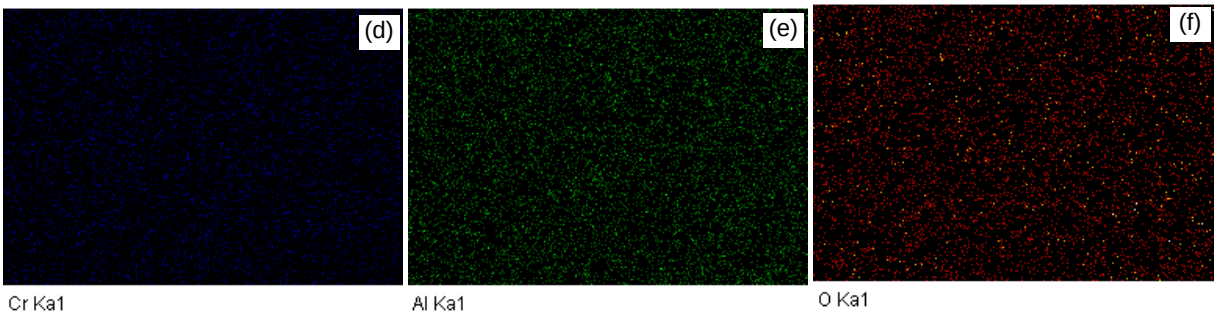


Figure 5: EDX mapping analysis of C-850 (a-f)

Table 1: Atomic percentage of elements from SEM-EDX

Sample	C	Fe	O	Al	Cr
SS	1.4	52.3	17.1	13.4	15.8
C-750	3.9	48.3	21.1	12.3	14.7
C-850	10.2	42.6	20.8	13.7	12.7
C-950	30.3	22.1	28.0	13.0	6.6

4. Conclusions

In this research, carbon formation from hydrolysis of stable structure - CH_4 over stainless steel catalyst was studied in CVD reactor under Ar flow. The reaction temperature is in the range of 750 - 950 ^oC. Noticeable effect of reaction temperature on formation of carbon morphologies was analyzed by SEM images and ESM-EDX technique . Our preliminary work proved that 850 ^0C is more suitable reaction temperature to produce carbon nanotubes from CH_4 in Ar flow. About 10 at.% of CNTs was detected at 850 ^0C and 30% of granular carbon was observed at 950 ^0C. Moreover, active site for the growth of nanotube carbon and granular carbon may regard with Fe-Cr sites on FeCrAl alloy substrate.

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Magnetite Fe_3O_4 nanoparticles: wet-chemical synthesis for therapeutic applications

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Among various superparamagnetic materials, magnetite Fe_3O_4 nanoparticles are the most frequently used for biomedical applications thanks to their excellent magnetic performance, biodegradability, biocompatibility and low toxicity. Fe_3O_4 nanoparticles possess a large surface area facilitating ready modifications with targeted agents which can be utilized for drug and gene delivery systems. In this field, small nanoparticles with uniform shapes correlated to various synthetic methods must be complied. Our efforts focus on the wet-chemical synthesis of Fe_3O_4 magnetic nanoparticles involving co-precipitation, thermal decomposition and solvothermal process with advantages and drawbacks of each method. In addition, requirements of Fe_3O_4 nanoparticles as nanocarriers for therapeutic purposes and achievements of magnetic drug targeting will be also described.

1. Introduction

Over the past few decades, magnetic nanoparticles consisting of metals (Fe, Ni, Co) and corresponding metal oxides, ferrites (MFe_2O_4 with M = Ni, Co, Cu) and Fe-based alloys have attracted intensive interests in catalysis (Govan and Gun'ko, 2014), pharmaceutical and biomedical applications (Reddy et al., 2012). Among these materials, Fe_3O_4 magnetic nanoparticles with appropriate surface chemistry have gained tremendous attractions in biomedicine, such as diagnostic imaging, tissue repair, therapeutic delivery, hyperthermia, and theranostics. Thanks to their unique capabilities, their biocompatibility, their stability, and their negligible side effects in cancer therapy, Fe_3O_4 nanoparticles with proper surface modification or conjugation with various targeting biomolecules have been extensively used nowadays for therapeutic applications.
The fabrication of magnetic nanoparticles can be categorized by either top-down or bottom-up approaches (Nikam et al. 2018). Top-down approach in which bulk materials are divided into nano-objects requires harsh conditions (high temperature and pressure) and produces non-uniform nanoparticles with surface defects. In contrast, the latter one mainly based on wet-chemical synthesis permits to tune size, morphology and crystalline structure of Fe_3O_4 nanoparticles associated to their instinct properties (such as optical, electronic, magnetic and surface properties). According to this approach, the reaction parameters are of vital importance including nature and concentration of precursor, temperature and heating method, ligands and additives, solvents, *etc.* This paper aims to discuss recent advances in the wet-chemical synthesis of Fe_3O_4 magnetic nanoparticles and their applications in drug and gene delivery systems.

2. Wet-chemical synthesis of Fe_3O_4 nanoparticles

Magnetite Fe_3O_4 nanoparticles have been frequently synthesized by physical, chemical or biological approaches. In general, the wet-chemical methods are preferred aiming at easily controlling size, shape and composition of nanoparticles, involving co-precipitation, thermal decomposition and solvothermal synthesis as discussed in this section.

2.1 Co-precipitation

Co-precipitation from an aqueous solution of Fe^{2+}/Fe^{3+} salts to prepare Fe_3O_4 nanoparticles is the most typical route, with the pioneering work of Massart as reported in 1981 (Massart, 1981). In this research, a mixture of ferric chloride and ferrous chloride was performed in aqueous ammonia solution. The black precipitates were separated from solution by centrifugation or an external magnet and then re-dispersed in alkaline or acidic media. In fact, the Fe^{2+} and Fe^{3+} salts in alkaline media react and then produce hydroxides precipitates. Simultaneously, Fe_3O_4 is yielded, accompanying with the loss of H_2O molecules as described in the Eq(1) (Mahmoud et al., 2013). Up to present, this method has been widely applied in the preparation of Fe_3O_4 nanoparticles thanks to its simplicity, reproducibility and the addition of less hazardous reagents.

$$[Fe^{3+}(OH)_3Fe^{2+}(OH)_2Fe^{3+}]n \rightarrow nFe_3O_4 + nH_2O \quad (1)$$

According to the similar strategy, Ghafoor and Ata synthesized Fe_3O_4 nanoparticles coming from $FeCl_2.4H_2O$ and $FeCl_3.6H_2O$ in deoxygenated water (adding concentrated HCl). The nanoparticles formation was performed by dropping NaOH solution under vigorous stirring at 80 ^oC under N_2 atmosphere. The as-prepared magnetic nanoparticles were isolated from solution by applying the external magnetic field (Ghafoor and Ata, 2017). Liu and co-workers used $FeSO_4.H_2O$ and $Fe_2(SO_4)_3$ (2:1 molar ratio) as iron precursors to fabricate Fe_3O_4 nanoparticles by adding ammonia aqueous solution under N_2 protection at 50 ^oC (Liu et al., 2016). On the other hands, the influence of ultrasonic auxiliary in co-precipitation process was studied as evidenced that obtained Fe_3O_4 nanoparticles have mean diameter of 24 nm without ultrasonic waves and 16 nm in the ultrasonic assistance (Rezayan et al., 2016). The effect of various iron precursors on particle size was observed (by XRD, SEM and TEM; see Table 1) that explained based on the double layer theory associated to the size of counterpart anions (Yazdani and Seddigh, 2016). Furthermore, the increase of saturation magnetization (Ms) (analyzed by VSM) exhibits the increase of particle size. However, morphology of obtained magnetite nanoparticles seems to be independent to such iron precursors.

Table 1: Specifications of Fe_3O_4 nanoparticles using various iron precursors (Yazdani and Seddigh, 2016)

Precursors	Ionic strength	Size (nm) XRD	Size (nm) SEM	Size (nm) TEM	Ms (emu/g) VSM
$FeCl_2.4H_2O$, $FeCl_3.6H_2O$	1.9	10.03	41.74	11.92	53.38
$FeCl_2.4H_2O$, $Fe_2(SO_4)_3$	2.8	6.6	36.64		35.1
$FeCl_2.4H_2O$, $Fe(NO_3)_3.9H_2O$	1.9	8.86	42.6		51.5
$FeSO_4.7H_2O$, $FeCl_3.6H_2O$	2.0	8.7	42.4		51.2
$FeSO_4.7H_2O$, $Fe_2(SO_4)_3$	2.9	5.1	26.32	5.12	30.5
$FeSO_4.7H_2O$, $Fe(NO_3)_3.9H_2O$	2.0	8.23	40.06		43.5

With the aim to simplify the co-precipitation method, Zhang et al. reported the similar procedure without inert gas protection: the aqueous solution of $FeCl_2.4H_2O$ and $FeCl_3.6H_2O$ (molar ratio of 1.6:1) was adjusted to pH 11 using 1 M NaOH solution and then dispersed in the presence of ultrasonic waves. Sodium citrate was employed acting as stabilizing agents permitting to obtain Fe_3O_4 magnetic fluid (Zhang et al., 2012). The use of stabilizers/modifiers in the preparation of metal nanoparticles is necessary to prevent the aggregation as reported by Radoń and co-workers. The magnetite Fe_3O_4 nanoparticles were modified by different organic compounds, indicating the different crystallite size (at the range of 2.9–12.2 nm as summarized in Table 2), structure, morphology and optical band-gap energy (Radoń et al., 2017). As clearly that the organic modifiers allow obtaining the smaller nanoparticles (except Fe_3O_4 - glycol) compared to unmodified Fe_3O_4 nanoparticles.

Table 2: The average crystallinity volume size and size of Fe_3O_4 nanoparticles (Radoń et al., 2017)

Sample	Crystallinity volume size (nm)	Particle size (nm)
Fe_3O_4	10.6	9.6 ± 2.0
Fe_3O_4 – glycol	12.2	10.8 ± 2.7
Fe_3O_4 – PVP	9.2	9.2 ± 2.3
Fe_3O_4 – tartrate	2.9	-
Fe_3O_4 – citrate	5.5	5.1 ± 0.8
Fe_3O_4 – dextrin	8.4	6.9 ± 1.4

5.1 Thermal decomposition

Normally, metal nanoparticles synthesized in aqueous and volatile solvents are poly-dispersive, agglomerative and poor in crystallinity. In contrast, thermal decomposition processes which metal precursors are heated up to their decomposition temperatures in high-boiling solvents, permit to obtain metal nanoparticles with mono-

crystallinity, mono-disperse in size and shape (Nikam et al., 2018). According to this approach, the preparation of metal nanoparticles generally coming from corresponding organometallic compounds has been employed in an organic solvent at high temperature and under inert atmosphere. Note that, the addition of surface stabilizing agents cannot be ignored.

Sun et al. reported the synthesis of Fe_3O_4 nanoparticles using iron (III) acetylacetonate ($Fe(acac)_3$) as starting precursors, oleic acid and oleylamine as surfactants in various organic solvents. The spherical nanoparticles were obtained with the mean size of 4 nm (phenyl ether, 265 ^oC, 30 min) and 6 nm (benzyl ether, 300 ^oC, 30 min), indicating that the increase of working temperature resulted in the increase of particle size (Sun et al., 2004). Similarly, $Fe(acac)_3$ was chosen as the iron precursor to prepare Fe_3O_4 nanoparticles using benzyl ether solvent and the capping ligands such as oleylamine (19 nm in mean diameter) (Saei et al., 2014), oleic acid and oleylamine (5.8 nm in mean diameter) (Ge et al., 2016). Thermal decomposition of $Fe(acac)_3$ in polyethylene glycol (PEG) and polyethylenimine (PEI) permits to achieve the stable colloidal solution of Fe_3O_4 nanoparticles coated by PEG and PEI (Huang et al., 2017). The diameter of nanoparticles (at the range of 9−14 nm) could be controlled by changing PEI amount, temperature and time. Besides $Fe(acac)_3$, Fe_3O_4 nanoparticles could be produced coming from iron(III) oxalate ($Fe_2(C_2O_4)_3.6H_2O$) in tri-*n*-dodecylamine (spherical nanoparticles of 6 nm in mean diameter) (Cavelius et al., 2012), iron oleate complex in 1-octadecene (spherical nanoparticles of 15.8 nm in mean diameter) (Huang et al., 2015), iron carboxylate in 1-octadecene (8−25 nm in the range of particle size) (Li et al., 2017) and pentacarbonyl iron in 1-octadecene (Lin and Doong, 2017).

Thermal decomposition process permits to prepare high-quality Fe_3O_4 nanoparticles; however, this method still possesses some drawbacks such as expensive metallic precursors and solvents, harsh reaction conditions, thus interfering to scale up the production.

2.2 Solvothermal synthesis

Chemical reaction carried out in organic solvents, under elevated temperature (100−1000 ^oC) and high pressure (1−10,000 bar) in a closed vessel (autoclave) is known as solvothermal synthesis (Nikam et al., 2018). In this method, metal nanoparticles are highly selective and reproducible in purity, crystallinity and morphology.

The pioneering work of Deng et al. reported the solvothermal method to synthesize single-crystalline Fe_3O_4 microspheres: $FeCl_3.6H_2O$ was dissolved in ethylene glycol (EG) and followed by adding sodium acetate (NaOAc) and polyethylene glycol (PEG), the mixture was then heated at 200 ^oC for 8−72 h in a Teflon-lined stainless steel autoclave (Deng et al., 2005). The particle size increases in the range of 200−800 nm with the increase of reaction time. Similarly, spherical magnetic Fe_3O_4 nanoparticles were also fabricated by a modified solvothermal method indicating mean diameters of 130 nm (Shi et al., 2011), 290 $\pm$ 20 nm (Li et al., 2016). According to the preparation of Fe_3O_4 nanoparticles from $FeCl_3.6H_2O$ in EG, the addition of some base anions such as NaOAc and NaOH facilitates the reduction of Fe^{3+}. In contrast, oxalate and citrate anions with strong coordination ability favor the stable iron complexes preventing the formation of $Fe(OH)_3$ and then Fe_3O_4 nanoparticles (Jamshidiyan et al., 2017). In this study, the effect of sodium salts including NaOAc, Na_2CO_3, a mixture of NaOAc and Na_3Cit (trisodium citrate), a mixture of NaOAc and $Na_2C_2O_4$ on the structure and optical properties of magnetic Fe_3O_4 nanoparticles was evaluated by XRF, XRD, FT-IR and SEM. Sizes and shapes of metal oxides can be controlled by reaction parameters (such as ratio of ligand and metal precursor, temperature, pressure, time, *etc.*) (Nikam et al., 2018). For instance, He and co-workers presented the sizes, morphologies and magnetic performances of sodium acetate oriented hollow/mesoporous Fe_3O_4 nanoparticles obtained by adjusting reaction time and molar ratio of reactants (He et al., 2018). Magnetic Fe_3O_4 nanoparticles were synthesized with different shapes as described in Table 3: cubes and octahedra (using $FeSO_4.7H_2O$, EG and KOH as a capping agent) and spheres ($FeCl_3.6H_2O$, EG and ammonium acetate as a capping agent). Besides, the effect of KOH concentration (0.5, 1.0, 1.5 and 5.0 M) on the shape of nanoparticles was found that the formation of octahedra is predominant at higher KOH concentration (Fatima et al., 2018). By the same approach, Ooi et al. reported the solvothermal synthesis of octahedral Fe_3O_4 nanoparticles coming from $FeCl_3.6H_2O$ in EG (solvent), oleylamine and 1,3-diaminopropane (organic surfactants) and NaOAc (steric stabilizing agent) (Ooi et al., 2015). The Fe_3O_4 octahedrons were produced in high yield (>85%) with edge lengths of 90 nm as evidenced by SEM, HR-TEM, XRD and XPS techniques.

Table 3: Morphologies of Fe_3O_4 nanoparticles depending on the reaction conditions (Fatima et al., 2018)

Synthesis conditions	Sphere	Cube	Transformation undergoing (Cube → Octahedron)		Octahedron
Iron precursor	$FeCl_3.6H_2O$	$FeSO_4.7H_2O$	$FeSO_4.7H_2O$	$FeSO_4.7H_2O$	$FeSO_4.7H_2O$
Ammonium acetate	1 M	-	-	-	-
KOH	-	0.5 M	1 M	1.5 M	5 M
Size	216.6 nm	158.5 nm	160 nm	581.2 nm	4.9 μm

Instead of using organic solvents, aqueous iron solutions could be applied to prepare metal nanoparticles under similar conditions as called for hydrothermal method. Lei and co-workers studied the synthesis of octahedral Fe_3O_4 (with edge lengths of 500 nm) by dissolving $FeSO_4.7H_2O$ in deionized water in the presence of α-propylene glycol and NaOH. The mixture was performed in a Teflon-lined stainless-steel autoclave by heating up 200 ^oC (a speed of 10 ^oC/min) and remaining this temperature for 20 h (Lei et al., 2017). Briefly, solvothermal/hydrothermal methods permit to synthesize mono-disperse and uniform shape Fe_3O_4 nanoparticles but being difficult in scaling up by reason of high temperature and high pressure.

3. Therapeutic applications of Fe_3O_4 nanoparticles: Magnetic drug targeting

3.1 Requirements of Fe_3O_4 nanoparticles as carriers in therapeutic applications

In general, for therapeutic purpose, the safety issues of nanocarriers depend on their physicochemical properties, such as surface charges, shape, size, composition, functional groups, and coating materials. These characteristics of magnetic nanoparticles need to be carefully investigated to ensure their biocompatibility, nontoxicity, biodegradability, and a good biodistribution in term of biomedical applications. The main advantages of using Fe_3O_4 nanoparticles for nanomedicine are their easy preparation, small sizes, good stability, low toxicity, facile surface modification and functionalization with biomolecules, strong biocompatibility in comparison to other inorganic nanoparticles, and their unique magnetic performance. There are several factors that strongly affect the ability of drug and gene delivery of Fe_3O_4 nanoparticles, such as the size, charge as well as surface chemistry (Chouly et al., 1996) although the iron oxide nanoparticles have already been approved by the Food and Drug Administration.

3.2 Fe_3O_4 nanoparticles for targeting drug/gene delivery systems

To enhance biocompatibility as well as to significantly increase the blood circulation time, Fe_3O_4 nanoparticles carriers can be coated either with natural polysaccharides, such as chitosan, dextran, starch, carbon, gelatin, or with synthetic polymers, amphiphilic polymeric surfactants such as polyethylene glycol (PEG) derivatives (Prabha and Raj, 2016), polyvinyl alcohol (Aliabadi et al., 2017), polyacrylic acid (Xu et al., 2012), polyethyleneimine (PEI) (Ma et al., 2017), *etc.* Moreover, by coating Fe_3O_4 nanoparticles with various compounds like polymer, silica, graphene oxide, carbon, *etc.*, the agglomeration of Fe_3O_4 nanoparticles which can lead to the increasing of size can be controlled. In term of biodegradability, after endocytosis, Fe_3O_4 nanoparticles can be degraded in endosomes, then releasing iron ions.

After being modified with a variety of coating materials, Fe_3O_4 nanoparticles will be conjugated with drugs or biomolecules, and then reach the target sites under the external magnetic field through the enhanced permeability and retention (Hudson, 2016). Thus, the drug doses and potential side effects to healthy tissues will be minimized with the improvement of treatment efficacy. Moreover, by modulating the coating surface, the Fe_3O_4 nanocarriers are used not only to targeted deliver drug but also to combine the chemophotothermal therapy (Du et al., 2016), magnetic resonance imaging (Rezayan et al., 2016), and other therapeutic methods (Ge et al., 2016). The loading drugs could be released under external stimuli, such as pH-responsive trigger (Saha et al., 2017), near-infrared laser-triggered (Deng et al., 2017), sensitivity with temperature, reactive-oxygen-species-mediated cytotoxicity, *etc.*

For example, doxorubicin (DOX), an anthracycline antibiotic, is a well-known drug model which is widely used in targeted drug delivery. Huang et al. reported a modified PEG and PEI coatings of Fe_3O_4 nanoparticles, and then folic acid (FA) was functionalized and DOX was then loaded on the nanoparticles to form DOX@FA– Fe_3O_4 magnetic nanoparticles (Huang et al., 2017).

In term of chemo-gene delivery, the group of Li has synthesized the magnetic Fe_3O_4@$mSiO_2$ nanoparticles and then modified them with PEI and FA with the purpose of co-delivery of plasmid-expressing small hairpin RNA (shRNA) against vascular endothelial growth factor (VEGF shRNA) and DOX (Li et al., 2016). The systems have a high biocompatibility and no toxicity on Hela cells or normal cells. The results showed that by combination of gene and drug factors, the uptake of DOX in tumor cells was increased, and a synergistic treatment effect was recorded. Moreover, the silencing effect of VEGF expression displayed a higher targeted gene – silencing effect

thanks to the presence of FA. Hence, Li et al. have produced an outstanding drug and gene co-delivery system that lead to a possibility of superimposed treatment for combination of gene and drug therapy for tumor cells.

4. Conclusions

Magnetite Fe_3O_4 nanoparticles are currently of great interest in pharmaceutical and biomedical applications thanks to their biocompatibility, low toxicity and magnetic property. Depending on the purposes, the coating materials on the surface of magnetic nanoparticles will be wisely chosen to proceed. Indeed, to be use in nanomedicine, especially in drug and gene delivery, the physicochemical properties and the pharmacokinetic profile of nanocarriers need to be investigated deeply. By physical or chemical interaction, a diversity of biomolecules can be attached or encapsulated or conjugated onto Fe_3O_4 nanoparticles together with the magnetic intrinsic property, this type of magnetic nanoparticles can provide maximum synergistic effects in biomedicine. Furthermore, nano-size of Fe_3O_4 should be controlled in the range of 10–200 nm permitting their steadily circulation in blood. Among various synthetic approaches of Fe_3O_4 nanoparticles as previously discussed, co-precipitation seems to be the most suitable method for the utilization in drug/gene delivery (obtaining small nanoparticles, using less hazardous reagents, low-cost and easily handling) compared to thermal decomposition (dangerous materials and harsh conditions) and solvothermal synthesis (large mean particle size and harsh conditions).

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Chemical Constituents and Bioactivities of Ethyl Acetate Extract from *Tacca leontopetaloides* (L) Kuntze Leaves

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This research was carried out to analyse total polyphenol, flavonoid, saponin and reducing sugar contents as well as *in vitro* bioactivities of ethyl acetate extract obtained from *Tacca leontopetaloides* leaves. The results indicated that this fractionated extract contained high level of bioactive compounds and showed high anti-inflammatory capacity *in vitro*. In addition, ethyl acetate extract was against all of the tested microorganisms, especially against *Fusarium oxysporum* and *Candida tropicalis*.

1. Introduction

Tacca leontopetaloides (L) Kuntze tubers have been used for years in An Giang province, Vietnam as source of starch but in-depth investigations on either its aerial parts chemical compositions or bioactivities remain limited.

Therefore, based on medico-ethnobotanical knowledge about possible uses of *Tacca leontopetaloides*, this study aimed at obtaining and evaluating biological properties of various extracts from *Tacca leontopetaloides* leaves and choosing the best fraction for further research.

2. Material and Methods

2.1 Materials

Fresh leaves of *Tacca leontopetaloides* were collected in Tinh Bien District, An Giang Province. The leaves were shade dried for three days before being grounded into powder.

2.2 Experimental Set-up

Preparation of the extracts

- An amount of 20 g *Tacca leontopetaloides* leaf powder was extracted three times, each time with 100 ml of ethanol 96% in 24 hours at room temperature. The ethanol extract (EtOH) was removed fats by shaking with n-hexane (n-Hex); then the undissolved part was extracted with different organic solvents in increasing polarity order [ethyl acetate (EtOAc), n-butanol (n-BuOH) and water] to get respective extracts. The solvent was then evaporated in a rotary evaporator at 40 ^{o}C for dried extracts which were stored at -20 ^{o}C in air-tight screw-capped glass vials until used for bioassays.

DPPH radical scavenging assay

- This assay was conducted according to the method prescribed by Rao (1996) with some modifications (Sreejayan & Rao, 1996). An amount of 100 µl DPPH solution (6 mM) and 100 µl of various concentrations of the extracts or standard solution (ascorbic acid) were added into 2800 µl of methanol. The mixtures were incubated at 37^{0}C for 30 min. Absorbance of each solution was measured at 517 nm by UV-VIS spectrophotometer. The radical scavenging activity of samples was calculated as the percent DPPH radical scavenging effect. IC50 value is calculated by GraphPad Prism via the base line of inhibition percentage (base line established from 6 different concentrations).

Scavenging of ABTS radical cation

- This assay was conducted according to the method prescribed by Rao (1996) with some modifications (Sreejayan & Rao, 1996). An amount of 100 µl DPPH solution (6 mM) and 100 µl of various concentrations of the extracts or standard solution (ascorbic acid) were added into 2800 µl of methanol. The mixtures were incubated at 37^0C for 30 min. Absorbance of each solution was measured at 517 nm by UV-VIS spectrophotometer. The radical scavenging activity of samples was calculated as the percent DPPH radical scavenging effect. IC50 value is calculated by GraphPad Prism via the base line of inhibition percentage (base line established from 6 different concentrations).

Antimicrobial bioassay

- Antimicrobial activities of extracts were tested against reference strains including Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella enterica* var Typhimurium ATCC 14028), Gram-positive bacteria (*Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923 and methicillin-resistant *Staphylococcus aureus* ATCC 43300), yeasts (*Candida albican*s ATCC 10231, *Candida tropicalis* ATCC 750) and moulds isolated from plants (*Aspergillus niger* and *Fusarium oxysporum*). The disk-diffusion agar method was used and inhibitory zone diameters of extracts were determined.

Anti-inflammatory activities

- Protein denaturation has been documented to correlate with the occurrence of the inflammatory response and employed as *in vit*ro screening model for anti-inflammatory compounds (Chandra *et al.*, 2012). Some modifications were applied to study anti-inflammatory activity of *Tacca leontopetaloides*. Egg albumin protein was used. The reaction mixture was consisting of 1 mL extracts' solution or standard solution (diclofenac sodium), 1 mL of 0.5% albumin and 1 mL of saline buffer (pH 6.4). The sample were incubated at 37 ºC for 15 min and then heated to 70 ºC for 5 min, after cooling the samples the turbidity was measured at 660 nm. The experiment was performed in triplicate. The Percentage inhibition of protein denaturation was calculated as follows: Percentage inhibition = [1 - (A1 - A2) / A0] x 100 where A0 was the absorbance of the control and A1 was the absorbance in the presence of the extract, A2 was the absorbance without albumin.

Total polyphenol content determination

- The amount of total phenols in EtOAc extract was determined using Folin-Ciocalteu reagent according to the method of Singleton with some modifications. Gallic acid was used as a positive control. Samples (40 µL) were introduced into test cuvettes, and then 0.2 mL of Folin-Ciocalteu's reagent and 0.6 mL of sodium carbonate (20%) were added. The absorbance of all samples was measured at 760 nm using the UV spectrophotometer after incubating at 30°Cf or 1.5 h. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g dry extract. Each assay was performed in triplicate.

Total flavonoid content determination

- Aluminium chloride colorimetric method was used for flavonoid determination (Zhishen, 1999) with some modifications. 0.5 mL of sample solution was mixed with 2 mL of distilled water. 150 µL of sodium nitrite was added. After 5 min, 150 µL of aluminium chloride (10%) was added and allowed to stand for 1 min. Then, 1 mL of sodium hydroxide was added and the mixture was shaken to mix well. The absorbance was measured at 510 nm using UV spectrophotometer.

Total saponin content determination

- Total saponins (SP) were determined by the method of Hiai et al. (1976) with some modifications. 0.1 mL of sample was mixed with 0.1 mL of vanillin reagent (8% vanillin in methanol) and then 1 mL of 72% aqueous H_2SO_4 was added. The mixture was heated in a water bath at 60◦C for 10 min followed by cooled in ice for 5 min and then it was kept at room temperature. The intensity of the colour developed due to the presence of saponin was measured as optical density in a spectrophotometer 538 nm. Oleanolic acid was used as a standard and the results obtained were expressed as mg oleanolic acid equivalent per 100 g of dried extract.

Total reducing sugars content determination

- Total reducing sugars content was determined by the method of Miller et al. (1959) using DNS with some modifications. The DNS method is a colorimetric technique that consists of a redox reaction between the 3,5-dinitrosalicyclic acid and the reducing sugars present in the sample. The reducing power of these sugars comes from their carbonyl group, which can be oxidized to the carboxyl group by mild oxidizing agents, while the DNS (yellow) is reduced to 3-amino-5-nitrosalicylic acid (red-brown) which can be quantified by spectrophotometry at 540 nm, wavelength of maximum absorbance. The intensity of the colour is proportional to the concentration of sugars. The reaction is carried out in an alkaline medium.

3. Results and Discussion

3.1 Bioactivities of extracts from Tacca leontopetaloides leaves

All of the three extracts from *Tacca leontopetaloides* showed significant antioxidant activity in two different methods of antioxidant assays tested (Table 1). The highest DPPH scavenging activity was observed in EtOAC (IC_{50} = 70.13 ± 2.2 µg/ml). Similar trend was observed in scavenging activity of ABTS: EtOAC extract was also the best (IC_{50} = 70.13 ± 2.2 µg/ml).

Table 1: Antioxidant activities of the fractionated extracts from Tacca leontopetaloides

	IC_{50} (µg/ml)	
	DPPH	ABTS
Hexane	633.91 ± 3.9	714.02 ± 4.0
EtOAc	70.13 ± 2.2	42.77 ± 0.1
n-BuOH	339.61 ± 13.2	239.49 ± 2.5
Water	612.76 ± 17.1	645.69 ± 9.9
Vitamin C	44.09 ± 0.8	27.05 ± 1.4

Antimicrobial activities of all extracts were relatively lower than that of standard antibiotic Gentamicin (Table 2). Only hexane and EtOAc extracts showed the inhibitory zone against bacteria. Hexane extracts could not resist two Gram-negative bacteria (*S. enterica* and *P. aeruginosa*) whereas the EtOAc extract did have activities on all tested bacteria though those were lower than the ones of Gentamicin.

Table 2: Inhibitory zone diameters of the fractionated extracts from Tacca leontopetaloides

	Diameter (mm)		
	Gentamicin	Hexane (0.5 mg/mL)	EtOAc
E. coli	22	-	11
P. aeruginosa	30	-	15
S. enterica	23	10	12
E. faecalis	20	10	18
S. aureus	26	11	15
MRSA	21	11	16

From the above data, it could be inferred that EtOAc extract had the highest bioactivity because it contained the most bioactive compounds. Therefore, this fraction was used for further study.

5.2 Bioactivities of EtOAc extract from *Tacca leontopetaloides* leaves

The data reported in Table 3 presents the antifungal activity of the EtOAc extract of *Tacca leontopetaloides* leaves. The results indicated that the EtOAc extract was less effective than Itraconazole in inhibiting 3 of 4 tested strains but performed rather good activity against *Fusarium oxysporum*.

Table 3: Inhibitory zone diameters of the EtOH extract from Tacca leontopetaloides

	Diameter (mm)	
	Itraconazole (4 mg/mL)	EtOAc
Candida albicans	23	12
Candida tropicalis	22	14
Aspergillus niger	19	12
Fusarium oxysporum	-	20

The *in vitro* anti-inflammatory effect of EtOAc extract was evaluated against denaturation of egg albumin. The results are showed in Table 4. The effect of diclofenac sodium was found to be 2.1 times less than that of EtOAc extract by comparing their IC50 values (23.60 ± 0.1 mg/mL and 11.11 ± 0.5 mg/mL respectively).

Table 4: Anti-inflammatory activity of EtOAc extract from Tacca leontopetaloides

	IC_{50} (mg/ml)
Diclofenac sodium	23.60 ± 0.1
EtOAc extract	11.11 ± 0.5

3.2 Total Phenolic, Flavonoid, Saponin and Reducing sugar Contents

The amounts of total bioactive compounds in the EtOAC were detected by colourimetric methods, and the results are summarised in Table 5. The total amount phenolics and another bioactive compounds could explaine the strong bioactivities of *Tacca leontopetaloides* EtOAc extract.

Table 5: Total bioactive compounds in EtOAc extract from Tacca leontopetaloides leaves

	% in EtOAC extract	Amount in dried weight (mg/100g)
Total phenolic content	77.89 ± 0.32	2441.8 ± 17.10
Total flavonoid content	12.28 ± 0.13	384.88 ± 4.12
Total saponin content	19.15 ± 0.57	600.33 ± 17.87
Total reducing sugar content	11.09 ± 0.51	347.66 ± 15.96

4. Conclusions

Among extracts obtained from *Tacca leontopetaloides* leaves, the ethyl acetate extract demonstrated the highest bioactivities especially significant anti-inflammatory activity and exhibited high total phenolic content.

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Catalytic Removal of Formaldehyde in Humid Condition Using Nano-Sized Noble Metal Supported on Ceria-Granular Carbon at Room Temperature

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Noble metal (Au, Pd, and Au-Pd) was supported on ceria/granular carbon (CeO_2/GC) by metal-sol method and used for adsorption and catalytic oxidation of formaldehyde which is a major indoor air pollutant. The crystal characteristic, surface properties, morphology and the actual metal content on the catalysts were evaluated. The catalyst's stability and the concentration of CO_2 in the outlet stream were also investigated. The 0.5%Au-0.27%Pd/CeO_2/GC catalyst exhibited superior catalytic activity and stability toward formaldehyde oxidation at room temperature. The formaldehyde removal efficiency was kept nearly at 83 % for a reaction time of 10 h with a high formaldehyde concentration of 3,278 ppmv, relative humidity of 77 % and GHSV of 30,600 h^{-1}.

1. Introduction

Formaldehyde (HCHO) is recognized as one of the major air pollutants in the indoor environment and widely derived from wooden furniture and decorating materials (Nie et al., 2016). Formaldehyde pollution has attracted much attention due to its carcinogenicity and teratogenicity (Lu et al., 2017). It is well known that long-term exposure to air containing HCHO, even from exposure to very low concentrations at ppm level, may cause serious health problems (Tan et al., 2015). Thus, the removal of HCHO is of significant practical interest to improve our indoor air quality. Catalytic oxidation of HCHO is an efficient and environmentally friendly approach to its removal (Huang et al., 2016).

Supported noble metals (e.g., Au, Pd, Pt) have been proposed as efficient heterogeneous catalysts for HCHO oxidation at low temperature (<100 °C). Several catalysts have been developed such as Au/TiO_2 (Ma et al., 2016), Au/MnO_2 (Pang et al., 2016), Pd/TiO_2 (Huang et al., 2011) and Pt/TiO_2 (Qi et al, 2016). Au-based catalysts have been found to have promising catalytic activities with HCHO conversion at low temperature (Li et al., 2011).

On the other hands, water vapor can be present in the gas stream from the atmosphere or formed as a reaction product. Wu et al. (2014) reported that the competitive adsorption between the organic compound and H_2O molecules on the active sites results typically in lower catalytic activity with nickel oxide catalysts supported on ɣ-Al_2O_3, SBA-15 and TiO_2. Soares et al. (2016) have reported that humidity has a significant effect on the oxidation of ethyl and butyl acetate with cryptomelane-type manganese oxide catalyst. Wu et al. (2000) concluded that water vapor reduced the activity only slightly due to the hydrophobicity of activated carbon (AC) with Pt/AC catalysts. The activated carbon can utilize for volatile organic compounds (VOCs) removal by adsorption (Iovino et al., 2013).

In this work, monometallic Au/Pd and bimetallic Au-Pd supported on CeO_2/Granular carbon catalyst were prepared using a metal sol method. The catalytic activity for formaldehyde removal by combined adsorption and catalytic oxidation at room temperature was investigated. The catalysts were characterized by XRD, BET, SEM, TEM methods. The catalyst's stability with/without moisture was also evaluated.

2. Materials and methods

2.1 Material preparation and characterization

The particles size of commercial granular carbon (GC) was comprised between 0.5 and 1 mm (supplied by TRABAC Joint Stock Corporation). CeO_2 supported on GC was prepared by a one-step-impregnation method with an excess of solvent (C_2H_5OH solution and distilled water, with molar ratio C_2H_5OH/H_2O of 5/3). An appropriate amount of GC used as support was stirred in 100 mL of solvent at room temperature in 15 min in the reaction vessel. The adequate amounts of $Ce(NO_3)_3 \cdot 6H_2O$ (99.5 %, from Acros) was dissolved in 60 mL solvent and was slowly added (2 mL/min) to the above reaction vessel to have CeO_2 loading of 20 wt%. The decomposition of the cerium precursor to having CeO_2/GC was practiced under flowing N_2 (50 mL/min) at 623 K for 5 h (heating rate of 5 K/min).

The monometallic Au and Pd and bimetallic Au-Pd supported on CeO_2/GC were synthesized by chemical reduction method (metal sol method). Firstly, a suitable amount of polyvinyl alcohol (PVA) solution (1 wt%) was added to an aqueous $HAuCl_4$ 0.4 mg/mL or/and $PdCl_2$ 0.4 mg/mL under vigorous stirring. Then, a freshly prepared solution of $NaBH_4$ (molar ratio $NaBH_4/M = 4$) was slowly added dropwise into the mixture. After that, 5 g of GC was added after the pH of the mixture was adjusted to 4 using HCl/NH_4OH solution. The mixture solution was stirred and vacuumed at 50 °C for drying, then the mixture washed until no Cl^- was detected by $AgNO_3$ solution. After that, the sample was dried at 100 °C for 12 h. The mass ratio of PVA/metal atoms of bimetallic Au-Pd catalysts was maintained and the molar ratio of Au:Pd was 1:1. All chemicals were purchased from Acros/Fisher and used without further purification.

The loading contents of Au on 0.5%Au/CeO_2/GC and 0.5%Au-0.27%Pd/CeO_2/GC catalysts were 0.5%. The loading contents of Pd on 0.5%Pd/CeO_2/GC and 0.5%Au-0.27%Pd/CeO_2/GC catalysts were 0.5% and 0.27%.

The crystalline structure of the samples was measured by powder X-Ray Diffraction (XRD, Advance D8 diffractometer), with Cu K_α radiation (λ = 1.5418 Å) operated at 40 kV. The surface morphology of each catalyst and GC was observed by scanning electron microscope (SEM, JEOL – JSM 5500) operated at 10 kV. BET (Brunauer-Emmett-Teller), the surface area data was recorded on NOVA 2200e, Quantachrome Instruments version 10.0. The size and the size distribution of noble metals particles were obtained using transmission electron microscopy (TEM, JEOL – JEM 1400) operated at 100 kV.

2.2 Formaldehyde removal test

The efficiency for formaldehyde removal of the combination adsorption-catalysis in this experiments was processed under atmospheric pressure in a U-shaped glass reaction vessel (internal diameter of 12 mm) and operated in continuous mode. The N_2 gas (99.99 %) and O_2 (99.9 %) purchased from Phu-Thinh company and formaldehyde (37 wt% in H_2O) purchased from Sigma Aldrich were used in the experiments. Catalysts (0.2 g) are deposited on a glass wool layer in the vessel. Each catalyst was pre-treated under flowing N_2 (6 L/h) at 200 °C in 1 h before use. The gaseous feed was received by passing model air (80 % N_2 and 20 % O_2, v/v) through two parallel saturators, one containing formaldehyde in a thermostat bath and the other containing distilled H_2O. Both were kept at the suitable temperatures to ensure that the formaldehyde concentration in the gaseous feed was 3,278 ppmv for the wet condition, and H_2O vapor content of 23.7 mg/L (relative humidity 77 % at 25 °C) for the wet condition. The total flow rate of gaseous feed was 150 mL/min, corresponding to gas hourly space velocity (GHSV) about 30,600 h^{-1}. The formaldehyde removal efficiency of the catalyst was evaluated at room temperature. The effluent gas was recorded by an online Gas Chromatograph HP-5890plus with a flame ionization detector (FID) and HP-Plot/Q (30 m x 0.53 mm) column. The CO_2 in the output stream was recorded by a CO_2 monitor (Alphasense sensor technology company, UK). In particular, the 6-way valve system allows the operating different processes without changing the pipeline of the system. The HCHO removal efficiency was calculated according to Eq(1) as follows:

$$\text{Formaldehyde removal}\,(\%) = 100 - \frac{[HCHO]}{[HCHO]_o} \times 100 \quad (1)$$

Where [HCHO] and $[HCHO]_o$ are the areas of the gas chromatograph result's peak of the formaldehyde at a reaction time (t) and the beginning (t = 0).

3. Results and discussion

3.1 Characterization results

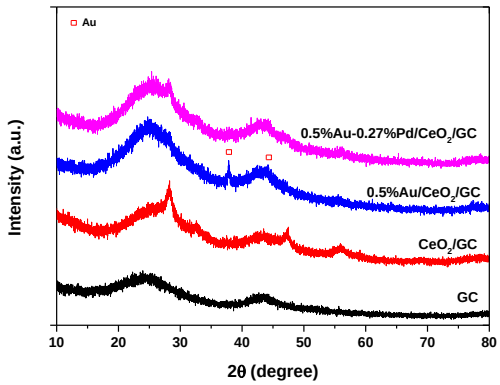

Figure 1: XRD patterns of GC, CeO_2/GC, 0.5%Au/CeO_2/GC and 0.5%Au-0.27%Pd/CeO_2/GC.

The X-ray diffraction pattern recorded for the as-prepared samples is shown in Figure 1. The GC exhibited a unique broad peak at 2θ from 24.3° to 26.3°, and peak at 43.4° corresponding to the Miller indices (002) and (101) in all samples (Li et al., 2007). All the reflections on the XRD pattern can be indexed to that of ceria according to the literature pattern (JCPDS card No. 34-0394). The diffraction peaks correspond to the (111), (200), (220), and (311) planes, represent the face-centered cubic structure (FCC) in all catalysts (Peng et al., 2005). For 0.5%Au/CeO_2/GC sample, the peaks at 37.9° and 44.2° corresponding to the Miller indices (111) and (200) represent the face-centered cubic structure of gold (JCPDS card No. 04-0784) (Jha and Prasad, 2011). Finally, for the 0.5%Au-0.27%Pd/CeO_2/GC sample, no peaks corresponding to Au and Pd can be observed in the XRD spectra, indicating that Au and Pd nanoparticles are high dispersion and their crystal size are very small, as observed in the TEM images (Figure 3).
The BET surface area of GC was 728 m^2/g whereas the BET surface area of CeO_2/GC, 0.5%Au/CeO_2/GC, 0.5%Pd/CeO_2/GC and 0.5%Au-0.27%Pd/CeO_2/GC were 685 m^2/g, 625 m^2/g, 593 m^2/g and 568 m^2/g. It can be seen that the chemical reduction method (metal sol method) can be used to deposit noble metal (Au, Pd, and Au-Pd) on the ceria/granular carbon. After adding noble metal (Au, Pd, and Au-Pd) on CeO_2/GC the BET surface area decreased (Liu et al., 2014).

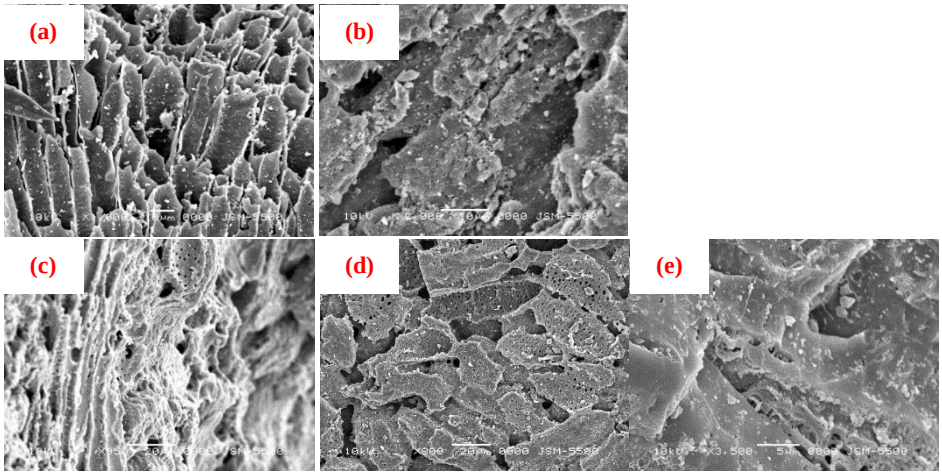

Figure 2: SEM images of (a) GC, (b) CeO_2/GC, (c) 0.5%Au/CeO_2/GC, (d) 0.5%Pd/CeO_2/GC and (e) 0.5%Au-0.27%Pd/CeO_2/GC

SEM images of noble metal (Au, Pd, and Au-Pd) synthesized on CeO_2/GC are shown in Figure 2. The GC surface is smooth and includes stacked carbon plates. The granular carbon image measured by this SEM method is similar to results reported by Yan et al., (2019). The well-dispersed metal layer was observed on the surface of M/CeO_2/GC (M = Au, Pd, and Au-Pd), which was due to the pore being blocked by surface deposition of ceria and metal M, thereby decreasing BET surface area.

TEM analysis of the monometallic Au/Pd and bimetallic Au-Pd catalysts supported on CeO_2/GC is shown in Figure 3. All the catalyst samples showed spherical particles dispersed on the support CeO_2/GC. The shape of the Au nanoparticles determined by TEM method has the similar results as previous study (Minh et al., 2018), while the shape of the Pd particles was also the similar as previous study (Tu et al., 2019). The Au nanoparticles of monometallic 0.5%Au/CeO_2/GC showed an average size of particles of 6 ± 2 nm. Pd nanoparticles of 0.5%Pd/CeO_2/GC revealed an average size of 30 ± 5 nm whereas Au-Pd nanoparticles of 0.5%Au-0.27%Pd/CeO_2/GC showed 3 ± 2 nm particles. These results indicate that the size of AuPd particles decreased when Au and Pd were both supported on the ceria/granular carbon in the bimetallic catalyst.

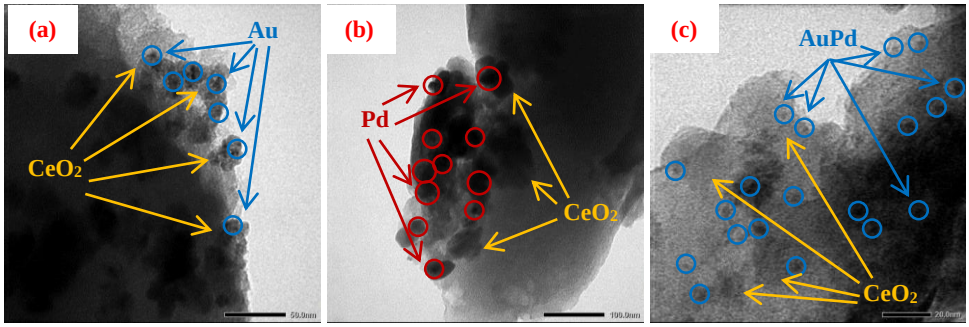

Figure 3: TEM images of (a) 0.5%Au/CeO_2/GC, (b) 0.5%Pd/CeO_2/GC and (c) 0.5%Au-0.27%Pd/CeO_2/GC

3.2 Formaldehyde removal efficiency

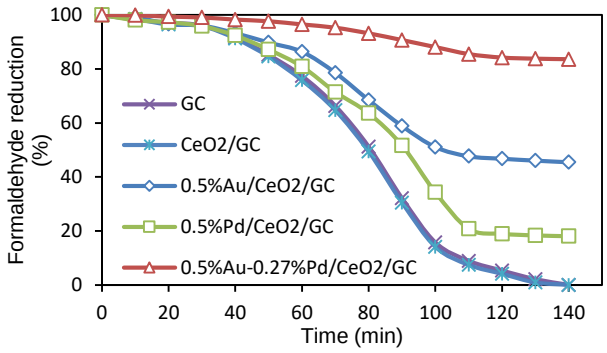

Figure 4: Formaldehyde removal efficiency by combined adsorption-catalysis over different materials at room temperature

The HCHO removal efficiency of samples GC, CeO_2/GC, 0.5%Au/CeO_2/GC, 0.5%Pd/CeO_2/GC and 0.5%Au-0.27%Pd/CeO_2/GC by dual-functional adsorbent/catalysts under high humidity condition (Relative humidity (RH) about 77 %), at room temperature are shown in Figure 4. The formaldehyde removal efficiency of the 0.5%Au/CeO_2/GC, 0.5%Pd/CeO_2/GC and 0.5%Au-0.27%Pd/CeO_2/GC samples by combined adsorption-catalysis are higher than the granular carbon and CeO_2/GC samples. Furthermore, formaldehyde removal efficiency by GC and CeO_2/GC was almost similar. Therefore, the HCHO removal efficiency of the 0.5%Au/CeO_2/GC, 0.5%Pd/CeO_2/GC and 0.5%Au-0.27%Pd/CeO_2/GC samples by combined adsorption-catalysis. While the HCHO removal efficiency of GC and CeO_2/GC samples was only adsorption. In particular, the HCHO removal efficiency of 0.5%Au-0.27%Pd/CeO_2/GC sample was 83.6 % at room temperature by adsorption and oxidation.

With the best formaldehyde removal efficiency, the 0.5%Au-0.27%Pd/CeO_2/GC catalyst was selected to analyze the stability, water-resistance test. It can be seen that despite the high humidity condition (RH = 77 %), the HCHO removal efficiency of 0.5%Au-0.27%Pd/CeO_2/GC was not significantly changed (Figure 5). The

hydrophobicity of GC may be suitable explanation for the phenomena observed in this work. This result is similar to results reported by Wu et al., (2000). Notably, the oxidation activity of the 0.5%Au-0.27%Pd/CeO_2/GC catalyst was almost constant after reaction for 10 h. The carbon dioxide concentration in the reactor's output stream corresponds to the formaldehyde concentration reduction. No other organic compounds (except HCHO) have been detected by our Gas Chromatograph.

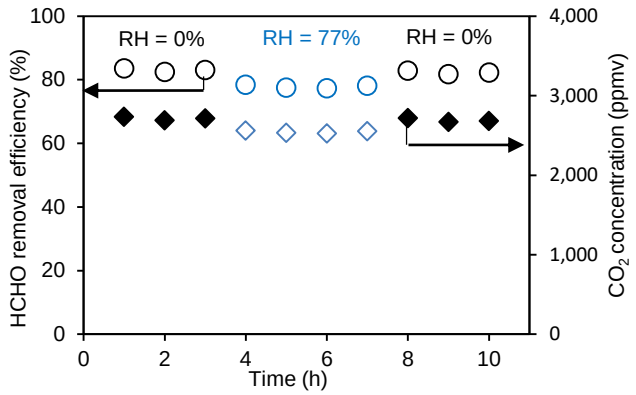

Figure 5: Stability, H_2O resistance test, and CO_2 concentration after the reaction of the 0.5%Au-0.27%Pd/CeO_2/GC for formaldehyde removal by catalytic oxidation at room temperature

4. Conclusions

The nano-sized monometallic Au/Pd and bimetallic Au-Pd supported on CeO_2/GC have been successfully synthesized by chemical reduction method (metal-sol method). The formaldehyde removal efficiency of materials by a combination of adsorption and oxidation at room temperature was investigated. The stability, water-resistance of the catalyst, and carbon dioxide concentration in the outlet stream after the reaction were studied. The catalyst of 0.5%Au-0.27%Pd/CeO_2/GC had the best formaldehyde removal efficiency in this study and the stable of good catalyst, this result may be due to the smaller size of AuPd particles when Au and Pd were both supported on the CeO_2/GC, and the water vapour reduced the formaldehyde removal efficiency only slightly due to the hydrophobicity of granular carbon.

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Investigation of activity of *Edwardsiella ictaluri* bacteriophages at different conditions

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White spots in the internal organs, caused by the bacterium *Edwardsiella ictaluri*, is among the most common and serious diseases in striped catfish in Mekong Delta, Vietnam. Bacteriophage or phage is virus infecting only bacterium. Phage therapy is the use of lytic phages as a treatment for pathogenic bacterial infections. Stability of phage to different conditions is one of the first considerations in phage therapy. Until now, there is no phage therapy is applied to treat bacterial diseases in striped catfish. In this study, activity of G1, G7 and G9.1 phages to factors such as organic solvents, temperature, and pH was investigated. Among these phages, G9.1 was found to be relatively the most stable in terms of temperature, pH, and organic solvents. The activity of G9.1 was retained to approximately 90% at 37 °C for 1 h. Its activity was maintained about 90% at pH 7–11 for 24 h and approximately 70–87% in organic solvents such as chloroform or diethyl ether, for 1 h. In conclusion, G9.1 was found to be promising in phage therapy for in vivo treatment of white spots in the internal organs in striped catfish.

1. Introduction

The annual cycle of bacterial pathogen infections significantly affects the sustainable development of the striped catfish industry in the region. The most common of which is white spots in the internal organs caused by Edwardsiella ictaluri. This disease first appeared among striped catfish in Vietnam in 1998, associated with a high mortality rate (up to 90%) (Dung et al., 2002; Dang and Nguyen, 2010). The use of antibiotics as the major preventative and curative treatment for this disease has since become common in the region. However, such control efforts have recently proven inadequate due to the development of antibiotic resistance by *E. ictaluri* (Quach et al., 2014). This has raised concerns regarding the long-term efficacy of antibiotic treatment in the commercial production of striped catfish. In addition, residual antibiotics at levels above approved limits in frozen striped catfish fillets may affect consumers' health. Due to these adverse impacts, there is an urgent need to identify more effective solutions to replace antibiotics. Bacteriophages (or phages) are virus infecting only bacteria. They were first discovered by Frederick W. Twort (Twort, 1915). Phage therapy involves the therapeutic use of phages to prevent and treat pathogenic bacterial infections. Such therapy has only gained serious attention in the aqua industry in the last 30 years due to the wide spread of antibiotic resistance in bacteria. Phage therapy has shown its efficacy in treatment of bacterial diseases in fish and shellfish (reviewed by Richards, 2014; Doss, 2017).
Currently, some phages G1, G7 and G9.1 infecting *E. ictaluri* in stripped catfish were isolated and characterized their latent period, burst size. However, phages are constructed relatively simply with a protein capsid and nucleic genome. Their activity is significantly affected by preservation and environmental conditions. Thus, phage stability should be clarified prior to *in vivo* trials. In this study, the stability of these phages with respect to temperature, pH, and organic solvents was investigated.

2. Materials and methods

2.1 Phage isolation

Striped catfish kidney and liver samples were obtained from markets in Ho Chi Minh City and transferred to the laboratory under cold conditions for phage isolation. The samples were homogenized and added to a log-phase *E. ictaluri* CT1 culture. The mixture was then incubated overnight at 30 °C with rotation at 150 rpm. An aliquot was subsequently taken and centrifuged at 9,727 ×g at 4 °C for 5 min. The resulting supernatant was passed through a 0.22-µm filter, and the filtrate subjected to a plaque assay. A single transparent plaque was picked from the plate, suspended in SM buffer, incubated overnight at 4 °C, and passed through a 0.22-µm filter. The resulting filtrate was subjected to the above protocol three times in succession to purify the phage. The purified phage stock was prepared as described elsewhere (Hoang et al., 2018).

2.2 Temperature stability test

Phage stocks of G1, G7, and G9.1 were prepared against *E. ictaluri* CT1. The stability of each phage at various temperatures (4, 20, 30, 37, 50 and 65 °C) was investigated by incubating the phage (~10^9 PFU mL^{-1}) at the respective temperatures for 1 h (Verma et al., 2009; Jun et al., 2013; Yamaki et al., 2014). The phage titer was estimated by serial dilution and the double agar-layer method (Hoang et al., 2014). The experiment was conducted in triplicate.

2.3 pH stability test

To determine the stability of the phages at various pHs, the pH of tryptone soya broth (TSB) was adjusted using either 1M HCl or 1M NaOH to attain solutions with pHs of 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13. Each phage suspension (~10^9 PFU mL^{-1}) was mixed with an equal volume of the TSB and incubated at 30 °C for 24 h (Verma et al., 2009; Jun et al., 2013; Yamaki et al., 2014). After incubation, the phage titer was estimated by serial dilution and the double agar-layer method, as described above. Phage suspension maintained at pH 7 was used as control. The experiment was conducted in triplicate.

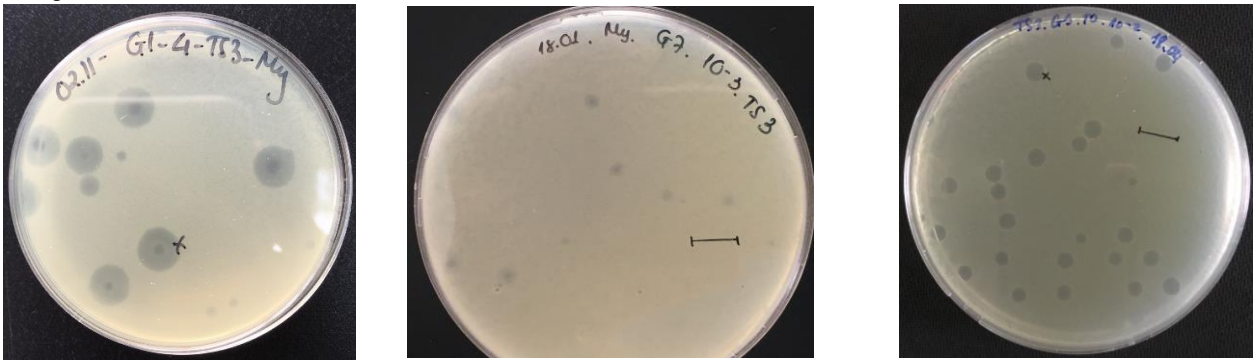
2.4 Organic solvent stability test

To assess the stability of the phages in organic solvents, a volume of each phage (~10^9 PFU mL^{-1}) was mixed with an equal volume of appropriate organic solvent (ethanol, chloroform, diethyl ether, SM buffer) and incubated at 30 °C for 1 h (Verma et al., 2009; Jun et al., 2013). The mixture was then centrifuged at 4 °C, 10,000 xg for 10 min. Phage titer was estimated by serial dilution and the double agar-layer method, as described previously. Phage suspension mixed with Phosphate Buffered Saline (PBS) was used as control. The experiment was conducted in triplicate.

3. Results

3.1. Phages isolated

Some phages G1, G7, and G9.1 were isolated, purified, and chosen. The other phages were not chosen since they resulted in small plaques (approximately 0.5 mm in diameter). Plaques of the chosen phages were shown in Figure 1.



G1 G7 G9.1

Figure 1. Phage plaques

3.2. Thermal stability of phages

Thermal and pH stability of phages were evaluated. G9.1 was found to be relatively thermostable at 20–37 °C (Figure 1), with phage activity being retained to approximately 80–95% compared to control. In the case of G7, phage activity was relatively similar to that of G9.1 at 20 °C, but, the activity was lower at 30 °C and 37 °C. On the other hand, activity of G1 phage decreased rapidly compared to that of the other phages when temperature increased. At high temperature of 50 °C, activity of all three phages decreased much to less than 50%. And at 65 °C, the activity completely appeared indicating that the phages were degraded totally. So, among three phages, G9.1 phage showed the highest stability at different temperatures. The phage activity of G9.1 was much greater than other published phages (Jun et al., 2013; Yamaki et al., 2014).

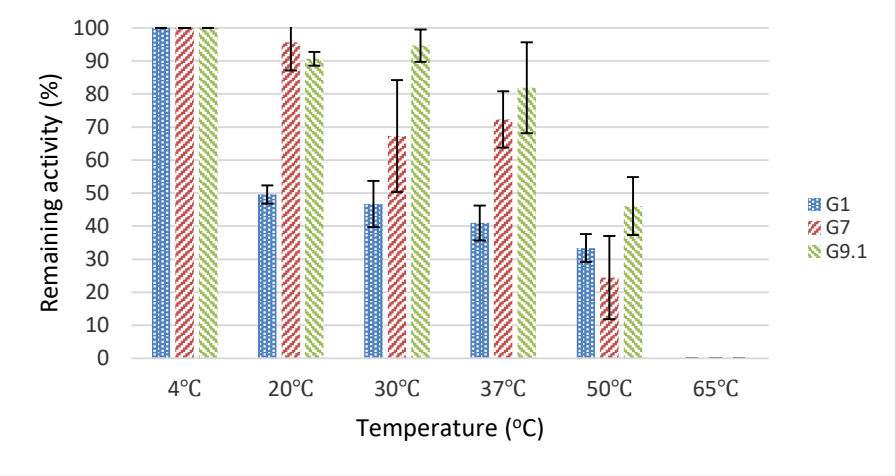


Figure 2. Effect of temperature on the viability of phages. Optimal condition at 4 °C was used as control. Error bars indicate 95% confidence intervals for the averaged values (n = 3).

3.3. pH stability of phages

A pH stability analysis showed that three phages were stable at pH 7–11, with relatively little difference in the phage titers with respect to control being observed (Figure 3). In contrast, significant reduction of phage activity was noted at acidic condition (lower than pH 7). Phage G7 showed a little higher stability than other phages at acidic condition. However, at pH 3.0, activity of all three phages losted completely. At alkaline condition, almost no difference of activity of three phages was observed.

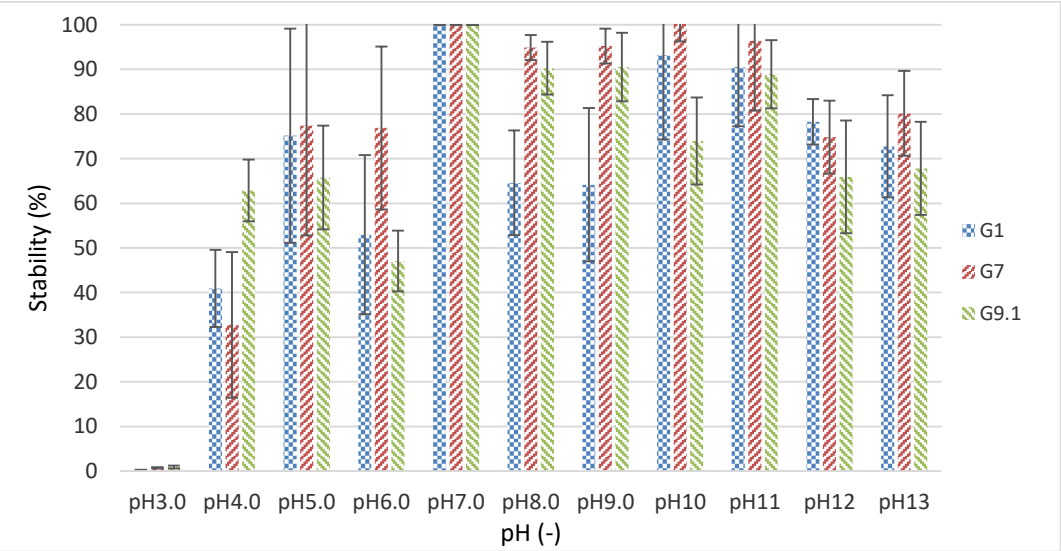


Figure 3. Stability of phages incubated at various pHs. Optimal condition at pH 7 was used as control. Error bars indicate 95% confidence intervals for the averaged values (n = 3).

3.4. Organic solvent stability of phage

No effect on phage activity of G7 and G9.1 was observed after 1 h of incubation with SM (Figure 4), while that of G1 phage decreased about 20%. In conditions, incubated either in chloroform or diethyl ether, activity of phages G1 and G7 much decreased to more than 50%. In contrast, activity of G9.1 phage still remained about 70% and 87%, either in chloroform or diethyl ether, respectively. At the incubation in ethanol, activity of all three phages completely losted. Many other published phages also completely lost their activity after treatment with ethanol (Verma et al., 2009; Jun et al., 2013). Thus, among three phages, G9.1 phage showed the highest resistance to organic solvents.

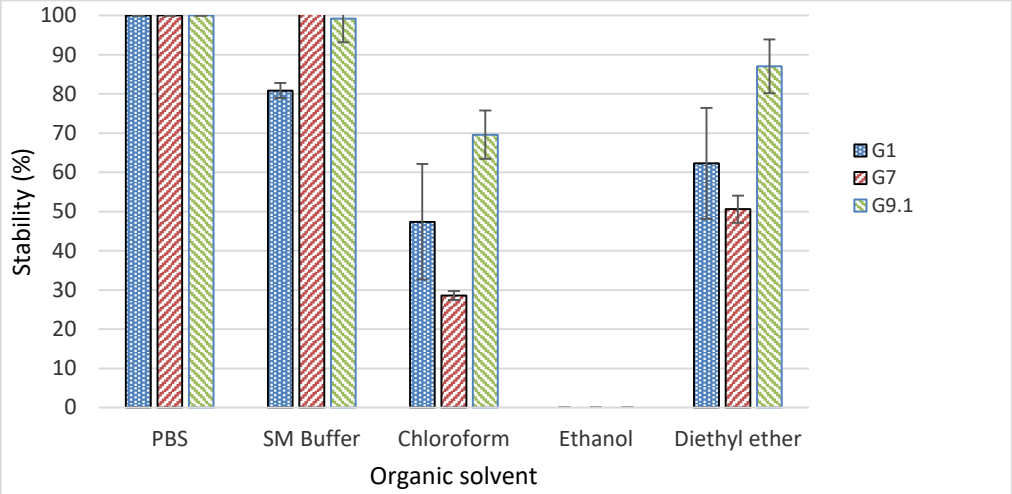


Figure 4. Viability of phages in the presence of various organic solvents. Incubation of phage in sterile PBS was used as control. Error bars indicate 95% confidence intervals for the averaged values (n = 3).

4. Discussion

E. ictaluri is one of the main causative agents of mass mortality among striped catfish. However, no effective method has been established to control *E. ictaluri* infections, except for the use of antibiotics. The high rates of resistance to antibiotics exhibited by this pathogen have led to a significant reduction in production. Moreover, increased levels of residual antibiotics have been detected in exported stock. Recently, many consignments from leading Vietnamese producers have been rejected by importers in markets such as the USA, Japan, South Korea, Canada, and Russia due to the presence of residual antibiotics in stock at levels higher than the approved limit. Notably, since August 2nd, 2017, all consignments of imported striped catfish have been tested for residuals of 89 types of antibiotics by the US Food and Drug Administration (2017). However, to the best of our knowledge, relatively little information regarding phage therapy in treatment of the disease in striped catfish has been reported. One of the first phages isolated from catfish farms in the Mekong delta against *E. ictaluri* was shown (Hoang et al., 2018).

To apply phage therapy at farm scale, phage-containing liquid or solid product should be investigated. To apply the phage-containing products at the farm scale, preservation condition of the products should be determined. Preservation of phages has been discussed previously. Generally, most phages maintain their stability when stored at low or freezing temperatures such as 4, -20, or -70 °C. Most research concerned phage preservation in dry or liquid buffer state for usage in the laboratory or medicine (Choiska-Pulit et al., 2015). However, phage preservation at ambient temperature is always challenging when the phage concentration decreases sharply in a period of days (Joczyk et al., 2011). In the current study, G9.1 was found to be quite thermostable, with its activity being maintained to approximately 85% at 37 °C. In addition, cryopreservatives also significantly support survival of phages (Ackermann et al., 2004). G9.1 showed a high resistance to organic solvents such as chloroform, and diethyl ether. These organic solvents will protect phage-containing products from contamination of microorganisms. Therefore, G9.1 is highly promising in the creation of low-cost phage-containing products stored at ambient temperature.

Striped catfish is relatively vulnerable to pond water conditions, with temperature and pH being two of the most important parameters. Temperature and pH ranges in pond water suitable for striped catfish are 25–32 °C (Duong, 2004) and 5.5–9.0 (Le et al., 2017), respectively. As investigated in the current study, activity of G9.1 was maintained at greater than 80% at these temperature and pH ranges. Therefore, this phage represents a highly appropriate antimicrobial agent against *E. ictaluri* on striped catfish farms. In future studies, this phage was selected to examine its stability and activity in *in vivo* control of *E. ictaluri* infection in striped catfish.

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Recovery of indium from waste streams by using supported liquid membranes with strip dispersion

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Two model systems of indium-containing waste stream were investigated. The first contained indium - the metal widely used to prepare indium – tin oxide (ITO) electrode for liquid crystal displays (LCDs), plasma displays and solar cells. The other also consisted of indium but in the presence of oxalic acid (2% v/v) which could be found in the industrial etching solution (Ishikawa et al., 2012).

We evaluated and compared the efficiency of SLMSD in separation of indium from the solution with and without oxalic acid. As the results, Indium could be recovered from the feed solution without presence of oxalic acid with very high purities. However, the efficiency of the membrane in case with 2%v/v oxalic acid decreased significantly. Therefore, some possible solutions to improve membrane permeability in later case will be proposed.

1. Introduction

Indium is widely used to prepare indium tin oxide (ITO) electrodes for liquid crystal displays (LCDs), plasma displays and solar cells. However, the reserve of indium on the earth is limited, estimated to be about 0.05 ppm and 0.072 ppm in the continental and oceanic crusts, respectively (Taylor and McLennan, 1985). Recovery of indium from industrial waste stream can be of great help for balancing the demand and supply of indium and as well for environmental pollution control. One of the viable resource for indium recovery is the etching waste solution from indium-consuming fabrication processes (Chou et al., 2016).

Liquid membrane, a process combining extraction and stripping (back-extraction), is the technique used in the present work for recovery of indium. For the process, extractant-containing oil is placed in between two aqueous solutions: the feed and strip solutions, and ions can be extracted from the feed to the oil and then be stripped from the oil to the strip solution. Because of its high selectivity for specific ions, the technique has attracted much research attention. However, practical applications of liquid-membrane processes are limited because of the difficulty in maintaining stable oil-water interfaces and in preventing extractant loss for long-term operations. To improve the stability of liquid membrane, various operation schemes have been introduced. One scheme that has been widely explored in recent years is the supported liquid membrane.

Supported liquid membrane (SLM) is an operation scheme that the extractant-containing oil is impregnated in the pores of a microporous hydrophobic membrane, which is placed in between the feed and strip solutions. Because of the water repellence of the hydrophobic membrane, the aqueous feed and strip solutions cannot penetrate into the membrane pores, and stable oil-water interfaces can thus form to allow for ion extraction and stripping. Though it is a promising scheme for practical applications, SLM still has a potential long-term stability problem of losing extractant-containing oil from the pores of the hydrophobic support. Therefore, the SLM operation scheme has been modified for further improvement of long-term operation stability. A promising technique is the SLM with strip dispersion (SLMSD) (Ho and Poddar, 2001), in which the aqueous strip solution is dispersed in extractant-containing oil and then introduced to contact with the hydrophobic membrane. Though the oil can penetrate into the membrane pores, the strip solution cannot because the size of the dispersed droplet of strip solution is much larger than the membrane pores. With the technique, the oil loss from the membrane pores to the feed can be greatly reduced by applying a pressure in the feed, and the extractant contained in the membrane pores can be replenished from the strip side in case it is lost to the feed due to its solubility in water or other reasons.

Conventional etchants are generally composed of inorganic acids (Graddon, 1956), such as HCl, HNO_3, the mixture of HCl, HNO_3 or H_2SO_4. In this case, indium can be recovered well by SLMSD using D2EHPA as the extractant. The extraction reaction can be described as following (Tsai and Tsai, 2012):

$$2In^{3+}_{(aq)} + 5(HR)_{2(org)} \rightleftharpoons In_2R_{10}H_{4(org)} + 6H^{+}_{(aq)} \quad (1)$$

with an equilibrium constant (K_{e1}) of 1.6×10^3, where HR represents the chemical formula of D2EHPA.
Over the past 20 years, organic acids have been used more often than inorganic acids, to avoid the problems of equipment corrosion and attack of metal layers. A widely used etchant in the current LCD industry is oxalic acid (Tsai and Wu, 2006) that forms complex with In^{3+} as described below:

$$In^{3+} + HC_2O_4^- \rightleftharpoons In(HC_2O_4)^{2+} \quad (2)$$

with an equilibrium constant (K_{e2}) of 1.2×10^3.
Because $K_{e1} \approx K_{e2}$, oxalic acid competes with D2EHPA for indium; therefore, the presence of oxalic acid slows down indium extraction. To improve the ion extraction efficiency, one can either decrease the oxalic-acid concentration to reduce the formation of indium-oxalate complex or increase the free D2EHPA content in the organic solution to enhance the formation of indium-D2EHPA complex.

2. Experimentals

2.1. Materials and solution preparation:

Feed solutions: $In_2(SO_4)_3$ (Sigma-Aldrich) was dissolved in water to prepare feed solution containing about 200 ppm of In^{3+}. To simulate the waste etching solution from LCD industries, 2 wt% of oxalic acid was added to the feed solution and H_2SO_4 (Sigma-Aldrich) was added to adjust the feed solution pH to 1.
Organic solutions: Di-(2-ethylhexyl) phosphoric acid (D2EHPA) (Merck) was used as extractant and Isopar-L (Exxon Mobil Chemical) was used as diluent. 2 vol% of 1-dodecanol (Acros) was added as modifier to increase the solubility of indium-D2EHPA complexes in Isopar-L.
Strip solutions: 5M of hydrochloric acid (HCl) (Sigma-Aldrich) aqueous solution was used as strip solution.
All the reagents were used as received without further purification, and the water used was all de-ionized.

2.2. Membrane modules:

Hydrophobic hollow-fiber modules with 6.35 cm in diameter and 20.3 cm in length were purchased from Membrana. The membrane surface area of the module was 1.4 m^2. The hollow fibers had outside diameters of about 300 μm and inside diameters of about 220 μm, and the fiber walls contained pores with an average pore size of 0.03 μm and a porosity of approximately 40%.

2.3. SLMSD

Figure 1 is the schematic presentation of the SLMSD set-up used in the present study. A commercial PP hollow fiber module (Liqui-Cel, membrane surface area 1.4 m^2) was used for the set-up. The feed solution was pumped into the tube side of the membrane module and the strip solution was dispersed in the extractant-containing oil and then pumped into the shell side.
The indium concentration in the aqueous phase was then assayed by using atomic absorption spectroscopy (Perkin Elmer AAnalyst200). All the extraction experiments were conducted with the same stirring rate at room temperature.

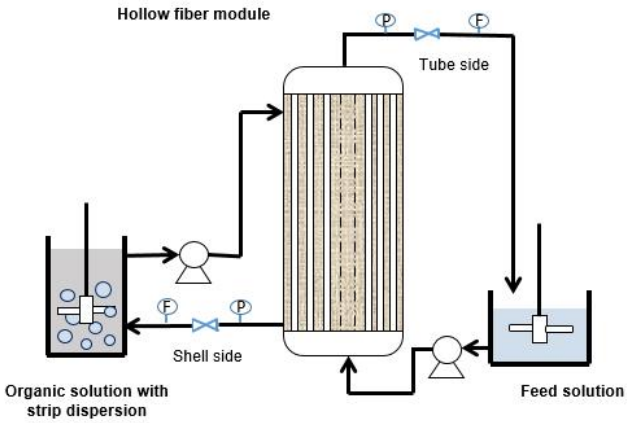

Figure 1: Schematic presentation of the experimental set-up

3. Results and discussions

3.1. SLMSD without oxalic acid

For the SLMSD from the solution without oxalic acid, the extraction rate was fast and almost all indium ions in the feed was extracted to the organic solution (Fig. 2) because of the high loading capacity of D2EHPA for indium (Sato and Sato, 1992).

3.2. SLMSD with oxalic acid

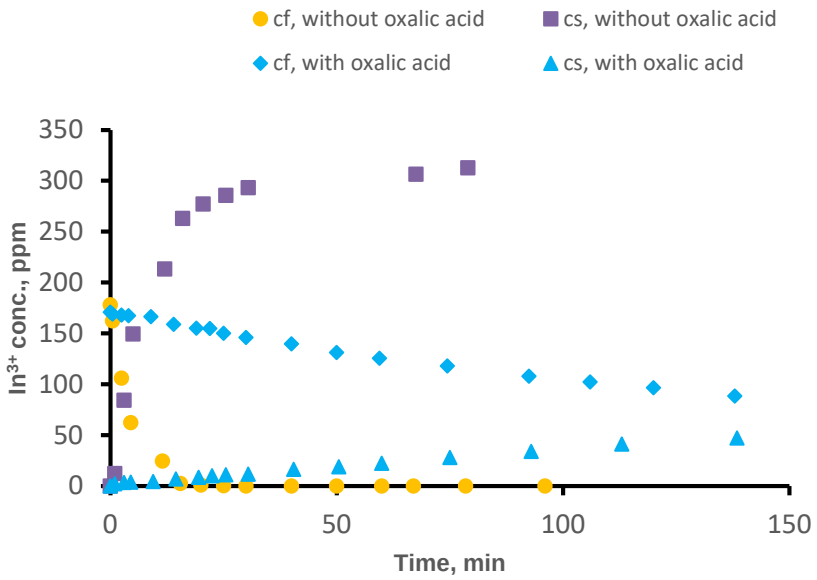

Figure 2: Time dependence of indium concentration in feed and strip solution (with oxalic acid)

In the presence of oxalic acid, indium can form complex with oxalic acid according to following equation:

$$In^{3+} + HC_2O_4^- \rightleftharpoons In(HC_2O_4)^{2+} \quad (3)$$

with an equilibrium constant (K_{e2}) of 1.2×10^3.

Because $K_{e1} \approx K_{e2}$, oxalic acid competes with D2EHPA for indium; therefore, the presence of oxalic acid slows down indium extraction (Figure 2).

To obtain indium permeability through the SLMSD, the following procedure were employed. With strong acidic solution as the strip solution, the ion concentration in the membrane at the interface of membrane and strip side is close to 0 and the ion flux through membrane can be described as $J = P.C$, where P the ion permeability and C represents the indium concentration in the feed.

Assume quasi – steady state and perform mass balance on the feed tank, one can obtain follow equation (Li et al., 2017):

$$\frac{dC}{dt} = -\frac{P.A}{V}C \quad (4)$$

Here t denotes the time, A represents the membrane area, and V the volume of feed cell.

The solution to above equation is $V * \ln\left(\frac{C}{C_0}\right) = -P.A.t$, where C_0 denotes the initial ion concentration in the feed tank. Plotting $V * \ln\left(\frac{C}{C_0}\right)$ versus t should give a straight line of which the slope is $-P.A$. After determining slope from the graph, P can be obtained from equation (4).

Replot data of Figure 2 in log scale we have Figure 3. As shown in this figure, for SLMSD, the whole process is a straight line.

As discussed above, the competition between oxalic acid and D2EHPA for indium complex formation slow down the extraction, therefore leads to the decrease of permeability in case with oxalic acid.

Table 1. Ion permeability in case without and with oxalic acid

	Permeability, m/min
Without oxalic acid	1.4×10^{-4}

With oxalic acid	3.1 x 10^{-6}

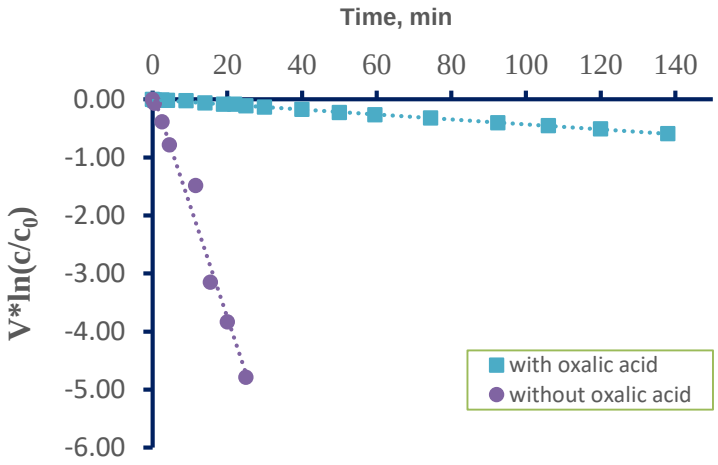

Figure 3: Replot data of Figure 2 in log scale

To increase permeability, one can either decrease the oxalic-acid concentration to reduce the formation of indium-oxalate complex or increase the free D2EHPA content in the organic solution to enhance the formation of indium-D2EHPA complex.

3.3. Improve indium recovery

3.3.1. Increase extractant concentration

When extractant concentration increases, extraction rate increases according to equation (1). However, the viscosity of solution also increases, leads to the decrease of diffusivity. Therefore, when D2EHPA concentration is raised from 0.2M to 0.6M, permeability increases 6 times, but from 0.6M to 1M, permeability increases only about 2 times.

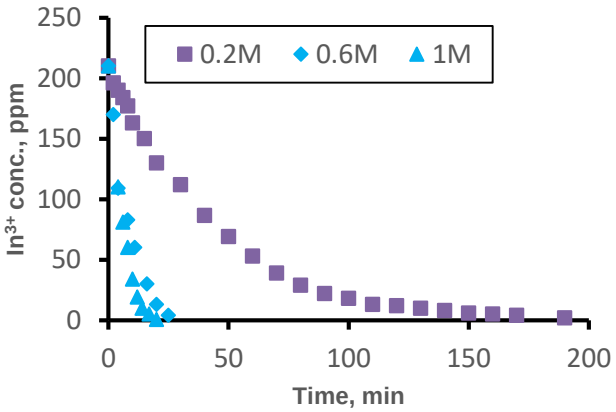

Figure 4: Effect of D2EHPA concentration on time dependence of indium in feed solution

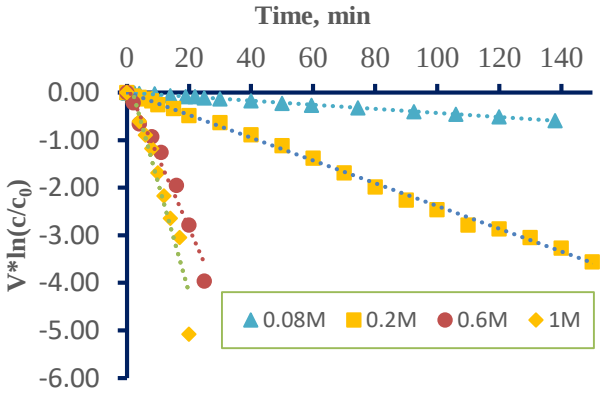

Figure 5: Replot data of Figure 4 in log scale

Table 2. Effect of D2EHPA concentration on ion permeability

D2EHPA concentration, M	Permeability, m/min
0.2	1.3×10^{-5}
0.6	8.0×10^{-5}
1	1.5×10^{-4}

3.3.2. Decrease oxalic acid concentration

The results discussed in the preceding section indicate that the presence of oxalic acid slowed down the extraction. To know how the concentration of oxalic acid affected the indium extraction performance, SLMSD experiments were performed with the feed solutions having different concentrations of oxalic acid. The results are presented in Figure 6.

The higher the oxalic acid concentration was, the lower permeability of indium. The results can be explained by that more $In(HC_2O_4)^{2+}$ was formed with higher oxalic acid concentration (according to reaction (2)), leading to less indium – D2EHPA complex formation.

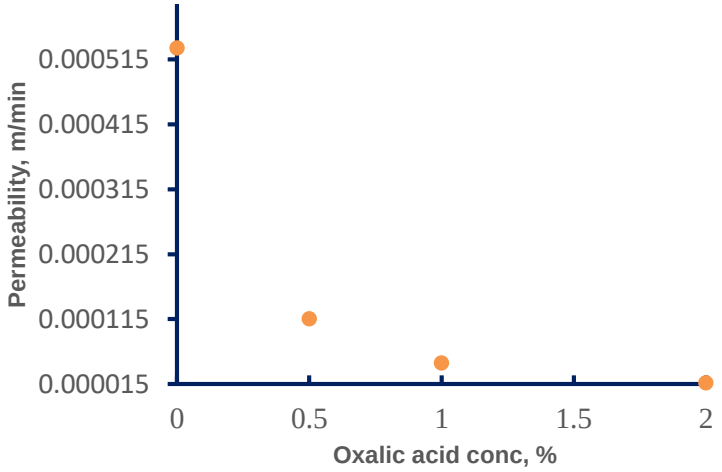

Figure 6: Effect of oxalic acid concentration on ion permeability

However, for etching waste stream, the concentration is fixed 2 wt% and can't be reduced because of the requirement of etching process.

Therefore, decomposition of oxalic acid was performed. Learning from previous research results reported in the literature (Mailen and Tallent, 1981), we oxidized oxalic acid with H_2O_2 according to equation (3), together with UV irradiation. Solvent extraction experiments were performed for the feed solutions that were treated with different oxidation conditions (various H_2O_2 concentrations and with or without UV).

Table 3. Indium extraction performance with different pretreatment conditions.

Pretreatment condition	Remaining [In^{3+}] concentration in the feed solution, ppm	Estimated oxalic-acid concentration, wt %
Without pretreatment	21	2.00
3 ml H_2O_2	18	1.69
3 ml H_2O_2 + UV (1 hour)	17	1.48
10 ml H_2O_2	11	1.13
10 ml H_2O_2 + UV (1 hour)	4	0.65
Without oxalic acid	<1	0.00

The results indicate that the pretreatment enhanced indium recovery efficiency. After the pretreatment, the lowest remaining indium concentration we obtained was about 4 ppm, requiring a volume ratio of 20% for H_2O_2/feed (10ml/50ml) and 1 hour of UV irradiation. Theoretically, higher degree of oxalic-acid degradation can be obtained with a pretreatment with higher volume ratio of H_2O_2/feed or longer UV irradiation. But, such high

H_2O_2 ratio or long UV irradiation may not be economically feasible for practical application. Therefore, SLMSD was investigated with 10 ml H_2O_2 and 1 hour UV irradiation as pretreatment.

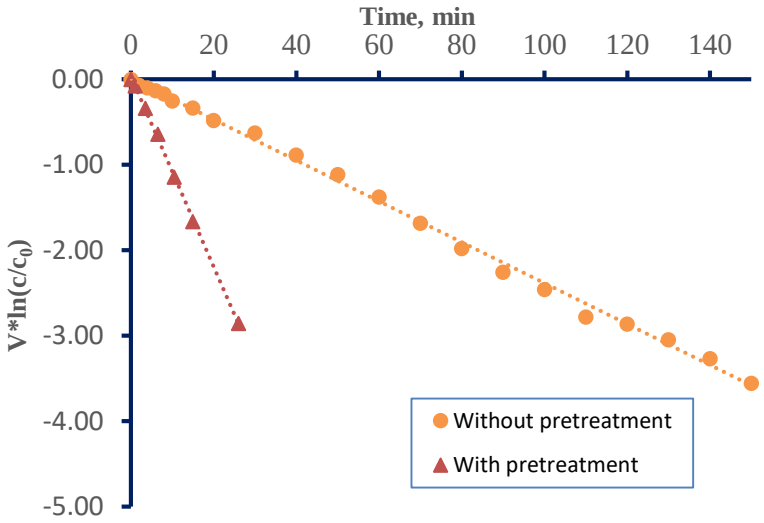

Figure 7: SLMSD performance with and without pretreatment ([D2EHPA] = 0.2M)

With pretreatment, the permeability of indium can obtain 7.8 x 10^{-5} m/min. (almost equal to the case D2EHPA concentration is 0.6M).

4. Conclusions

- Recovery of indium without oxalic acid and in the presence of oxalic acid were investigated
- The presence of oxalic acid slows down the permeability of indium and the effect of oxalic acid concentration on SLMSD performance was investigated
- Two possible solutions to improve ion permeability in the presence of oxalic acid were proposed, including raising extractant concentration and reducing oxalic acid concentration

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Pretreatment of Lignocellulosic Biomass for Value-Added Products: A Brief Review

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Today, the development of sustainable energy based on renewable biomass materials is one of the biggest global challenges. Moreover, biomass can be converted to various products having a wide range of applications. However, the natural structure of lignocellulosic biomass is highly resistant to outside impacts. Thus, the conversion of biomass into value-added products is ineffective without pretreatment processes. Pretreatment methods were proposed to disrupt the structure of lignocellulose in several pathways depended on their chemical nature. The advantages as well as disadvantages, the potential for industrial application of various pretreatment technologies are discussed in this review article.

1. Introduction

The concern over the environment protection and depletion of petrochemical feedstock have spurred research and development toward new technologies. Thus, the modern industrial production has to meet strict requirements such as friendly to the environment, high sustainability, using renewable materials. The gradual transition towards a bio-based economy is one of the most potential solutions for the problems mentioned earlier. Lignocellulosic biomass is a renewable and extraordinarily abundant resource, which includes a wide range of bioresources such as agricultural residues, forest-industrial residues, energy crops, and municipal solid waste. Lignocellulosic biomass is composed of carbohydrates (cellulose and hemicellulose), lignin, and other extraneous components (proteins, lipids, and inorganic substances). Recently, a concept of biorefinery is integrated with the conversion of lignocellulose feedstocks into various products with a primary focus on biofuels (bioethanol, biodiesel, and biogas).
Moreover, biorefinery also produces other value-added bioproducts (food, feed, platform chemicals, and materials) and bioenergy (heat and power). However, several difficulties are associated with the conversion of lignocellulosic biomass. The main factor is the recalcitrance due to the complex integration of main components in biomass structure. As a result, pretreatment technologies have been developed to overcome the physical and chemical barriers of the native recalcitrance in lignocellulose. The paper aim is to provide a comprehensive review of most common pretreatment methods.

2. Structure of lignocellulosic materials

Lignocellulosic biomass consists of carbohydrates (cellulose and hemicellulose), lignin, and other constituents (Wertz et al. 2017). The fractions of these main components depend on species, environment, position, growth, and maturity (Table 1). Besides, other constituents such as protein, pectin, and ash make up a minor proportion (Sun 2010).

2.1 Cellulose

Cellulose is the most abundant polymer containing about 30-50% of lignocellulosic biomass (Ramesh et al. 2017). The cellulose molecule is a linear homopolymer of D-anhydroglucopyranose units linked together by β-(1,4)-glycosidic bonds (Brodeur et al. 2011). In the cellulose structure, the partitions between crystalline and amorphous zones are non-uniform (Wertz et al. 2017). The crystalline structure of cellulose inhibits the enzymatic hydrolysis (Kim et al. 2016).

Table 1: Composition of some lignocellulose

Source(%)	Cellulose	Hemicellulose				Lignin	Extract
		Xylan	Mannan	Galactan	Arabianan		
Spruce wood	41,9	6,1	14,3	-	1,2	27,1	9,6
Pine wood	37,7	4,6	14,3	-	-	27,5	10,8
Birch wood	38,2	18,5	7,0	-	-	22,8	4,8
Poplar wood	49,9	17,4	1,2	1,2	1,8	18,1	-
Corn waste	36,4	18,0	4,7	1,0	3,0	16,6	7,3
Wheat straw	38,2	21,2	0,3	2,5	23,4	13,0	
Straw	34 - 38	32 - 40				12	

2.2 Hemicellulose

Hemicellulose is a chemically heterogeneous polymer, which is predominantly composed of 5-carbon and 6-carbon monosaccharides. Although the composition of hemicellulose varies in different species, xylose is the principal constituent of hemicellulose fractions. The content of hemicellulose in lignocellulosic biomass is in a range of 20-35%. Due to the branched structure and low degree of polymerization, hemicellulose is easily degraded comparing with cellulose (Yang et al. 2013).

2.3 Lignin

Lignin is a complex amorphous polymer, which is constructed from three predominant monolignols (p-courmaryl, coniferyl, and sinapyl alcohol) and a minor of other compounds. Precursors in the complex matrix of lignin link to others in various linkages. The β-aryl ether bonding is the most popular linkage in the lignin structure (Yang et al. 2013). Lignin in higher plant cell has no amorphous region. Lignin also associates with carbohydrates to form lignin carbohydrate complex (LCC) (Van Soest 2006). Also, lignin acts as a cement for the binding between cellulose and hemicellulose to form a three-dimensional structure of the cell wall. Hence, it provides the rigidity and stiffness of cell walls (Calvo-Flores et al. 2015).

2.4 The role of pretreatment

In order to enhance the efficiency of conversion processes, “pretreatment” was proposed to disrupt the recalcitrance of lignocellulosic biomass. Some typical results of pretreatment can be obtained such as the removal of lignin, reduce the crystallinity of the biomass, and expose the larger surface area (Kim et al. 2016). Therefore, pretreatment plays a critical role in the valorization of lignocellulosic biomass. Pretreatment is expected to meet the following requirements: (1) reasonable capital and operating costs, (2) high lignin recovery, (3) minimize the formation of inhibitors, (4) avoiding the loss of sugars (Kim et al. 2016).

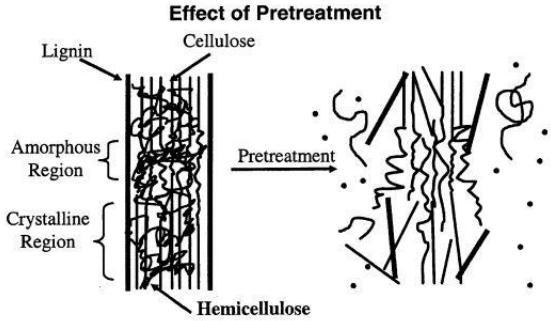

Figure 1. Convert biomass into fuel through pretreatment, reproduced with permission from (Mosier et al. 2005)

3. Pretreatment methods

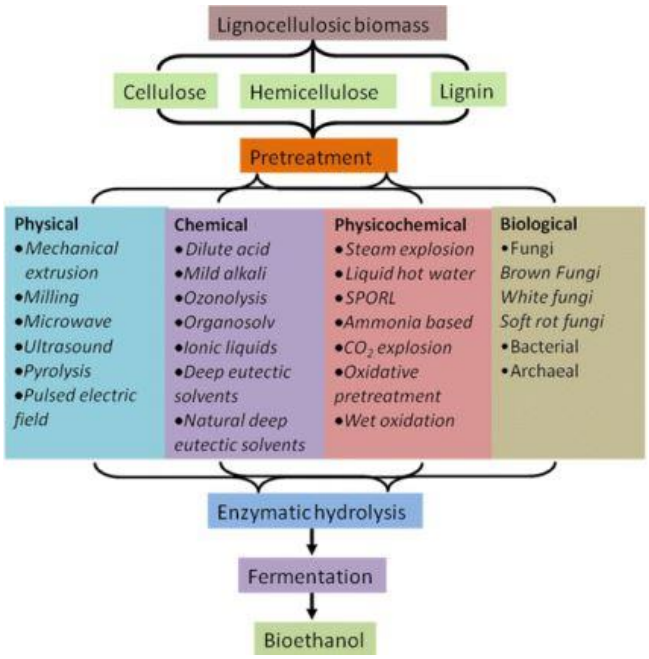

Figure 2. Overview of pretreatment processes, reproduced with permission from (Kumar and Sharma 2017)

3.1 Physical pretreatments

In general, physical methods disrupt the structure of lignocellulosic biomass by the reduction of particle size and crystallinity. Hence, the mass transfer characteristics, surface area, and enzyme penetration are noticeably improved. However, physical alteration is inefficient to cleave the linkages among three main constituents, which creates a closely enmeshed structure in biomass (Che Kamarludin et al. 2014). Moreover, the cost of physical pretreatments is usually expensive due to energy consumption (Brodeur et al. 2011). As a result, physical methods are not preferred. In some cases, mechanical pretreatments can be applied as a preliminary pretreatment followed by chemical pretreatments (Che Kamarludin et al. 2014).

Physical pretreatments include conventional mechanical methods (grinding and milling) and nonconventional methods (i.e. microwave, ultrasound, and pulsed electric field) (Kumar and Sharma 2017).

3.2 Chemical pretreatments

3.2.1 Acid

Acid pretreatments occur with the presence of mineral acids (i.e. H_2SO_4, HCl, and H_3PO_4) or organic acids. Acids disrupt biomass structure due to the hydrolysis of carbohydrates, especially hemicellulose (Liu and Wyman 2004). Both dilute and concentrated acids can be used to pretreat lignocellulosic biomass (Yang and Wyman 2008). The higher concentrations require milder conditions. This method is not suitable for lignin recovery since it only target on sugar-based components (Jung and Kim 2015). Besides, the formation of toxic compounds and corrosion are most significant disadvantages of acid pretreatment (Pedersen et al. 2010).

3.2.2 Alkaline

Alkaline pretreatment is more effective in herbaceous and agricultural plants than woody plants due to the difference in lignin quantity. Alkaline pretreatment is traditionally applied to treat nonwood biomass such as straw, sugarcane bagasse, and flax. The commonly used alkalis for pretreatment are NaOH, KOH, NH_3, and $Ca(OH)_2$. Among these alkali mediums, NaOH is the most effective chemical to delignify biomass (Sindhu et al. 2015). Depolymerization action occurs principally by the removal of α and β-aryl ether bonds and saponification of intermolecular ester bonds cross-linking hemicellulose and lignin (Calvo-Flores et al. 2015). In mild conditions, no further chemical modification occurs beyond the two mechanisms mentioned above. The considerable amount of free phenolic groups in grass increases the solubility of lignin into alkali solution. The type of alkali,

the concentration of alkali in pretreatment solution, biomass loading, temperature, and pretreatment time are important factors affecting the yield of alkaline pretreatment (Sun 2010).

3.2.3 Organosolv

Organosolv can extract lignin from biomass with mixtures of organic solvents and a small amount of catalysts (Calvo-Flores et al. 2015). Ethanol organosolv pretreatment is suite for a wide range of biomass feedstocks such as softwoods, hardwoods, and agricultural residues (Wyman 2013). Both low-boiling-point solvents such as ethanol, methanol, or acetone and high-boiling point solvents such as glycols can be used. The most preferred solvents are primary alcohols and carboxylic acids (Johansson et al. 1987). Acids and bases, which are common catalysts, added in organosolv to achieve higher yield. Under alkali-catalyst conditions, the cleavage of β-ether bonds is a priority, whereas it is required a robust acidic environment where the removal of α-ether bonds are more referred. Organic solvents can be recycled several times (Sarkanen and Hoo 1981). Lignins generated from this method are relatively pure, undegraded, and free of sulfur. Some organic solvents are flammable and toxic (Galbe and Zacchi 2007).

3.2.4 Ionic Liquids

Ionic liquids (ILs) are defined as organic salts with no vapor pressure or fused salts liquid containing only ions. ILs have several unique properties, such as high recyclability, low volatility, high thermal stability, less toxic formation, and nonflammability. A wide range of solubility and miscibility is the most useful property of ILs, which make ILs become potential alternatives for traditional flammable and volatile solvents (Negi and Pandey 2015).

3.2.5 Deep Eutectic Solvents

Deep eutectic solvents have many characteristics similar to ionic liquids. The difference between DESs and ILs is that DESs are not entirely composed of ionic species as ILs. A deep eutectic solvent is defined as a fluid containing two or three components that are able to self-association through hydrogen bonds. These components form a eutectic mixture with a melting point lower than that of each individual constituent (Kumar and Sharma 2017).

3.2.6 Ozonolysis

Ozone treatment mainly targets lignin but negligibly affects the carbohydrates (Kumar et al. 2009). Ozonolysis is carried out in ambient temperature and pressure. Also, this method does not produce any toxic inhibitors. Ozonolysis is not preferred in large scale due to the high cost of ozone (Kumar and Sharma 2017).

3.3 Physicochemical pretreatments

3.3.1 Liquid hot water

Hot water pretreatment (also called hydrothermal pretreatment) is usually carried out at high temperature (160-240°C and pressure (1-3.5MPa), in a period of few minutes to hours. The pH condition should be stable in the range of 4-7 to reduce the degradation of sugars (Jeong and Lee 2015). The hydronium ions, which are generated from water auto-ionization, promote xylan depolymerization and remove the acetyl groups. Subsequently, removed acetyl groups catalyze the hydrolysis of hemicellulose. At high temperature, part of acetyl groups are converted into acetic acid support to the hydrolysis of glycosidic bonds in hemicellulose and the β-ether linkages in lignin (Yang et al. 2013) .Because hot water pretreatment does not use any chemicals excepts water. It cannot be denied that this hydrothermal treatment is an environmentally friendly process, compared with other pretreatments. Moreover, corrosion and chemical recycling problems are eliminated. Acid-insoluble lignin, which make up a substantial proportion of total lignin, remain in the biomass (Jeong and Lee 2015).

3.3.2 Steam explosion

The mechanism in steam explosion is similar to the hot-water pretreatment. Saturated steam under high pressure penetrates the cell walls by diffusion, and then the pressure is suddenly released to ambient pressure (Agbor et al. 2011). This leads to the evaporation of condensed moisture in the cell walls and consequently alter the macrostructure of biomass (Wang et al. 2015). Steam explosion causes degradation of hemicellulose and partial hydrolysis of lignin (Calvo-Flores et al. 2015). This method does not use any chemicals except water, thus offer a mild pH of reaction media, which minimal the equipment corrosion (Calvo-Flores et al. 2015). A typical condition of steam explosion is at 200-450psig for 10 minutes. The high-pressure activates an autohydrolysis reaction as described above. The reaction temperature and retention time are important parameters in this pretreatment method (Yang et al. 2013). Besides a significant fraction of hemicellulose is

removed, there are other issues such as the generation fermentation inhibitors during the process and low delignification yield (Wang et al. 2015).

3.3.3 Ammonia-based methods

Ammonia pretreatments are similar to steam explosion excepting that water in steam explosion is replaced by ammonia(Rabemanolontsoa and Saka 2016). The pressure is released rapidly at the end of this pretreatment process resulting in decrystallization in cellulose, hydrolyzation and alteration of lignin structure. Ammonia Recycle Percolation and Ammonia Fiber Explosion are commonly applied among various methods employing ammonia (Jeong and Lee 2015).

Ammonia Recycle Percolation (ARP): The ARP has been developed to remove lignin as well as increase the susceptibility of biomass to enzymes. The typical condition of this process is 5-15% ammonia, at a temperature of 150-180 °C, and under a pressure of 2.3 MPa (Yang et al. 2013). Ammonia solution is passed through a vessel packed with biomass with a flow rate of 1-5 mL/min, and the residence time of 10-90 min. ARP is capable of removing over 80% lignin and solubilizing up to 50% of hemicellulose in woody materials (Wang et al. 2015).

Ammonia Fiber Explosion (APEX): The pretreatment conditions of this method are mild; 60-90°C in liquefied anhydrous ammonia having the dosage of 1-2 kg of ammonia/kg of dry biomass, and the residence time is 10-60 min (Wang et al. 2015). The primary effect of APEX is the depolymerization of hemicellulose and lignin. The used ammonia can be recovered. The degradation of sugar-contents is minimized because all the products after pretreatment remain in a solid phase. APEX proceeds under high pressure and involves phase changes. Consequently, the investment for pretreatment reactors is expensive and hard to control. Thus, it is not feasible to implement APEX at commercial scale due to the aforementioned reasons, which might cause some operating issues and significantly increase both investment and operating costs (Yang et al. 2013). Besides, AFEX pretreatment is not as substantial as NaOH or lime pretreatment in lignin removal (Sindhu et al. 2015).

3.3.4 Carbon dioxide explosion

The supercritical CO_2 is pumped to a high pressure and temperature reactor containing biomass (Kim and Hong 2001). The high-pressure CO_2 forms carbonic acids to hydrolyze the hemicellulose. Then the release of pressure also causes physical alteration which increase accessibility to biomass structure (Zheng et al. 1995). The moisture of biomass is the controlling factor in this pretreatment (Kim and Hong 2001). The outstanding of this pretreatment is low cost of carbon dioxide, low operating temperature, high solid capacity, and no byproducts formation. However, CO_2 explosion is unlikely to reach the industrial scale because of the high capital and maintenance cost of reactor (Agbor et al. 2011).

3.3.5 Wet oxidation

Wet oxidation involves the use of oxygen to oxidize compounds in biomass structure. There are two possible reactions during wet oxidation process: low temperature hydrolysis and high temperature oxidation reaction. A typical condition of this method is 12 bar, 195 °C for 10 to 20 minutes. Besides, the size of lignocellulosic materials has to be reduced to about 2 mm in length (Pedersen and Meyer 2009). Wet oxidation alters the biomass structure by solubilizing of hemicellulose and decomposition of lignin to carbon dioxide, water, and carboxylic acids (Banerjee et al. 2009). The most setback of this pretreatment is the formation of inhibitors such as succinic acid, glycolic acid, acetic acid, phenolic compounds. Therefore, the combination between wet oxidation and another pretreatment is applied to enhance the pretreatment effect. For instance, the presence of alkaline medium is proved to reduce the byproducts formation (Banerjee et al. 2011).

3.4 Biological pretreatments

Biological pretreatment is the use of microorganisms (mainly fungi) to fractionate biomass (Capolupo and Faraco 2016). Some fungi species such as white, brown, and soft-rot selectively degrade lignin leading to the disruption in biomass structure and minimizing the loss of carbohydrate (Brodeur et al. 2011). The main advantages of biological pretreatments are mild conditions, high selectivity, environmental-friendly. In contrast, the drawbacks of microorganisms such as long pretreatment time, high price, strict condition maintenance, and low rate of hydrolysis may limit its application in large scale (Palmqvist and Hahn-Hägerdal 2000).

4. Conclusions

The increasing use of biofuels will contribute to sustainable development by reducing greenhouse gas emissions as well as using non-renewable resources. Lignocellulosic biomass, composed of agricultural and forestry residues, is a good source of abundant sugar to ferment into a biofuel known as bioethanol. Various pre-treatment technologies for lignocellulose biomass have been studied and implemented to improve ethanol production. A difficult problem in these technologies is the presence of lignin, a major inhibitor of cellulose and

hemicellulose hydrolysis. This has led to extensive research on various pretreatment methods, which are based on the principles of physical, chemical, physicochemical, and biological. This paper analyzes different methods with various advantages and disadvantages. Therefore, depending on the needs, raw materials, and equipment available to choose the method that is appropriate for the purpose of production.

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The Induction of *Beta vulgaris* L. Adventitious Roots In In Vitro Culture for Betalains

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Betalains have gained the attention as natural food colorants because of their high anti-oxidative and free radical scavenging activities. This study focused on the process to induce the adventitious roots from *Beta vulgaris* L. seedlings. IAA 0.5 mg/L was the best treatment to induce adventitious roots from hypocotyls and cotyledons in agar culture. In liquid culture, growth index of adventitious roots were highest at the fourth week of cultivation but betalains contents were highest at the second week of cultivation. 5 g/L inoculum density of adventitious roots produced the highest of growth index (2.27) and betalains concentration (0.33 mg/g FW). Sucrose concentration at 20 g/L and 30 g/L showed little significant difference in growth index as well as betalains contents.

Keywords: adventitious roots, *Beta vulgaris* L., betalains, cotyledons, hypocotyls

1. Introduction

For decades, plant cell culture has been utilized as a method to produce valuable secondary metabolites. Betalains from *Beta vulgaris* L. (beetroot) are water-soluble nitrogen-containing pigments, which comprise the red-violet betacyanins and the yellow betaxanthins (Strack et al., 2003). They have the bright red color and are used commercially as food colorants in Europe and USA (Castellar et al., 2006). Betalains also attract the attention of researchers because of their antioxidant and radical scavenging properties for protection against certain oxidative stress-related disorders (Strack et al., 2003). Beside hairy root cultures, adventitious roots which developed from different parts of plants are used to produce secondary metabolites. For instance, Fazal et al. (2014) optimized adventitious root cultures of *Prunella vulgaris* for production of phenolics and flavonoids; adventitious roots were successfully induced from *Panax ginseng* by Sivakumar et al. (2005) for ginsenosides; Betsui et al. (2004) obtained anthocyanins from adventitious roots of *Raphanus sativus* L. *in vitro*.

However, there are lacks of scientific information about beetroot adventitious roots culture for betalains production. People used to focus on micropropagation of beetroots (Sullivan et al., 1993; Bekheet et al., 2007; Ghosh et al., 2013) or hairy root cultures (Escribano et al., 1998; Kanner et al., 2001; Pavlov et al., 2003). So, in this research, we studied on the induction of adventitious roots at various concentration of auxins (NAA, IBA and IAA) from different types of explants, and liquid culture for biomass and betalains.

2. Materials & methods

2.1 Materials: cotyledons, hypocotyls and roots of *Beta vulgaris* L. seedlings.

2.2 Methods

Seeds sterilization. The *Beta vulgaris* L. seeds were immersed in 2% sodium hypochlorit) with 2 -3 drops of Tween 20 in 15 minutes before rinsing with distilled water. After that, the incubation in distilled water at 60^{o}C in 20 minutes was required to shorten germination time. The sterilized seeds were aseptically cultured on growth regulator-free MS medium (Murashige and Skoog, 1962) with 2% sucrose, 6 g/L agar agar, pH 5.8 and incubated in dark at 25^{o}C for germination. 1- week old seedlings were used as sources of explants in further experiments.

Root induction. Sections of cotyledon, hypocotyl and root (1 cm in length) from *in vitro* seedlings were used as explants for adventitious roots induction by transferring to MS medium supplemented with different types and concentrations of auxins. The culture condition was maintained with 16h photoperiod, 4000 lux, at 25^{o}C.

Liquid cultures. 14 day-old adventitious roots from root explants were transfered to liquid medium for biomass and betalains. The factors were tested to determine optimal condition for biomass as well as betalains yield included: inoculum density and sucrose concentrations (20 and 30 g/L). Each glass bottle contained 40 mL of liquid MS medium with 0.2 mg adventitious root as initial inoculum weight. For inoculum density treatment, the inoculum size varied among 0.12 g, 0.20 g and 0.28 g of roots in 40 mL of liquid medium (3, 5 and 7 g/L respectively). The cultures were shaked at 110 rpm at 25^{0}C under light (4000 lux) in 5 weeks.

Biomass and betalains accumulation. The proliferation of adventitious roots was assessed by growth index in fresh weight after each week. The adventitious roots were collected to dertemine betalains by mass spectrometry of Nilsson (1970).

Data analysis. Statistical analysis was performed with SPSS Version 20.0 statistic software package. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1 Effect of auxins on adventitious root induction

Adventitious roots formed from all kinds of explant even on free plant growth regulator medium. The response of root explants with auxins was better than the others. Hypocotyl explants were more suitable than cotyledon explants in adventitious root formation. The numbers of root per explant were different with the different kinds and concentrations of auxins. In MS medium with NAA 0.5 mg/L, the numbers of root per explant from hypocotyls were 16 and 15.83 from cotyledons. Whereas, IAA at various concentrations were suitable for root induction from root explants (21.0, 26.2 and 24.8 roots per explant on medium with IAA 0.5, 1.0 and 2.0 mg/L respectively) (Table 1). Roots on medium with NAA were red with many root hairs, roots with IAA treatments appeared with a thicker shape and brighter red color (Figure 1). However, callus could be observed in hypocotyl and cotyledon explants and shoots formed from any treatments in hypocotyl explants.

Table 1. Effects of auxins on adventitious root induction from explants after 3 weeks of culture

Auxin (mg/L)			Root explants		Hypocotyl explants		Cotyledon explants	
NAA	IBA	IAA	Response (%)	Avg. no of roots	Response (%)	Avg. no of roots	Response (%)	Avg. no of roots
-	-	-	100.00	9.00^{d}	50.00	1.20^{e}	42.42	5.80^{c}
0.5	-	-	100.00	20.70^{b}	83.33	16.00^{a}	55.00	15.83^{a}
1.0	-	-	100.00	15.30^{c}	87.50	5.80^{cd}	68.00	16.74^{a}
2.0	-	-	100.00	25.00^{a}	71.43	2.60^{e}	34.62	9.50^{b}
-	1.0	-	100.00	20.00^{b}	71.43	2.60^{e}	70.83	8.70^{bc}
-	2.0	-	100.00	16.00^{c}	71.43	9.40^{b}	42.86	5.60^{d}
-	3.0	-	100.00	10.20^{d}	73.33	9.80^{b}	57.14	9.80^{b}
-	-	0.5	100.00	21.00^{b}	92.31	5.80^{cd}	58.33	8.90^{b}
-	-	1.0	100.00	26.20^{a}	77.78	9.20^{b}	64.71	5.40^{d}
-	-	2.0	100.00	24.80^{a}	86.67	8.60^{bc}	68.75	5.80^{c}

Values not sharing a common letter are significantly different at P<0.05 based on Duncan method

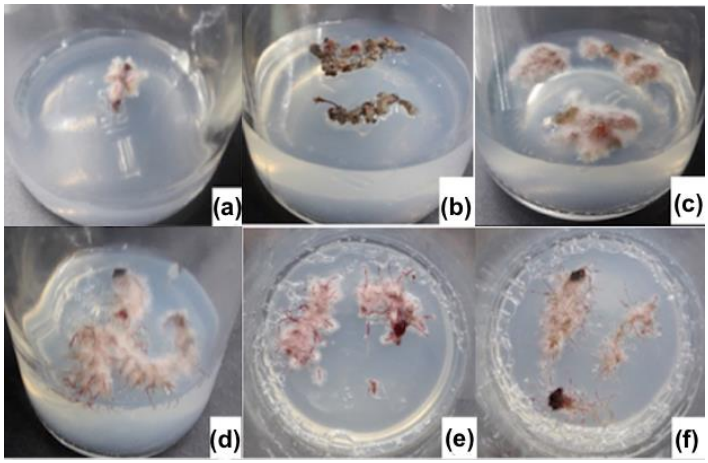

Figure 1: Adventitious roots from root explants after 3 weeks of culture on MS medium with auxins (a) NAA 0.5 mg/L; (b) NAA 1.0 mg/L (with callus); (c) NAA 2.0 mg/L; (d) IAA 0.5 mg/L; (e) IAA 1.0 mg/L; (f) IAA 2.0 mg/L

Adventitious roots induction in vitro depends on exogenous auxins and explants. All kinds of explant from Beta vulgaris L. seedlings responsed with the addition of auxins in adventitious roots induction, especially IAA and NAA. The same as research of Sullivan et al. (1993) on Beta vulgaris L., cotyledon explants had less rooting percentage than hypocotys, IAA (1.0 mg/L) and NAA (1.0 mg/L) were more effective in inducing roots. The number of roots per explant (hypocotyls and cotyledons) in our experiments were higher, which may be due to the different cultivars and the age of seedlings

3.2 Liquid culture

3.2.1. Effects of initial inoculum density

In liquid cultures, growth index of roots with inoculum density at 5 g/L and 7 g/L significantly increased in week 2 and week 3 and both reached maximum at week 4 (2.27 and 1.76 respectively) then started to decline from week 5. Whereas the growth index of roots in the treatment with initial inoculum density at 3 g/L continued to increase until week 5 (1.49) (Figure 2).

Betalains concentration increased at week 2, dropped at week 3, increased again at week 4 and then reduced in the experiments with 3 and 7 g/L of initial roots. In the experiment with root initial inoculum density 5 g/L, betalains concentration kept stable from week 4 to week 5. The highest concentration of betalains was at the second week of cultures (0.32, 0.33 and 0.33 mg/g FW respectively with initial inoculum density 3, 5 and 7 g/L) (Figure 3).

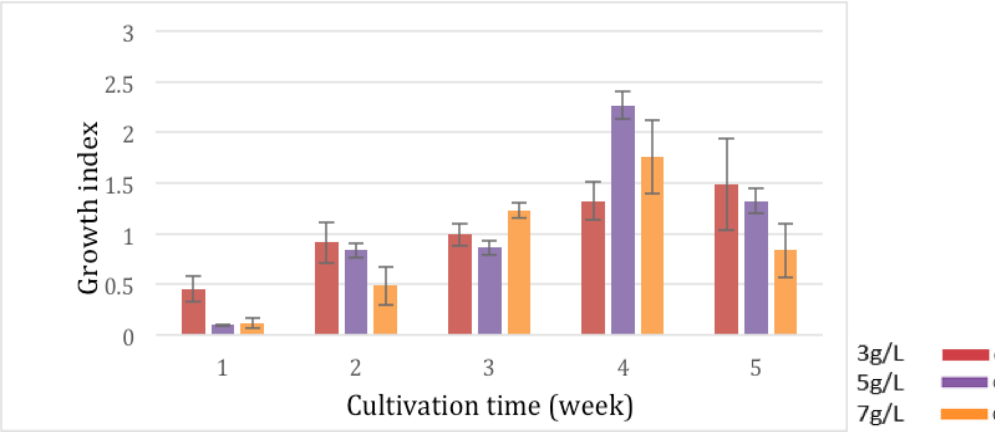

Figure 2: Growth index of Beta vulgaris L. adventitious root in liquid culture with different initial inoculum density

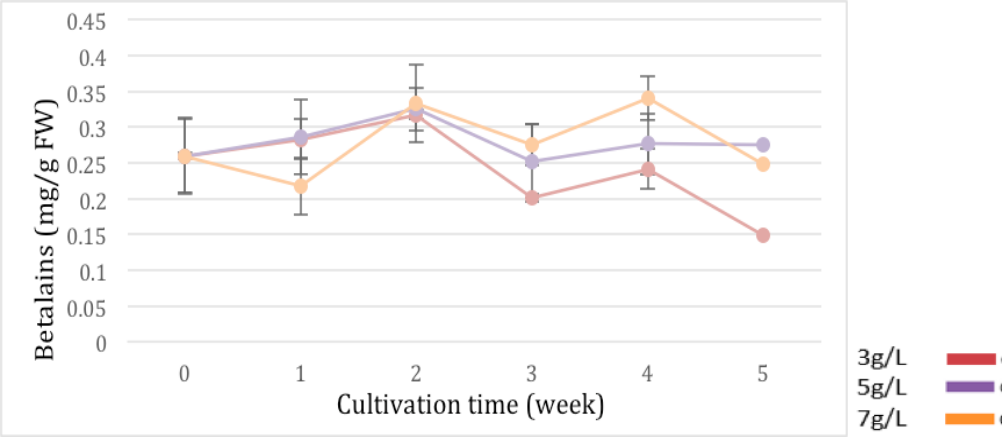

Figure 3: Betalains concentrations of Beta vulgaris L. adventitious roots in liquid culture with different initial inoculum density

The initial inoculum density and sucrose concentration were factors that do effect on biomass and betalains accumulation of B.vulgaris L. roots in liquid culture. The inoculum density 3 g/L seemed be so low that did not sufficiently maintain betalains biosynthesis after the first two weeks of culture while 5 g/L and 7 g/L inoculum density almost showed more appropriate for root proliferation as well as betalains accumulation.

3.2.2. *Sucrose concentration*

For the first three weeks, samples supplemented with 20 g/L sucrose had a higher growth index compared with that of 30 g/L sucrose. However, biomass accumulation at week 4 (1.92) was lower than samples with 30 g/L sucrose (2.27) and both two treatments of sucrose concentration stopped growing at week 5.

Betalains concentration from roots in experiment with 30 g/L sucrose was stable during culture time, roots were dark red (Figure 4). However, roots in the treatment with 20 g/L sucrose synthesized a significant amount of betalains in week 2 and 3 (0.4 and 0.34 mg/g FW respectively) (Figure 5).

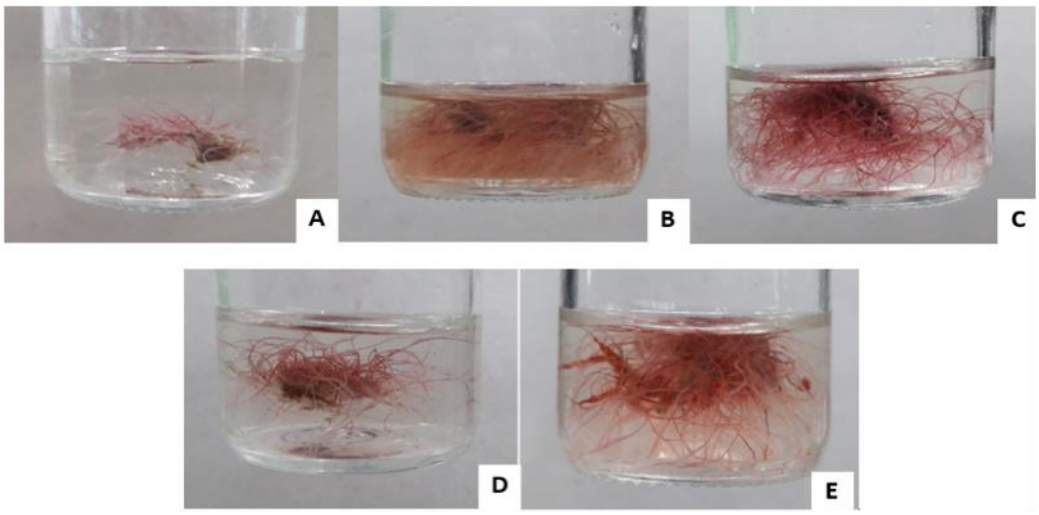

Figure 4. Adventitious roots in quid culture
A, B and C: Initial inoculum density at 3 g/L, 5 g/L and 7 g/L respectively
D and E: Sucrose concentration at 20 g/L and 30 g/L respectively

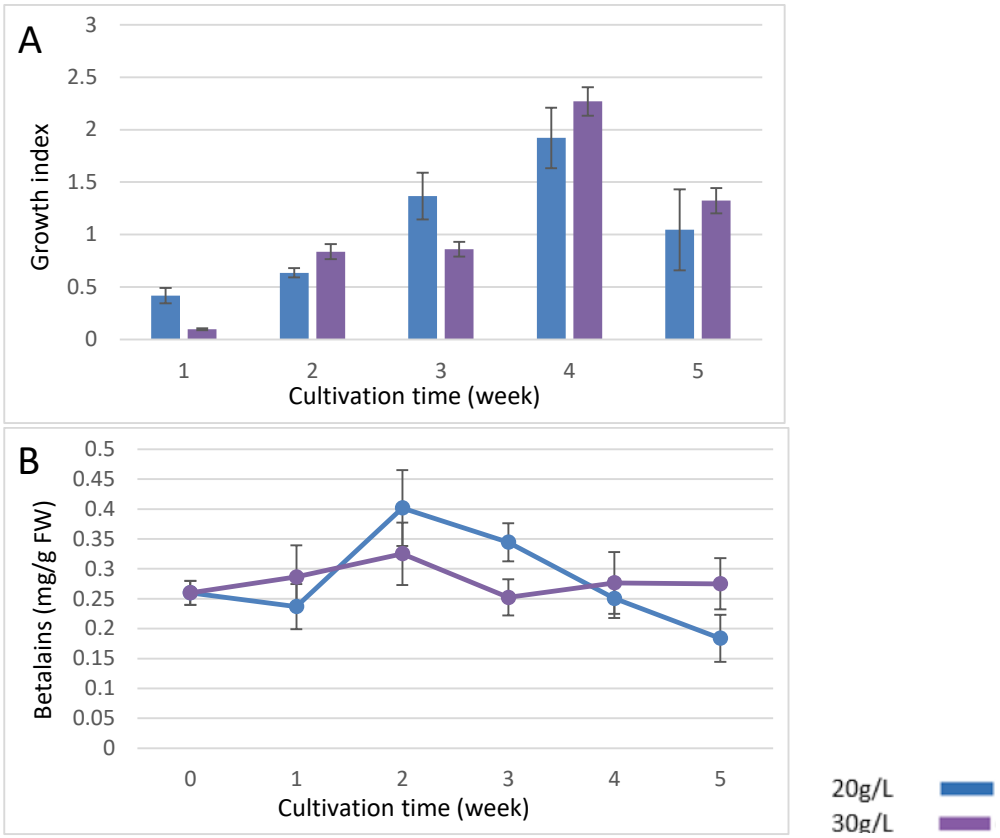

Figure 5: Growth index (A) and betalains concentration (B) of Beta vulgaris L. roots in liquid culture with sucrose

Plant cells use sucrose to make energy in cellular respiration. However, sucrose is a source of carbon for metabolite reactions in the cells. With 30 g/L of sucrose, adventitious roots of Beta vulgaris L. proliferated and accumulated betalains better than sucrose of 20 g/L. That indicated that the concentration of 20 g/L sucrose was not enough for roots proliferation and pigment biosynthesis.

4. Conclusions

Auxins at the appropriate concentration could enhance the rooting percentages and number of roots per explant, more specifically, IAA 1 or 2 mg/L for root explants, NAA 0.5 mg/L for hypocotyl explants and NAA 0.5 or 1 mg/L for cotyledon explants. In liquid culture, sucrose at the concentration of 30 g/L was better than 20 g/L and 7 g/L of roots was the appropriate initial inoculum density for root proliferation and betalains accumulation.

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