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Pakistan Academy of Sciences

Research Article

Quality of Prenatal-care Utilization in Bangladesh: Socioeconomic Factors

Rana Ejaz Ali Khan[1*] , and Sara Noreen[1]

[1]Department of Economics,
The Islamia University of Bahawalpur, Bahawalpur, Pakistan

Abstract: The delivery of prenatal-care services and its quality reduces mortality and morbidity in mothers and neonates. The current study has attempted to explore the socioeconomic determinants of quality of prenatal-care utilization in Bangladesh. Micro-data set comprising 22437 observations has been taken from Bangladesh Demographic Health Survey (BDHS) 2004 and 2007. Ordered logistic regression is applied to estimate the correlation between quality of prenatal-care utilization and explanatory variables. The quality of prenatal-care utilization is measured by simple additive index by taking six components of prenatal-care. The results have revealed that the woman's current age improves the probability of quality of prenatal-care utilization while age square decreases it. The number of died children and ratio of male to female children of a woman negatively influences the likelihood of quality of prenatal-care utilization. Age of the woman at first marriage, education of woman and her partner, household's wealth status and urban locality of the household and large/ capital city raise the probability of quality of prenatal-care utilization. The results about administrative divisions of Bangladesh have revealed statistically insignificant impact on quality of prenatal-care utilization which demonstrates that there exists no disparity among these divisions in the perspective of use of quality prenatal-care services by women.

Keywords: Healthcare quality, Woman health, Maternal health, child health, newborns, prenatal health-care

1. INTRODUCTION

The maternal mortality and morbidity due to complications of pregnancy in women may be reduced by good maternal health-care [1]. Maternal health-care comprised of prenatal-care, natal-care, postnatal-care and family planning. Prenatal-care is the first episode of maternal health-care. It is significant not only for the woman in duration of pregnancy but woman health in the whole of next life. It deters maternal morbidity and ultimately infant and maternal mortality [2]. The quality of prenatal-care availed by the mother during pregnancy is important for neonates and mothers. Prenatal-care is basically a preventive measure comprising of food and nutrition recommendations, advice for rest, basic awareness about the biological process and how to avoid pregnancy complications. The quality of services provided at prenatal visits fosters the woman's health during pregnancy. It is evident from the empirical literature that good quality provided during prenatal-care substantially improves not only the neonate' and mother's health but supports the growth of the neonates in coming years [3]. Nyamtema et al. concluded that twenty percent severe maternal morbidities are attributed to substandard quality of services provided during prenatal-care [4].

WHO acknowledged the requisite for standardization of contents of prenatal-care. The standard quality of prenatal-care is comprised of three components: (i) assessment, that is history taking, physical examination and laboratory tests, (ii) health promotion, that includes advice on nutrition, planning the birth, information regarding pregnancy, subsequent contraception and breastfeeding, and (iii) care provision that is comprised of folate supplements, tetanus toxoid

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*Corresponding author: Rana Ejaz Ali Khan; E-mail: ranaejazalikhan@yahoo.com

immunization, psychosocial support and record-keeping) [5]. The developing economies are much behind developed economies in utilization of prenatal-care services. In these economies maternal mortality rate is fifteen times higher than their counterparts in developed economies. For instance in Bangladesh maternal mortality ratio is 176 per 100,000 live births as compared to developed economies of France, Germany and Greece having the ratio of 8, 6 and 3 respectively. On average the ratio is 12 in developed economies. The country is even much behind Iran, Turkey and Oman having the ratio of 25, 20 and 22 respectively. The quality of prenatal-care in Bangladesh is also in a bad shape. For example, only 54.6 percent women receive prenatal-care, a small ratio of the births, i.e. 37 percent go through the skilled birth attendant, and 61 percent of women ages 15-49 years are using contraceptive. As a result lifetime risk of maternal death is reached to 0.58 percent. The socioeconomic determinants that keep quality of prenatal-care utilization lower are needed to be probed and that is the emphasis of current analysis.

The quality of prenatal-care has been assessed by researchers in various ways. Most prevailing measures of quality of prenatal-care are the number of prenatal visits and the components covered during prenatal-care. The researchers have quarried the number of prenatal visits as a measure of quality of prenatal-care [6]. They stressed that number of visits is not sufficient but package of the component of prenatal-care is important [3]. Globally and regionally the number of optimal visits is varied. It is not entirely evidence-based. The optimum number of visits is lacking the consensus. It is as high as fifteen in Finland and six in Netherlands. Both of the countries are developed in medical-care of their population. Similarly, in Belgium ten prenatal visits are advised by the medical experts for primiparae and for multiparae seven visits are advised. In the developing economies Indonesia and Vietnam stresses on three to four visits for their expected mothers. There is an evidence of source of quality prenatal that has been taken as the public sector health institutions [7]. It may also be questioned as the private sector health institutions may also provide the quality services for prenatal-care. So the proper measure of quality of prenatal-care may the utilization of prenatal components. If a woman receives all the components of prenatal-care either from public or private sector health institution she is utilizing quality service of prenatal-care. In the current study the same criterion is used for quality utilization of prenatal-care service.

The principal objective of the current analysis is to scrutinize the socioeconomic determinants of quality use of prenatal-care services in Bangladesh taking index of components of prenatal-care as measure of quality of prenatal-care.

2. DATA AND METHODOLOGY

2.1 Data Set

For the estimation of socioeconomic determinants of quality use of prenatal-care services in Bangladesh, a data set comprising a sample of 22437 observations about ever married women in the reproductive age group (15-49 years) has been extracted from micro-data of BDHS 2004 and 2007.

2.2 Measuring Quality of Prenatal-care Utilization

Donabedian [8] proposed the assessing of quality of health-care by categorizing the indicators of quality as structure of care, the process of clinical-care and the process of interpersonal-care. The current study assessed the quality of prenatal-care utilization through questions asked during the BDHS regarding prenatal-care services provided to women during pregnancy. Due to data constraint they partially cover the Donabedian's [8] categorization as well WHO's [5] recommended contents. BDHS asked the respondent (woman) whether each of following services of prenatal-care was availed by her in the last pregnancy at least once during her visits. The questions about services of prenatal-care include: whether the woman was weighed, her blood pressure was measured, her blood sample was taken for test, her urine sample was taken for test, and she was told about the signs of pregnancy complication and whether she was told about where to go for pregnancy complication if she had. Same type of indicators has been included by Melo et al. for quality of prenatal-care [9].

The response of each question was coded as 1 if the woman availed that specific service and 0 if she did not. These responses were added to compute the additive index for the quality of prenatal-care utilization [3, 10, 11]. The value of index ranged 0-6.

2.3 Selection of Explanatory Variables

Grossman introduced the concept of demand for medical-care that is theoretically derived from the demand for good health [12]. We are mainly concerned with the quality of health-care that is derived from the idea of Grossman and framed on health behavior model presented and developed by Anderson and Newman [13, 14]. The model gives the theoretical foundation for the behavior of individual and/or household for health-care. The components of health-care (in the care of prenatal-care) are external environment, predisposing factors, enabling factors and need factors. The McCarthy and Maine approach for estimating the factors responsible for utilization of maternal health-care utilization overlaps these determinants but categorized as individual, household, community, and health system factors. Individual level factors contain age of the woman, woman's educational level and employment status, etc.

Table 1. Operational definitions of the variables.

Variables	Definitions
Dependent Variables	
QPNC (Quality of prenatal-care utilization)	Quality index of prenatal-care having values 0-6
Explanatory Variables	
Demographic Characteristics	
WAGE (Woman's age)	Woman's age in completed year
WAGESQ (Woman's age squared)	Woman's age squared
PAGE (Partner's age)	Partner' age in completed years
PAGESQ (Partner's age squared)	Partner's age squared
WAGEMAR (Woman's age at first marriage)	Woman's age at first marriage in completed years
HHSIZE (Household size)	Number of household members
RASD (Ratio of sons to daughters)	Ratio of sons to daughters in the household
Socioeconomic Characteristics	
WEDU (Woman's education)	If the woman has no education =0, Primary=1, Secondary=2, Higher=3
PEDU (Partner's education)	If the partner has no education =0, Primary=1, Secondary=2, Higher=3
WWS (Woman's work status)	If woman is working =1, otherwise=0
PWS (Partner's work status)	If Partner is working =1, otherwise=0
WINDX (Wealth Index)	Wealth index, i.e. Poorest=1, Poorer=2, Middle=3, Richer=4, Richest=5
GENHH (Gender of head of household)	If gender of head of household is male = 1, female = 0
Health Characteristics	
WBMI (Woman's Body Mass Index)	Woman's Body Mass Index
BORD (Birth-order)	Birth-order of the child of last pregnancy
DICHIL (Died children)	Number of died children of the woman
Regional Characteristics	
LOC (Locality of the household)	If the household is urban =1, rural = 0
LCITY (Large city)	If household is situated in large city=1, otherwise =0
BRSAL (Barisal)	If the household is situated in Barisal = 1, otherwise = 0
CHITG (Chittagong)	If the household is situated in Chittagong = 1, otherwise = 0
DHAKA (Dhaka)	If the household is situated in Dhaka = 1, otherwise = 0
KHULN (Khulna)	If the household is situated in Khulna = 1, otherwise = 0
RAJSH (Rajshahi)	If the household is situated in Rajshahi = 1, otherwise = 0
SYLHT (Sylhet)	If the household is situated in Sylhet = 1, otherwise = 0

Household level factors include household wealth and size, etc. Community level factors are comprised of locality of the household (urban/rural) or geographic location in an economy and the health system available to the woman represented by birth-order of child and woman's body mass index [15].

The explanatory variables for the current analysis have been selected from the theoretical support from model presented by Andersen and Newman's model [13] but they partially overlap with those given by McCarthy and Maine [15].

2.4 Model Specification

The dependent variable i.e. quality index of prenatal-care utilization is ordinal or rank ordered so the ordered logit model is employed to investigate the probability for quality of prenatal-care utilization. The general model of the quality of prenatal-care utilization is expressed as:

Quality of prenatal-care = f (Demographic characteristics, Socioeconomic characteristics, Health Characteristics, Regional characteristics)

The functional form of the quality of prenatal-care utilization is given as below:

$$QPNC = f(\beta_0 + \beta_1 WAGE + \beta_2 WAGESQ + \beta_3 PAGE + \beta_4 PAGESQ + \beta_5 WAGEMAR + \beta_6 HHSIZE + \beta_7 RASD + \beta_8 WEDU + \beta_9 PEDU + \beta_{10} WWS + \beta_{11} PWS + \beta_{12} WINDX + \beta_{13} GENHH + \beta_{14} WBMI + \beta_{15} BORD + \beta_{16} DICHIL + \beta_{17} LOC + \beta_{18} LCITY + \beta_{19} BRSAL + \beta_{20} CHITG + \beta_{21} DHAKA + \beta_{22} KHULN + \beta_{23} RAJSH + \beta_{24} SYLHT) \quad \ldots\ldots \quad (1)$$

The operational definitions of the variables have been given in Table-1.

3. RESULTS AND DISCUSSION

The ordered logit model results about quality of prenatal-care have been expressed in table-2.

Majority of the results are supported by theoretical interpretation of the variables. They have been discussed in the following.

3.1 Demographic Characteristics

In social perspectives, age of a woman influence the behavior of woman regarding health-care. Age of the woman is basically a proxy of awareness, information and mobility within the society. The results of ordered logit model expressed that woman's age increases the likelihood of quality use of prenatal-care in Bangladesh. It explains that increase in awareness, information and mobility of woman increases the use of quality use of prenatal-care [16]. Our estimation has shown non-linear relationship between the woman's age and quality use of prenatal-care. It is inverted U-shaped, that is by incremental change in the woman's age, the likelihood of quality use of prenatal-care services first increases but after specific age it decreases. It explains that the argument of over-confidence and ignorance of pregnancy complications by women becomes valid at later ages.

It is found that age of the woman at first marriage also enhance the quality use of prenatal-care services. It may be explained on the fact that the women who marry comparatively in higher age group are equipped with better fertility and motherhood awareness, knowledge about the health and maternal system of woman, and information regarding maternal health-care as well as components of prenatal-care and pregnancy complication. It increases the probability of quality of prenatal-care utilization. It is evidenced in literature that highly educated women generally marry in higher age group. The phenomenon is connected with the use of quality prenatal-care services. The women married at comparatively higher age group are assumed highly educated and educational status of these women transforms them to have good quality consultation from the medical experts and choose the best quality hospitals confidently. Another explanation of the relationship between age at first marriage and quality use of prenatal-care services may be that the women who marry later age are those who are generally employed in paid work particularly in the formal sector of employment. It makes their socioeconomic and financial status better resulting into stronger empowerment at household level. They have comparatively higher mobility within the society. The situation enhances the accessibility of these women to better quality medical consultants for the purpose of medical check-ups generally and prenatal-care particularly. They have better access to the good quality high cost hospitals and clinical labs. All such types of elements directly and indirectly amplify the likelihood for quality of prenatal-care utilization when women marry at comparatively later ages. In

Table 2. Result of ordered log it regression for quality of prenatal-care utilization in Bangladesh.

Dependent Variable: QPNC
Method: ML - Ordered Logit (Quadratic hill climbing)
Sample (adjusted): 2 10996
Number of Ordered Indicator Values: 7
Convergence Achieved after 8 Iterations

Variable	Coefficient	Std. Error	z-Statistic	Prob.
WAGE	0.076396	0.046335	1.648783	0.0992*
WAGESQ	-0.004850	0.000767	-6.327076	0.0000**
PAGE	-0.011457	0.020081	-0.570539	0.5683
PAGESQ	3.12E-05	0.000200	0.155639	0.8763
WAGEMAR	0.185529	0.015567	11.91844	0.0000**
HHSIZE	0.033696	0.011268	2.990411	0.0028**
RASD	-0.141270	0.050916	-2.774589	0.0055**
WEDU	0.383094	0.066282	5.779781	0.0000**
PEDU	0.184239	0.044079	4.179716	0.0000**
WWS	-0.008319	0.081896	-0.101583	0.9191
PWS	0.501747	0.179043	2.802379	0.0051**
WINDX	0.170855	0.033764	5.060204	0.0000**
GENHH	-0.177525	0.111377	-1.593915	0.1110
WBMI	1.63E-05	3.88E-05	0.420148	0.6744
BORD	0.408507	0.044000	9.284161	0.0000**
DICHIL	0.155883	0.086527	1.801557	0.0716*
LOC	0.225485	0.084127	2.680279	0.0074**
LCITY	0.117038	0.112131	1.043765	0.2966
BRSAL	-0.547399	0.542407	-1.009203	0.3129
CHITG	-0.556463	0.542776	-1.025217	0.3053
DHAKA	-0.561933	0.549432	-1.022753	0.3064
KHULN	-0.488850	0.550554	-0.887925	0.3746
RAJSH	-0.349743	0.548845	-0.637233	0.5240
SYLHT	-0.489495	0.553936	-0.883666	0.3769
Limit Points				
LIMIT_1:C(25)	2.721744	0.826798	3.291910	0.0010
LIMIT_2:C(26)	2.947079	0.826760	3.564612	0.0004
LIMIT_3:C(27)	3.446420	0.826933	4.167713	0.0000
LIMIT_4:C(28)	3.854416	0.827305	4.659004	0.0000
LIMIT_5:C(29)	4.845762	0.828726	5.847245	0.0000
LIMIT_6:C(30)	5.309897	0.829731	6.399539	0.0000

Pseudo R-squared = 0.148928	Akaike info criterion = 2.191080
Schwarz criterion = 2.234296	Log likelihood = -4836.388
Hannan-Quinn criter. = 2.206317	Restr. log likelihood = -5682.703
LR statistic = 1692.630	Avg. log likelihood = -1.088786
Prob(LR statistic) = 0.000000	

* Significant at 10 percent level of significance
**Significant at 5 percent level of significance

Bangladesh (as well as South Asia) marriage in adolescent is still widespread affecting more than one third of girls. It is consequence of the tradition, culture and social set up which force the parents to marry off their girls at early age. The girls married in the earlier life cycle remained least empowered at household level. Due to their lower empowerment and say in the decision-making of their health-care they cannot avail the quality services of prenatal-care.

Conceptually, the household size may affect the quality of health-care including the prenatal-care negatively. The household economics explained the income dilution effect of larger households on quality of health-care. Such type of effect has another aspect that is of household composition. If the household is composed of larger number of children instead of adults the negative affect may emerge. The larger number of children not only dilute per-capita expenditures for health but it also results into diminishing marginal utility of quality services of prenatal-care. The quality concept is related with cost of the services. If the household is comprised of more adults and they are earning hands for the household the impact of household size may be positive. Our results have shown that the larger family size increases the likelihood for use of quality services of prenatal-care. The explanation may be that larger households or larger families may pool the household resources which cover the burden of use of quality services for prenatal-care. It increases the probability of quality of prenatal-care. The larger household size is connected with combined family system in Bangladesh as the fertility rate has declined drastically in the last three decades reaching almost at one. The combined family is prevalent in South Asia and pooling of income is one of the characteristics of combined family system.

The utilization of quality services of prenatal-care may be influenced by composition of sons and daughters in the household. In the South Asian nations there is high preference for sons. If there are more daughters in the household as compared to sons the mothers demand high quality prenatal-care for the coming baby hoping for a son. To detangle the puzzle of quality of prenatal-care utilization and composition of sons and daughters in the household we have included the explanatory variable of ratio of sons to daughters in the analysis. The results from ordered logit estimation have revealed that ratio of sons to daughters negatively impacts the likelihood of quality of prenatal-care utilization. The phenomenon explains the preference for sons as the hope of son in the presence of more number of daughters have increased the quality of prenatal-care utilization.

3.2 Socioeconomic Characteristics

The women's education as a categorical variable has a substantial impact in household welfare including household health, education, nutrition and environment. The regression results have revealed that woman's education expands the probability of use of quality services of prenatal-care. It explains that educated women obtain good quality of prenatal-care and take all necessary components of prenatal-care as they have better capability for utilization of good health-care inputs [16, 17, 18]. The educated women have the capacity to take decisions about their own and their children health-care. It enhances the chances of quality of prenatal-care utilization.

In South Asian economies the social and cultural norms which are basically determined by the religion and myths make the male members of the household dominant in household decision-making. In this scenario the educational status of the male heads/husbands and their working status act as catalyst for rational decision-making. These decisions also include the health-care of women. To which source of health-care provider the woman will go for check-up or even the woman will go for check-up or not is determined by the male head that is influenced by his educational status and work status. In our results the age of the partner has shown insignificant effect. However, the probability for quality use of prenatal-care services improves through the incremental change in the educational level of the partner. It explained that education of the partners helps their wives for attaining good awareness, information and know how about different components of the prenatal-care as well as the quality source of these components. They themselves have feelings of carefulness of their wives during pregnancy. The wives of the educated husbands feel relaxed while discussing the prenatal affairs with their husbands. These women have comparatively high social and physical mobility so they have more exposure to the social world and ignore the bad social and household norms. The adaptation of modern technology regarding health-care is generally associated with education of the head of household. The positive approach of the educated partner helps to adopt modern health-care facilities [16]. It increases the use of quality of prenatal-care services. The partner's work status has been found to affect the utilization of quality of prenatal-care positively. The household's financial status and income risk depends upon the working status of the partner. On the other hand quality utilization of prenatal-care services and generally the quality use of health-care services are costly. They include the hospitalization charges, consultant's fee and lab tests. It established the link between the working status of the partner and the quality utilization of prenatal-care services. The public sector employees and employees of big corporations and organizations receive the medical funds for health-care of themselves and their families. It also augments the use of quality of prenatal-care services. By working status of partner the woman has higher probability to have quality use of prenatal-care [1, 17].

The results regarding the wealth index as a factor of quality use of prenatal-care revealed that it enhances the likelihood of the phenomenon. It explained that good socioeconomic status households have approach to good quality health-care providers. The members of families from good socioeconomic status households are generally well-educated and are well aware of the quality services of health-care. These families are financially strong so they can easily afford the good quality services of consultants and hospitals. The women from good socioeconomic status households have strong financial status and stability in the income. These women can face the fluctuations in income without disturbing the utilizations of health-care services. The situation boosts the utilization of all components of prenatal-care [3, 11]. Due to their financial status they use all basic components of prenatal-care along with optional components from medically trained health-providers.

3.3 Health-related Characteristics

The need factors as described by the Alderman and Newman's model [12] have been transformed into health-related factors. The results of current analysis have revealed that child's birth-order positively influences the probability of quality of prenatal-care utilization. It seems hard to explain that why the woman having higher birth-order have good quality utilization of prenatal-care. During the earlier pregnancies the women give good attention in seeking the prenatal-care services and due to the higher birth risk during the pregnancy of earlier birth-order children they should obtain the high quality prenatal-care services. The explanation of the positive effect of birth-order on quality of prenatal-care utilization is particularly supported by the results of age of woman in section 3.1. This section explains that initially the increase in age results into high utilization of quality of prenatal-care. It may be inferred that increase in the awareness and mobility of the woman at the time of higher birth-order children enhances the likelihood of quality utilization of prenatal-care. Furthermore by the experience of the pregnancies they got more awareness and knowledge of the quality of prenatal-care. It explains the process of having experience and knowledge about prenatal-care and its quality components and well as source of quality prenatal-care by having higher number of pregnancies. It results into increased quality of prenatal-care utilization.

In the health-related characteristics it has been found that the women who have suffered the death of their children previously are more likely to have quality prenatal-care. The explanation is based on the fact that increased number of died children enhances the demand for safe pregnancies. Furthermore, the died children confound the woman's health and affect the woman psychologically. The phenomenon increased the demand for quality of prenatal-care utilization.

3.4 Regional Characteristics

Among the regional variables of the model which fall in the external environment component of the Anderson and Newman's model, only a single variable has shown significant result. It revealed that urban women are more likely to have good quality utilization of prenatal-care. The rural urban disparity is one of the major characteristic of developing economics. Women belonging to urban areas have options to choose good quality service [1]. The urban women have higher level of information and knowledge [19]. They are generally more educated as compared to their rural counterparts and have well awareness about quality and basic components of prenatal-care. Formal sector employment particularly for the women is more prevalent in urban areas. These women have easy access to quality source of prenatal-care. National health promotion programs are generally more frequently existed in urban areas. Urban women have more access to general and particularly electronic media in the form of television and internet and have better information, awareness, and knowhow about quality of prenatal-care as compared to their rural counterparts [20].

In Bangladesh there occurs rural-urban disparity in the perspective of quality of prenatal-care utilization but the current analysis has resulted that there exists no regional disparity among the divisions of the country. It is established on the results of ordered logit regression that the dummy variable of each division has shown statistically insignificant effect on the probability of use of quality of prenatal-care.

4. CONCLUSIONS AND POLICY IMPLICATIONS

The focus of the study was to determine the factors of quality of prenatal-care utilization by the women in the age group of 15-49 years in Bangladesh.

The clues of gender preference in children emerged from the analysis regarding quality of prenatal-care utilization. The mother having comparatively higher number of daughters are found more inclined towards the use of quality of prenatal-care. Their desire for sons instigates them for this act. The gender discrimination in children regarding health, nutrition and education is a problem not only in Bangladesh but in majority of the developing economies, and particularly in South Asia. The education and awareness along with provision of social security may alleviate the problem. However to enhance the utilization of quality of prenatal-care, the information that the quality is necessary for both mother and newborns' health should be propagated.

Some of the policy proposals have been suggested from the empirical evidence of the current study. Firstly, the legal age of marriage for girls has been established in law, but it is not properly implemented. It should be strongly materialized that under age marriages are penalized. Secondly education programs for females should have the priority in the policy framework. It has the spillover effects not only on the general health of women but children's health and education as well. Thirdly the media, more precisely the electronic media and social campaign can impart their role for awareness rising about maternal health-care generally and quality components of prenatal-care particularly.

The wealth index of the household that basically captures the socioeconomic status of the household has shown encouraging effect on the use of quality service of prenatal-care. A number of studies have proposed the poverty alleviation, increase in income of the household, boosting the resources of the household, improving the financial status of the household and the woman for better maintenance of maternal health-care. All such type of measures need a long-time span but some immediate and urgent measures may also be adopted particularly for the prenatal-care. For instance the provision of public sector low cost quality service of prenatal-care, the introduction of the insurance, conditional distribution of child's and woman's supplementary nutrition to have full components of prenatal-care may be introduced. So fourthly it is proposed to the policy makers to work for such type of measures.

Fifthly we have seen in our results that birth-order of child enhances the utilization of quality of prenatal-care. For the earlier birth-order particularly for first birth there should be specific incentives like the first delivery would be free of cost if all the components of the prenatal-care (quality of prenatal-care) are covered. Furthermore, by covering the complete set of components of prenatal-care services the first-born child may have the free medical checks up in the first year. Bangladesh is a developing economy, the international donors specifically the WHO and the World Bank should play their part for the purpose.

The analysis demonstrated the existence of inequality in quality use of prenatal-care in rural and urban communities. Sixthly, health department may decrease this disparity by providing all components of quality of prenatal-care through mobile health units in rural areas. For the rural areas of the country awareness about the quality components of prenatal-care needs attention of policy makers.

Last but not the least we have analyzed the demand side determinants but the supply side factors are equally important. In the further research the supply side factor should also be the part of the analysis. The demand side incentives may only be the successful if supply side elements of prenatal-care are available at satisfactory level.

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Research Article

Effect of Dilute Sulphuric Acid Pretreatment on Cellulase Production by *Bacillus subtilis* (K-18) through Response Surface Methodology

Anam Arooj[1], Muhammad Irfan[2]*, Fouzia Tabbasum[1], Hafiz Abdullah Shakir[1], and Javed Iqbal Qazi[1]

[1]Microbial Biotechnology Laboratory, Department of the Zoology,
University of the Punjab, Lahore, Pakistan
[2]Department of Biotechnology, University of Sargodha, Sargodha, Pakistan

Abstract: The present study investigated the optimization of dilute sulphuric acid pretreatment to maximize cellulase production from banana peduncle waste through Box-Behnken design of response surface methodology. Cellulase production was carried out in 250ml capacity Erlenmeyer flask using pretreated banana peduncle as substrate in submerged fermentation by *Bacillus subtilis* K-18 incubated at 50°C for fermentation period of 24 h. Results indicated that chemical pretreatment using sulphuric acid favored cellulase production as compared to thermochemical pretreatment using sulphuric acid followed by autoclaving at 121°C for 15 min and 15 psi. Maximum Filter Paper activity of 0.958 IU/ml/min was observed at optimal pretreatment conditions of 0.4 N H_2SO_4 concentration, 15% substrate concentration and residence time of 6h with chemical pretreatment. For thermochemical pretreatment optimal FPase activity of 0.63 IU/ml/min was recorded at 0.4 N H_2SO_4 concentration, 10% substrate concentration and residence time of 4 h. The proposed regression model for both types of pretreatments was found significant as revealed by *F-value*, *P-value* and coefficient of determination. These results indicated that banana peduncle can be successfully utilized as solid substrate in submerged fermentation for cellulase enzyme production.

Keywords: Cellulase, RSM, pretreatment, *Bacillus subtilis*, submerged fermentation

1. INTRODUCTION

Cellulose being the most abundant organic polymer from plant biomass can act as an inexhaustible and inexpensive raw material for a number of value added products like ethanol, organic acids and various chemical solvents, etc. [1]. Cellulose is a polysaccharide of repeated β-D-glucopyranose units interlinked by β-1,4-glycosidic bonds. Therefore, it needs to be depolymerized into its monomer glucose units which are further subjected to microbial fermentation leading to the production of various valuable products. The breakdown of glycosidic bonds in cellulose is done either by chemical or enzymatic hydrolysis. Since chemical breakdown of cellulose using acids under harsh conditions generates byproducts toxic to microbes, enzymatic hydrolysis through the activity of cellulases is more attractive. Complete hydrolysis of cellulose into its glucose monomers is done by synergistic activity of three different cellulases belonging to Glycoside Hydrolase (GH) family of enzymes [2].

These enzymes hydrolyze the glycosidic bond by acid/base catalysis method [3].Endo- β-1,4-glucanase also called CMCase randomly cuts glycosidic linkages particularly at internal amorphous sites of cellulose chain, generating long chain oligomers [2, 4]. These oligomers are further depolymerized by Exoglucanase or β-1,4-Cellobiohydrolase. Exoglucanases can hydrolyse both reducing and non-reducing ends in a highly processive manner producing Cellobiose units [2]. Finally β-Glucosidases which have a pocket shaped active site specifically bind to non-reducing glucose ends of cellobiose, hydrolyse it

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*Corresponding author: Muhammad Irfan; Email: m.irfan@uos.edu.pk, irfan.biotechnologist@gmail.com

and liberate both glucose units [5].

Cellulases are produced from a vast diversity of microorganisms mainly Bacteria and Fungi. Most extensively studied fungal genera for cellulolytic activity include *Aspergillus, Trichoderma, Fusarium, Penicillium*. Some bacterial genera well known for cellulolytic activity *are Clostridium, Pseudomonas, Bacillus,* etc. *Streptomyces, Cellulomonas*, and *Thermomonospora* are major cellulase producing actinomycetes [1, 4]. Fungal Cellulases are commercially more attractive as they are robust and extracellular. They are having simple structure consisting of a Cellulose binding domain (CBD) and a Catalytic domain (CD) interlinked by a linker peptide. *Trichoderma reesei* is the most extensively used fungus for cellulase production [4]. Bacterial cellulases are present in the form of cellulosomes attached to the cell wall of bacterial cell [2].

Cellulases are known to have diverse industrial applications as they are being used in textile, food, brewing, pulp and paper industry as well as additives in detergents. Growing concerns over the depletion of fossil fuels have led the increased demand of cellulases to be used in lignocellulose based biorefinery [2, 4]. The high costof cellulases is the major bottleneck in commercialization of these biorefineries. A number of lignocellulosic wastes have been used to produce cellulases from various microbes using either solid state or submerged fermentation that leads to not only cost effective enzyme production but also waste management [6]. Solid state fermentation utilizes solid substrates like bagasse, bran, rice straw and is most applicable for Fungi and microbes requiring little water content. Submerged fermentation technology is based on using free flowing liquid substrates such as broth and is suited for bacteria requiring high water potential [7]. More than 70% of commercial enzyme production has been reported through the use of submerged fermentation technology due to the advantages of better monitoring, handling, ease of product purification and its greater extent to support the use of genetically modified organisms [2, 7, 8].

Different strains of *Bacillus subtilis* have been used to produce cellulases using a variety of lignocellulosic wastes [1, 6, 9]. Most of *Bacillus* species have shown to produce high cellulases on sugarcane bagasse [10], rice husk [8] and Corn stover [11]. Several studies have shown that Banana fruit stalk and other wastes as pseudostems found abundantly in tropical and subtropical regions have a great potential to be used as solid substrate for commercial production of cellulases employing *Bacillus subtilis*, *Trichoderma viride*, *Aspergillus niger*, *Neurospora sitophila and Pleurotus sp.* [6, 12-16]. The present study investigates the cellulolytic potential of *Bacillus subtilis* using pretreated banana peduncle.

2. MATERIALS AND METHODS

2.1. Microbial Strain

The bacterium *Bacillus subtilis* K-18 was obtained from Microbial Biotechnology Laboratory, Department of Zoology, University of the Punjab, New Campus, Lahore, Pakistan. The culture was maintained on nutrient agar slants and was used for production of cellulase in submerged fermentation.

2.2 Pretreatment of Banana Peduncle

Pretreatment of Banana Peduncle was done as described in our earlier reports [17]. For chemical pretreatment, the powdered banana peduncle samples were soaked in 0.24 N, 0.32 N, 0.4 N H_2SO_4 solutions with substrate loading of 5%, 10%, 15%w/v and pretreatment time of 4, 6, 8 h. Likewise thermochemical pretreatment was carried out by autoclaving the soaked biomass for 121°C, 15 psi, 20 min. After pretreatment the samples were filtered and solid residues were washed up to neutrality.

2.3. Enzyme production

Enzyme production was done in 250ml Erlenmeyer flask capacity having 25ml of fermentation medium containing 2% pretreated substrate and 1% yeast extract with initial medium pH of 5 was autoclaved at 121^{o}C, for 15 minutes and 15 psi pressure. After sterilization, the flasks were allowed to cool at room temperature and 2% (v/v) of the vegetative cell culture was transferred aseptically to each of the fermentation flasks. After inoculation, the flasks were incubated at 50^{o}C with agitation speed of 120 rpm for 24 h of fermentation period. After completion of the fermentation period, the fermented broth was filtered through muslin cloth followed by

Table 1. Coded and actual levels of the factors for three factor Box-Behnken design.

Independent variable	Symbol	Coded and actual values		
		-1	0	+1
Acid concentration (N)	X_1	0.24	0.32	0.40
Substrate concentration (%)	X_2	5	10	15
Time (h)	X_3	4	6	8

Table 2. Cellulase production by chemical treated banana peduncle using Box-Behnken design.

Run #	X_1	X_2	X_3	CMCase activity (IU/ml/min)			FPase activity (IU/ml/min)		
				Observed	Predicted	Residual	Observed	Predicted	Residual
1	0.32	10	6	0.456657	0.456657	0.000000	0.551704	0.551704	-0.00000
2	0.40	10	8	0.431824	0.515637	-0.08381	0.860741	0.845833	0.014907
3	0.40	15	6	0.471833	0.448035	0.023799	0.958222	0.911556	0.046667
4	0.40	10	4	0.560130	0.515637	0.044493	0.698963	0.722944	-0.02398
5	0.40	5	6	0.376639	0.361118	0.015521	0.634667	0.672259	-0.03759
6	0.24	15	6	0.237296	0.252817	-0.01552	0.526815	0.489222	0.037593
7	0.32	5	4	0.531157	0.591171	-0.06001	0.522667	0.461093	0.061574
8	0.24	10	8	0.441481	0.485975	-0.04449	0.514370	0.490389	0.023981
9	0.32	15	8	0.641528	0.581514	0.060014	0.474963	0.536537	-0.06157
10	0.24	10	4	0.500806	0.416993	0.083812	0.572444	0.587352	-0.01490
11	0.24	5	6	0.404231	0.428030	-0.02379	0.556889	0.603556	-0.04666
12	0.32	5	8	0.550472	0.482181	0.068292	0.395111	0.372426	0.022685
13	0.32	15	4	0.335250	0.403542	-0.06829	0.399259	0.421944	-0.02268

Table 3. Cellulase production by thermochemical treated banana peduncle using Box-Behnken design.

Run #	X_1	X_2	X_3	CMCase activity (IU/ml/min)			FPase activity (IU/ml/min)		
				Observed	Predicted	Residual	Observed	Predicted	Residual
1	0.32	10	6	0.21	0.218	0.00	0.55	0.55	0.00
2	0.40	10	8	0.24	0.24	-0.00	0.45	0.45	0.00
3	0.40	15	6	0.29	0.28	0.00	0.51	0.50	0.01
4	0.40	10	4	0.18	0.18	0.00	0.63	0.66	-0.02
5	0.40	5	6	0.19	0.20	-0.00	0.50	0.49	0.01
6	0.24	15	6	0.22	0.21	0.00	0.29	0.30	-0.01
7	0.32	5	4	0.14	0.13	0.00	0.39	0.37	0.01
8	0.24	10	8	0.15	0.15	-0.00	0.35	0.33	0.02
9	0.32	15	8	0.19	0.20	-0.00	0.30	0.31	-0.01
10	0.24	10	4	0.19	0.18	0.00	0.32	0.32	-0.00
11	0.24	5	6	0.18	0.18	-0.00	0.22	0.23	-0.01
12	0.32	5	8	0.15	0.14	0.00	0.34	0.35	-0.01
13	0.32	15	4	0.18	0.19	-0.00	0.50	0.49	0.01

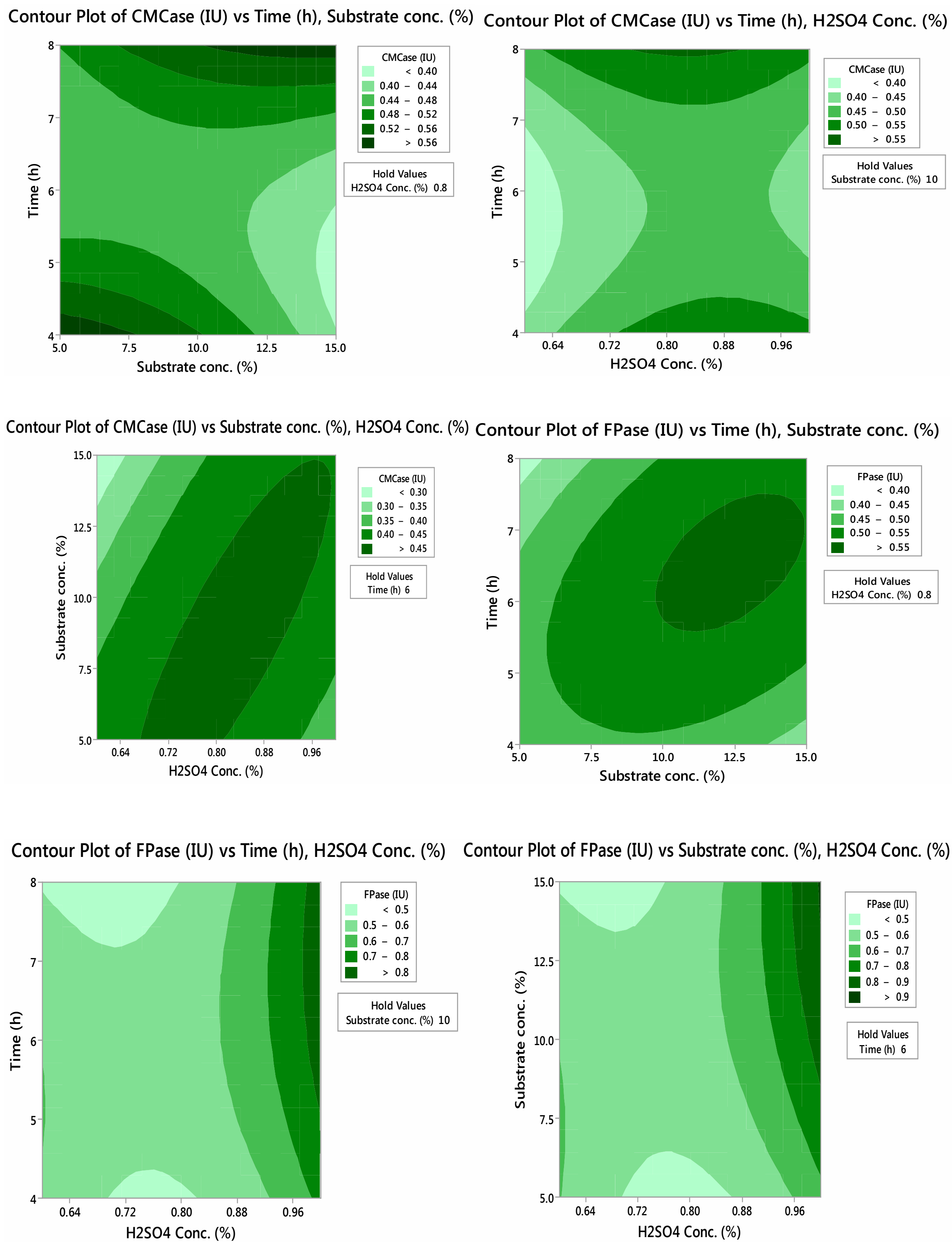

Fig. 1. Contour plots for CMCase (IU/ml/min) and FPase (IU/ml/min) production from sulphuric acid treated banana peduncle by *Bacillus subtilis* K-18 in submerged fermentation.

centrifugation (Sigma 2-16 PK) for 10 minutes at 10,000 x g and 4°C for the removal of cell mass and unwanted particles. The clear filtrate obtained after centrifugation was used as a crude source of enzyme. Triplicate readings were taken for each of the experiment.

2.4. Cellulase assay

CMCase and FPase activity was determined as described in our earlier reports [18]. One unit of CMCase or FPase activity defined as the amount of enzyme required to liberate one micromole of glucose from substrate per milliliter per minute under standard assay conditions.

2.5. Experimental design

In order to optimize different pretreatment conditions for cellulase production, Box-Behnken design (BBD) was used in this study. The independent variables used were H_2SO_4 concentration (X_1), substrateconcentration, (X_2) and residence time (X_3) and their levels are mentioned in Table 1. This design is most suitable for quadratic response surface and generates second order polynomial regression model. The relation between actual and coded values was described by the following equation;

$$x_i = \frac{X_i - X_o}{\Delta X_i} \qquad \text{Eq. (1)}$$

Where *xi* and *Xi*are the coded and actual values of the independent variable, *Xo* is the actual value of the independent variable at the center point and Δ*Xi* is the change of *Xi.* The response is calculated from the following equation using STATISTICA software (99th edition).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \qquad \text{Eq. (2)}$$

Y is the response, X_1, X_2 and X_3 are the independent variables, $ß_0$ is the intercept, $ß_1$, ß2 and $ß_3$ are linear coefficient, $ß_1^1$, $ß_2^2$ and $ß_3^3$ are square coefficients, $ß_{12}$, $ß_{13}$ and $ß_{23}$ are interaction coefficients.

3. RESULTS AND DISCUSSION

The present study investigated the effect of different pretreatment conditions for cellulase production from banana peduncle waste by *Bacillus subtilis* K-18 under submerged fermentation. Before carrying out enzyme production, biomass was pretreated chemically using H_2SO_4 and thermochemically using H_2SO_4 followed by autoclaving at 121°C for 15 min and 15 psi. Three experiment factors of H_2SO_4 concentration, substrate loading and residence time were optimized to maximize cellulose production. Second order polynomial equations were used to calculate enzyme production as shown in Eq. 3-6. Maximum Filter Paper activity of 0.958 IU/ml/min was observed at optimal conditions of 0.4 N H_2SO_4 concentration, 15% substrate concentration and residence time of 6 h with chemical pretreatment. For thermochemical pretreatment optimal FPase activity of 0.63 IU/ml/min was recorded at 0.4 N H_2SO_4 concentration, 10% substrate concentration and residence time of 4h. Sulphuric acid pretreatment resulted in higher values of enzyme production than sulphuric acid pretreatment followed by autoclaving. The results of cellulase production using Box-Behnken design for both types of pretreatments were shown in Table 2, 3.

Equations for CMCase and FPase production from acid treated substrate

$$\text{CMCase activity (IU)} = 3.58 - 4.58 X_1 - 0.0543 X_2 - 0.357 X_3 + 2.19 X_1^2 + 0.00023 X_2^2 + 0.0203 X_3^2 + 0.0552 X_1{*}X_2 + 0.054 X_1{*}X_3 - 0.00014 X_2{*}X_3 \qquad \text{Eq. (3)}$$

$$\text{FPase activity (IU)} = 0.983 - 0.354 X_1 - 0.0409 X_2 - 0.1710 X_3 + 0.240 X_1^2 + 0.000902 X_2^2 + 0.01381 X_3^2 + 0.0207 X_1{*}X_2 - 0.0376 X_1{*}X_3 + 0.00036 X_2{*}X_3 \qquad \text{Eq. (4)}$$

Equations for CMCase and FPase production from acid followed by steam treated substrate

$$\text{CMCaseactivity (IU)} = 0.291 - 1.009 X_1 + 0.00275 X_2 + 0.0792 X_3 + 0.395 X_1{*}X_1 - 0.000392 X_2{*}X_2 - 0.01042 X_3{*}X_3 + 0.01300 X_1{*}X_2 + 0.0591 X_1{*}X_3 + 0.000100 X_2{*}X_3 \qquad \text{Eq. (5)}$$

$$\text{FPase activity (IU)} = -2.678 + 3.811 X_1 + 0.1331 X_2 + 0.2915 X_3 - 1.433 X_1{*}X_1 - 0.004667 X_2{*}X_2 - 0.01430 X_3{*}X_3 - 0.0150 X_1{*}X_2 + 0.1325 X_1{*}X_3 + 0.00396 X_2{*}X_3 \qquad \text{Eq. (6)}$$

Statistical significance of data was evaluated by applying F-test in ANOVA. For chemical pretreatment, regression model for CMCase production was found to be insignificant with

Table 4. Analysis of variance of chemical treated banana peduncle.

	Source	DF	Adj SS	Adj MS	F value	P value
CMCase (IU/ml/min)	Model	9	0.098	0.010	1.51	0.339
	Linear	3	0.014	0.004	0.67	0.607
	X_1	1	0.008	0.008	1.14	0.335
	X_2	1	0.003	0.003	0.54	0.496
	X_3	1	0.002	0.002	0.33	0.591
	Square	3	0.044	0.014	2.06	0.224
	X_1^2	1	0.012	0.012	1.69	0.250
	X_2^2	1	0.002	0.002	0.36	0.575
	X_3^2	1	0.026	0.026	3.65	0.114
	2 Way interaction	3	0.038	0.012	1.80	0.265
	X_1*X_2	1	0.017	0.017	2.38	0.184
	X_1*X_3	1	0.001	0.001	0.16	0.702
	X_2*X_3	1	0.020	0.020	2.85	0.152
	Error	5	0.036	0.007		
	Lack of fit	3	0.036	0.012		
	Pure error	2	0.000	0.000		
	Total	14	0.134			
FPase (IU/ml/min)	Model	9	0.312	0.034	9.99	0.010
	Linear	3	0.128	0.042	12.34	0.010
	X_1	1	0.120	0.120	34.67	0.002
	X_2	1	0.007	0.007	2.25	0.194
	X_3	1	0.000	0.000	0.10	0.768
	Square	3	0.130	0.043	12.48	0.009
	X_1^2	1	0.101	0.101	29.09	0.003
	X_2^2	1	0.008	0.008	2.46	0.178
	X_3^2	1	0.011	0.011	3.28	0.130
	2 way interaction	3	0.053	0.017	5.14	0.055
	X_1*X_2	1	0.031	0.031	8.99	0.030
	X_1*X_3	1	0.012	0.012	3.47	0.121
	X_2*X_3	1	0.010	0.010	2.97	0.145
	Error	5	0.017	0.003		
	Lack of fit	3	0.017	0.005		
	Pure error	2	0.000	0.000		
	Total	14	0.329			

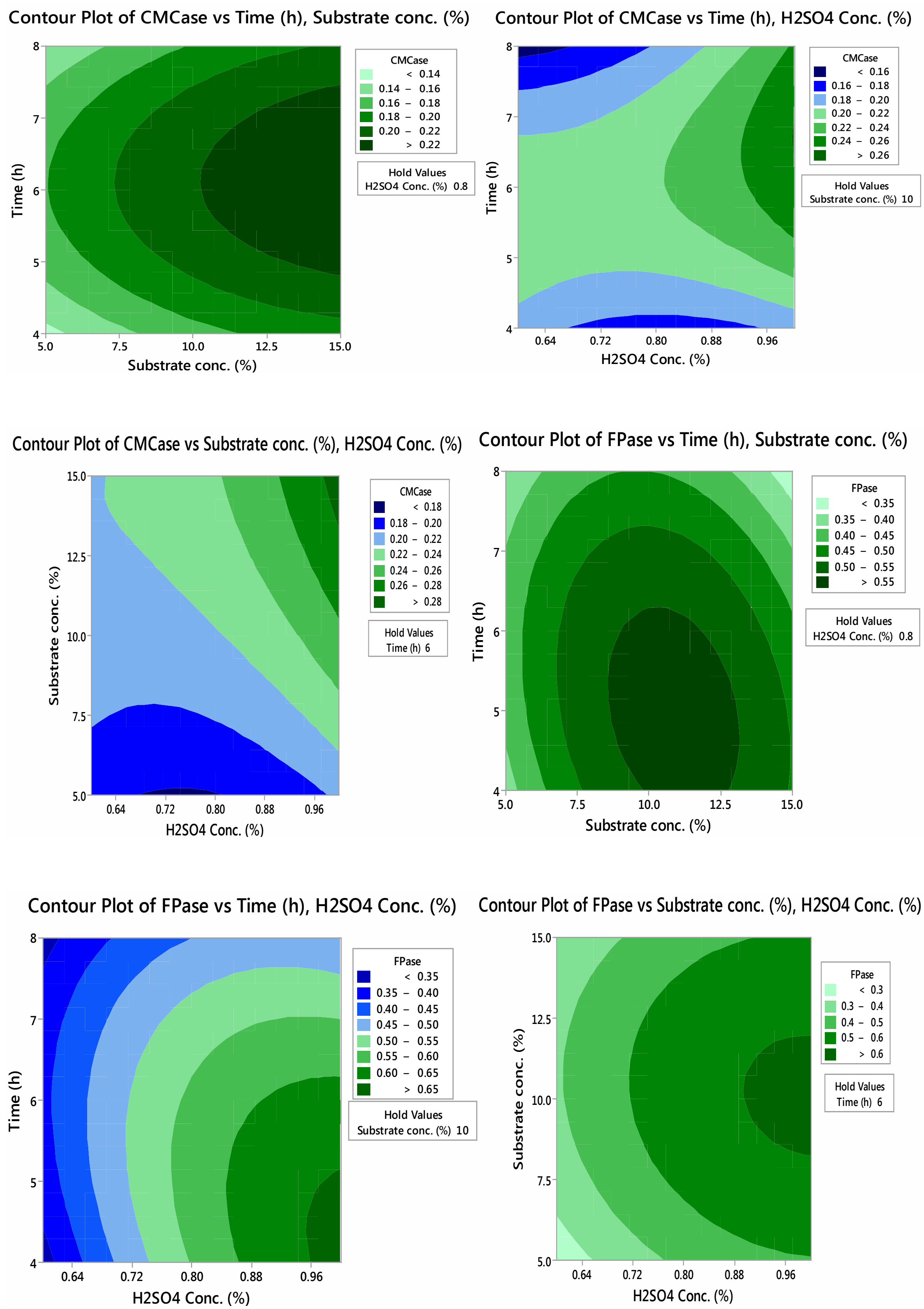

Fig. 2. Contour plots for CMCase (IU/ml/min) and FPase (IU/ml/min) production from sulphuric acid followed by steam treated banana peduncle by *Bacillus subtilis*K-18 in submerged fermentation.

Table 5. Analysis of variance of thermochemical treated banana peduncle.

	Sources	DF	Adj SS	Adj MS	F value	P value
CMCase (IU/ml/min)	Model	9	0.02	0.00	25.72	0.00
	Linear	3	0.01	0.00	38.51	0.00
	X_1	1	0.00	0.00	39.54	0.00
	X_2	1	0.00	0.00	74.01	0.00
	X_3	1	0.00	0.00	1.97	0.21
	Square	3	0.00	0.00	28.34	0.00
	X_1^2	1	0.00	0.00	9.81	0.02
	X_2^2	1	0.00	0.00	3.78	0.10
	X_3^2	1	0.00	0.00	68.22	0.00
	2 Way interaction	3	0.00	0.00	10.32	0.01
	X_1*X_2	1	0.00	0.00	7.19	0.04
	X_1*X_3	1	0.00	0.00	23.73	0.00
	X_2*X_3	1	0.00	0.00	0.04	0.84
	Error	5	0.00	0.00		
	Lack of fit	3	0.00	0.00		
	Pure error	2	0.00	0.00		
	Total	14	0.02			
FPase (IU/ml/min)	Model	9	0.21	0.02	50.84	0.00
	Linear	3	0.12	0.04	92.17	0.00
	X_1	1	0.10	0.10	224.84	0.00
	X_2	1	0.00	0.00	6.70	0.04
	X_3	1	0.02	0.02	44.96	0.00
	Square	3	0.06	0.02	47.26	0.00
	X_1^2	1	0.01	0.01	25.89	0.00
	X_2^2	1	0.05	0.05	107.31	0.00
	X_3^2	1	0.01	0.01	25.77	0.00
	2 way interaction	3	0.01	0.00	13.10	0.00
	X_1*X_2	1	0.00	0.00	1.92	0.22
	X_1*X_3	1	0.01	0.01	23.98	0.00
	X_2*X_3	1	0.00	0.00	13.41	0.01
	Error	5	0.00	0.00		
	Lack of fit	3	0.00	0.00		
	Pure error	2	0.00	0.00		
	Total	14	0.21			

Fisher's F-test value of 1.51 and p-value of 0.339. The proposed model for FPase production was found to be significant with p-value of 0.010 and F-value of 9.99. Sulphuric acid concentration (X_1) with p-value of 0.002 was the only linear term to influence FPase production significantly. Among square terms, $H_2SO_4$was significant factor as p-value was 0.05. Two way interaction between acid concentration and substrate loading was found to significantly influence results as p-value of 0.030 was lower than 0.05 (Table 4).The model fitness was further checked by coefficient of determination (R^2 value) which showed that the predicted model 94.73% and 73.07% accurately explained the predicted response for FPase and CMCase respectively for sulphuric acid pretreatment. Furthermore, the adjusted R^2 value supported the model with values of 85.25% and 24.61% for FPase and CMCase respectively.

The regression model for CMCase production by thermochemical pretreatment was significant with F-value of 25.72 and p-value of 0.00. The linear terms X_1, X_2, the quadratic terms, X_1^2, X32and interaction terms X_1X_2, X_1X_3 were found to be significant as probability value for all these was less than 0.05. High R^2 value of 97.89% and adjusted R^2value of 94.08% showed that there was a close agreement between experimental values and those predicted by model. A large F-value of 50.84% and the corresponding p-value of 0.00 implies that regression model for FPase production from sulphuric acid pretreatment followed by autoclaving was highly significant. X_1, X_2, X_3, X_1^2, X_2^2, X_2^2, X_1X_3, X_2X_3 were the linear, square and interaction terms to be significant with probability values of less than 0.05 as shown in Table 5. The coefficient of determination (R^2) of the model was 98.92% and adjusted R^2 value was 96.97%, which indicated that the model adequately represented the real relationship between FPase production and the tested variables. Fig. 1 and 2 depicted the contour plots for experimentally observed values of CMCase and FPase versus results predicted by quadratic model from H_2SO_4 treated and H_2SO_4 followed by steam treated banana peduncle waste.

Cellulase production in this study was higher as compared to Sreena *et al*. [6] who reported CMCase activity of 0.133 IU/ml from banana rachis incubated with 1% inoculum of *Bacillus subtilis*at 40°C for 48h. Krishna et al.[15] reported optimal filter paper activity of 2.8 $IUgds^{-1}$ and CMCase activity of 9.6 $IUgds^{-1}$ from banana fruit stalk pretreated by autoclaving at 121°C for 60min. Pretreatment by 2 N H_2SO_4 for soaking period of 6h resulted in FPase and CMCase activity of 1.04 and 2.30 $IUgds^{-1}$ respectively. In a comparative study of cellulase production using rice husk, banana peels, wheat bran, Millet bran, saw dust and coir waste, banana peels gave highest values of FPase and CMCase activities as 12.4IU/ml and 11.3 IU/ml, respectively, with *Aspergillis niger* at 30° C and incubation time of 4 days [19]. Kumar *et al*. [8] reported 100U/ml, 45U/ml and 3.5U/ml of CMCase, FPase and B-glucosidase by *Bacillus* sp. in submerged fermentation using rice husk as substrate. Shafiq et al. [16] reported that solid state fermentation of banana peduncle using *Bacillus subtilis* at 35°C, pH 7, for 72h generated FPase activity of 3.48IU/ml/min. This study indicates successful utilization of banana peduncle waste for the production of highly active cellulases. Sharma et al. [20] employed submerged fermentation of coconut water by *A. niger* to optimize cellulase production. Maximum value of FPase obtained was 0.531 IU/ml for 3 days of incubation period, 0.07% w/v glucose and 8% waste paper. The enzyme produced was then used for hydrolysis of acid and alkali treated mixture of cotton stalk and wheat straw. In one study submerged fermentation of corn husks using *Bacillus cereus* strain resulted to maximum cellulase activity of 0.213 IU/ml for temperature of 30°C, pH 5 and substrate concentration of 1% [21]. Vijayaraghavan et al. [22] used an RSM based experimental design to optimize the simultaneous production of CMCase and protease from solid state fermentation of cow dung with *Bacillus subtilis*. The resulted values were 2.1 and 2.5 fold higher for CMCase (473.01 U/g) and protease (4643 U/g protease) respectively than using non optimized medium, suggesting RSM as an effective methodology to enhance enzyme productions using cost effective substrates.

4. CONCLUSIONS

Results of this study revealed that dilute sulphuric acid pretreatment of banana peduncle effectively improved cellulase production by *Bacillus subtilis* K-18 under submerged fermentation. The produced cellulase enzyme could be industrially exploited with special emphasis on saccharification and bioethanol production.

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Research Article

Allometric Study of *Urotheutis (Photololigo) duvauceli* (d'Orbigny 1835) from Northern Coast of Java, Indonesia

Norma Afiati*, Dewi Megapuspa Nusari, and Subagiyo

Faculty of Fisheries and Marine Science, Diponegoro University,
Jl. Prof. Soedarto, Tembalang, Semarang 50275, Indonesia

Abstract: This study presents some basic biology of *Uroteuthis* (*Photololigo*) *duvauceli*, the most often caught species during sampling in the area of study, such as the sex ratio and length-frequency, allometric growth of various parts of the body, with particular interest in allometric relationship of eye shape against other parts of the body. Samples were collected from Cirebon in West Java, Kendal and Semarang in Central Java and Tuban waters in East Java. During 4 mo sampling from May to August 2015, four species were identified including *Uroteuthis (Photololigo) duvauceli* (d'Orbigny, 1835), *Octopus* sp, *Sepiella inermis* (Ferussac &'d'Orbigny, 1848), and *Sepioteuthis* cf. *lessoniana* (Ferussac, 1830). In all four populations, males dominated in number, the sex ratios ranged from 1:1.21 to 1:1.95 in favour of male individuals. Allometric growth of juveniles and mature individuals (60 mm to152 mm dorsal mantle length, DML) showed that *U. (P.) duvauceli* grew its length faster than any other part of the body except its fin ($P < 0.01$). Eyes grew according to its long axis than to its height. Head length and eye shape (eye length and height) grew significantly slower compared to the body length ($P < 0.01$). This means that since early juveniles *U. (P.) duvauceli* concerned more to the fin growth and development than to its eye size, even though newly hatch squid seemed to have shown big eyes compare to its overall body size. Previous studies on this species elsewhere substantiated that the length-weight relationship of *U. (P) duvauceli* do not follow ideal cube law. The fact in this study that wet body weight was always negatively allometric ($P < 0.01$) compared to any part of the body, not only its length, suggesting that *U (P.) duvauceli* is indeed a real swimmer, shaping a very light, slim and slender body with fully developed fin since early juveniles, balance feature for a predator escaping predation.

Keywords: Allometric growth, northern coast of Java, *Urotheutis* (*Photololigo*) *duvauceli* (d'Orbigny, 1835)

1. INTRODUCTION

The contribution of cephalopods to fisheries has increased worldwide, since in proportion input of traditional finfish stocks have started to decline in various regions [1, 2]. Hunsicker et al. [3], while studying 28 LMEs (Large Marine Ecosystem) commodity and supportive services provided by cephalopods, found that the group contributed as much as 55 % of fishery landings (t) and 70 % of landed values (USD). Other study reported that as overexploitation of finfish stocks continues, cephalopod populations seemed to take over niches of finfishes in the marine ecosystem and become dominant in terms of world fisheries resources [4].

Squid was known to exhibit polymorphism and possibly is species complex, which is not surprising, considering their wide distributional range in the sea. Considering their restricted niche, *i.e.*, only in saline waters with a very few species extended into estuarine habitats, also neither in the freshwater nor on land, cephalopods fisheries should be treated with more careful and assessed with direct primary data source to conserve the stock.

The family Loliginidae is comprised of the mostly neritic squids inhabit majority of the continental shelf seas with an exception of the very cold polar region [2, 5]. A certain species, *i.e.*, the big fin squid, *Sepioteuthis lessoniana* is being used

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Corresponding author: Norma Afiati; Email: normaafiati.na@gmail.com

for biomedical research [6] as also of economic value as commercial mariculture species [7]. Indeed that *P. duvauceli* as a species has yet having regulated fishery worldwide, neither have broad ecological review nor environmental relationship studied, apart from partial population identity recognised and researched [8]. As an ecologically important component of many near-shore ecosystems either as prey or as predators [2, 5], squid in general is known to have a very advance sight and nerve systems as also shown by their big sized eyes, in particular for the swimmer loliginid.

This study aims to collect basic information about *P. duvauceli* from northern coast of Java, such as the sex ratio in length-frequency relationship, various allometric measurements, with particular interest in allometric relationship of growth in eye size against other parts of the body, since this species is both predator and prey on various other species.

2. MATERIALS AND METHODS

Taking into consideration time limit of this study to the rough water during prolonged dry season in the year 2015, the availability of the fishermen, thus, sample collection was conducted four times; each were for regency Cirebon, West Java, regency Kendal and the city of Semarang, Central Java, and regency Tuban, East Java, from May to August 2015.

About 50 to 60 specimens for population structure analyses from each trawling station along the coast of Cirebon, Kendal, Semarang and Tuban were randomly collected from the catch on the deck. Assorted trawled specimens for ordinary collections were preserved in ethanol 70 % until further identification analyses. Fresh squid were sexed externally to assess the presence of the hectocotylus. No small immature animals that could not be sexed externally. Further, morphological characteristics of the specimens, such as mantle, fin, tentacle club, beaks, hectocotylus shape, arm sucker and arrangement, as well as number and shape of sucker teeth were examined. Whereas mantle width and length, head, gladius, tentacle and fin length, eye height and width were measured to the nearest 0.1 mm; total number and wet weight (to the nearest 0.1 g) of each specimen measured.

Allometric growth represents the growth rate of one parameter relative to that of another part of the body or to the whole organism, since body shape does not always change uniformly with an absolute increase in the size of the whole organism. Analysis of relative (allometric) growth of various shell parameters of the body normally used for bivalve mollusk was applied to this soft-bodied organism, urging the specimen evenly treated all way through the process. The relationship of any two parts of the body can be expressed by a non-linear exponential equation:

$$Y = ax^b \qquad (1)$$

Which can be linearized as the following:

$$\log_{10} Y = \log_{10} a + b \log_{10} x \qquad (2)$$

For which, x and Y are the measurements of parts of the body to be compared. The exponent b is the growth coefficient, which illustrates the relative growth rate of the two variables under consideration; while the constant a is the value of Y when x is unity [9–11]. For example, if Y is a weight or volume (g or cm^3) and x is a fin coverage area (cm^2), then b equal to 3/2 would correspond to isometry. If b is greater than 3/2 is positively allometry, whilst if less than 3/2 is negatively allometry. This simple statistic test was used to verify the deviation of b from isometry β [9, 10]:

$$t\ \text{obs}\ (n-1)\text{df} = \frac{(b-\beta)}{S\ \text{error}\ b} \qquad (3)$$

3. RESULTS AND DISCUSSION

Four species were identified during the course of this study, including *Uroteuthis (Photololigo) duvauceli* (d'Orbigny, 1835), *Octopus* sp and *Sepiella inermis* (Ferussac & d'Orbigny, 1848), whereas the fourth, big-fin squid, *Sepioteuthis lessoniana* (Ferussac, 1830) was rarely found during the trawling survey, *i.e.*, with only one individual recorded in Kendal during particular months of field work. This finding may be related to the gear selectivity and the ecology of the species. The low numbers of *S. lessoniana* obtained in the trawl survey may therefore be a result of not sampling in its main habitat.

Species composition of coastal trawling and light luring surveys conducted in Thailand [12, 13] showed similar findings to the present study. There, the catches were dominated by *U. (P.) duvauceli* comprising over 60 % of total cephalopod catches, followed by *U. (P.) chinensis*

Table 1. Descriptive statistics of dorsal mantle length (DML, mm) of *P. duvauceli* from four localities on the northern coast of Java, May to August 2015.

Locality	South	East	n	Min.	Max.	Median	Mean	SD
Cirebon	06° 38’ 12.04”	108° 37’ 47.95”	65	60.12	105.10	77.68	80.03	11.06
	06° 38’ 32.97”	108° 39’ 15.09”						
	06° 40’ 41.04”	108° 40’ 42.29”						
Kendal	06° 49’ 56.55”	110° 17’ 19.19”	62	72.90	152.08	90.67	92.43	13.71
	06° 49’ 32.24”	110° 16’ 53.93”						
Semarang	06° 55’ 42.80”	110° 24’ 30.92”	53	64.32	144.02	94.86	92.29	15.27
Tuban	06° 46’ 08.77”	111° 43’ 44.18”	31	62.12	129.02	81.62	85.42	14.54
	06° 45’ 14.54”	111° 44’ 05.04”						

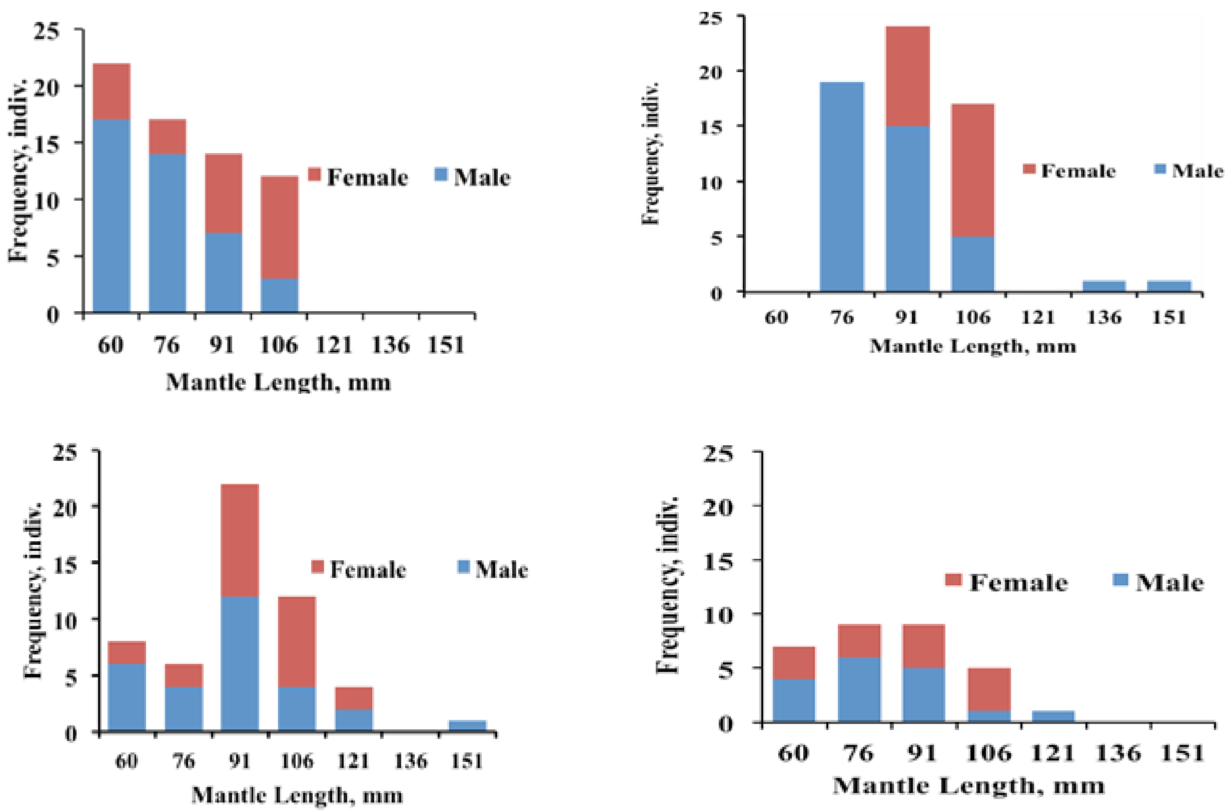

Fig. 1. Size frequency distribution based on DML of male and female *P. duvauceli*: top left A. Gebang, Cirebon (May 2015), B. Bandengan, Kendal (June 2015), bottom left C. Tambak Lorok, Semarang (July 2015), and D. Bulu, Tuban (August 2015).

at 18 % and *Loliolus* sp. (*L. sumatrensis*) which represented about 12 % of total catches. The catch of *S. lessoniana* was relatively minor, only at about 7 %. The high percentage of *U. (P.) chinensis* catches in Thailand may be due to the depth of trawling, which was between 10 m to 60 m in comparison to the present study *i.e*., 12 m to 18 m depth. Furthermore, *U. (P.) chinensis* were caught in more off shore regions (from 30 m to > 50 m depth [13]. *U. (P.) duvauceli* occurs at depths between 30 m and 170 m, and it forms large aggregations during the spawning season [14].

Maximum dorsal mantle length (DML) of *U. (P.) duvauceli* is indeed varies with geographical location, being the largest found in India ranged from 228 mm [16] up to 355 mm DML [15], or 238 mm for males and 162 mm for the females in the northwestern Red Sea [17], whereas in Thailand it attained a maximum size of 300 mm DML [13] whereas in Hong Kong, the maximum size of 155 mm DML for this species was recorded. In the present study, however, the maximum size attained was the smallest amongst the other four places of previous studies, *i.e*., 152.08 mm from Kendal waters. Seasonal variations of water temperature and productivity, which in turn affected food availability for the growth of aquatic organisms, might be the cause of these variations in maximum body size of the cephalopods [16]. Within the localities studied, specimen from Kendal waters was in average the largest (92.43 mm ± 3.71 mm; Table 1), whereas those from Cirebon were the smallest (80.03 mm ± 1.06 mm). Besides food availability, this difference in maximum attainable length could be attributable to seasonal reproduction, sampling size [16, 17] and perhaps lack of effort to apply an open and close season for the caught of squid in Indonesia, unlike in South Africa [18, 19].

Fig. 1B revealed that specimen from Kendal consists of the largest-in-average-size specimen (92.43 mm; Table 1) and was mostly male. Choi [14] reported that in the Gulf of Thailand size at 50 % maturity ranges between 90 mm and 130 mm mantle length for females and 70 mm to 150 mm for males. Therefore, in general, all samples in this study were consists of juvenile to adult size (Table 1). Subsequently, sex ratio for population in Cirebon and Kendal was 1:1.7 and 1:1.95 in favor of males, whereas population from Semarang and Tuban shared the same sex ratio, *i.e.,* 1:1.21 for male individuals. Further, observations on growth after sexual maturity support a suggestion that an extended reproductive phase existed within the life cycle of *U. (P.) duvauceli*, *i.e.,* not a strictly semelparous reproduction, as is the case in other squid [14]. This finding was supported by recent study of the same species from northwestern part of the Red Sea [17], which reported that this species been experiencing longevity ranged from 3.08 yr to 3.54 yr.

In natural food web, squid is a main prey for large carnivorous fish [20], for which the consumer includes at least 19 species of fish, 13 species sea bird and six sea mammals [21] yet, squid also a robust predator themselves. Thus, despite its advance vision, innervation and pigmentation, it is thought that morphologically their eyes grow quickly to escape predation and to prey – since, hatchlings, about 1 mm to 1.8 mm mantle length, have big eyes and are planktonic.

Table 2 shows 54 allometric growth coefficients (*b*) measured from various parts of *U. (P.) duvauceli* body. In general, Kendal and Cirebon populations showed more similarity to each other compared to the other two populations, *i.e*. Tuban and Semarang. It can be seen in those four populations that growth rate of mantle length was always relatively faster than the width, resulting a slender body shape of the organism. Length and width of the fin grow comparably at the same rate or even faster (isometrically or positively allometric) than the length and width of the body. It is also shown that head length and eye shape (length and height of the eye) grow significantly slower compared to the body length means that *P. duvauceli* concerned more to the fin growth and development than to its eye size, even though newly hatch squid seemed to have shown big eyes compare to its overall body size. It does not mean though that the sight is less developed [22]. Eyes grow according to the long axis than to its height. Likewise gladius width that grow isometrically to its length, fin length and width grow more or less isometrically to each other to get its perfect shape. Fins developed significantly faster ($P < 0.01$) than the mantle, the head, the eyes, tentacles, gladius and even to the overall weight of the animal. These measurements then suggest that *P. duvauceli* is indeed puts balance into priority as a true swimmer.

Table 2. Allometric coefficient noted as slope or *b* value in logarithmic regression equation ($Y = ax^b$; a is the intrecept) of *P. duvauceli* from Semarang, Tuban, Kendal and Cirebon. All isometry, positive (+) and negative (-) allometric growth were significant at P < 0.01.

Variable														
Ind.	Dep.	β	*b*-Smrg	Allo.	R	*b*-Tuban	Allo.	R	*b*-Kendal	Allo.	R	*b*-Crbn	Allo.	R
ML	MW	1	0.42	-	0.762	0.72	-	0.883	0.50	-	0.678	0.74	-	0,757
	FL	1	1.19	+	0.962	1.28	+	0.981	1.11	iso	0.942	1.40	+	0.864
	FW	1	1.27	+	0.878	1.43	+	0.939	1.04	iso	0.838	1.12	iso	0.728
	HL	1	0.38	-	0.445	0.46	-	0.79	0.44	-	0.437	0.50	-	0.402
	EL	1	0.26	-	0.359	0.49	-	0.821	0.29	-	0.385	0.38	-	0.371
	EH	1	0.35	-	0.496	0.30	-	0.533	0.31	-	0.383	0.22	-	0.147
	TL	1	0.65	-	0.651	0.97	iso	0.805	0.79	-	0.763	0.81	iso	0.552
	GL	1	0.95	iso	0.949	0.99	iso	0.998	0.94	iso	0.966	0.78	-	0.78
	GW	1	0.86	iso	0.735	0.98	iso	0.896	0.83	iso	0.744	0.79	iso	0.355
	WWT	3	2.05	-	0.938	2.37	-	0.969	2.13	-	0.928	2.30	-	0.813
MW	FL	1	1.60	+	0.714	1.38	+	0.869	0.99	iso	0.613	1.18	iso	0.707
	FW	1	1.86	+	0.708	1.58	+	0.849	0.99	iso	0.575	0.86	iso	0.541
	HL	1	0.93	iso	0.603	0.53	-	0.75	0.47	-	0.345	0.48	-	0.379
	EL	1	0.44	-	0.343	0.53	-	0.737	0.32	-	0.306	0.25	-	0.238
	EH	1	0.67	iso	0.526	0.33	-	0.468	0.40	-	0.365	-0.10	-	0.061
	TL	1	1.13	iso	0.628	1.05	iso	0.719	0.68	iso	0.485	0.87	iso	0.579
	GL	1	1.37	iso	0.758	1.07	iso	0.882	0.87	iso	0.657	0.61	-	0.591
	GW	1	1.49	+	0.7	1.19	iso	0.889	0.73	iso	0.483	0.50	iso	0.22
	WWT	3	3.32	iso	0.84	2.72	iso	0.91	1.96	-	0.628	1.87	-	0.641
FL	FW	1	0.94	iso	0.798	1.10	iso	0.936	0.89	iso	0.848	0.59	-	0.625
	HL	1	0.27	-	0.387	0.36	-	0.792	0.32	-	0.372	0.21	-	0.272
	EL	1	0.24	-	0.409	0.38	-	0.827	0.21	-	0.328	0.19	-	0.306
	EH	1	0.30	-	0.534	0.22	-	0.489	0.27	-	0.402	0.17	-	0.183
	TL	1	0.57	-	0.707	0.71	-	0.769	0.63	-	0.720	0.42	-	0.469
	GL	1	0.73	-	0.905	0.75	-	0.983	0.77	-	0.932	0.42	-	0.67
	GW	1	0.66	-	0.695	0.75	-	0.885	0.71	-	0.755	0.41	-	0.298
	WWT	3	1.60	-	0.902	1.81	-	0.959	1.73	-	0.891	1.28	-	0.735
FW	HL	1	0.29	-	0.484	0.31	-	0.819	0.20	-	0.250	0.38	-	0.477
	EL	1	0.21	-	0.419	0.30	-	0.786	0.24	-	0.392	0.24	-	0.36
	EH	1	0.24	-	0.504	0.16	-	0.424	0.30	-	0.461	0.23	-	0.235
	TL	1	0.33	-	0.474	0.64	-	0.814	0.58	-	0.697	0.39	-	0.407
	GL	1	0.60	-	0.875	0.61	-	0.94	0.65	-	0.829	0.46	-	0.711
	GW	1	0.56	-	0.691	0.61	-	0.851	0.63	-	0.698	0.48	-	0.331
	WWT	3	1.33	-	0.885	1.48	-	0.924	1.51	-	0.810	1.13	-	0.614
HL	EL	1	0.36	-	0.429	0.83	iso	0.813	0.30	-	0.392	0.19	-	0.227
	EH	1	0.39	-	0.473	0.55	-	0.566	0.21	-	0.261	-0.22	-	0.176
	TL	1	0.40	-	0.343	1.41	iso	0.685	0.35	-	0.342	0.41	-	0.348
	GL	1	0.57	-	0.488	1.33	iso	0.784	0.43	-	0.448	0.27	-	0.334
	GW	1	0.54	-	0.392	1.39	iso	0.738	0.13	-	0.121	0.24	-	0.133
	WWT	3	1.38	-	0.542	3.42	iso	0.815	1.19	-	0.521	0.83	-	0.362
EL	EH	1	0.66	-	0.673	0.53	-	0.544	0.48	-	0.453	0.44	-	0.295
	TL	1	0.27	-	0.19	1.55	iso	0.763	0.52	-	0.382	0.52	-	0.359
	GL	1	0.60	iso	0.424	1.38	iso	0.824	0.58	-	0.456	0.40	-	0.408
	GW	1	0.70	iso	0.42	1.44	iso	0.777	0.50	-	0.344	0.89	iso	0.406
	WWT	3	1.29	-	0.42	3.55	iso	0.857	1.29	-	0.428	0.95	-	0.341
EH	TL	1	0.61	iso	0.424	0.87	iso	0.415	0.47	-	0.359	-0.02	-	0.022
	GL	1	0.72	iso	0.504	0.91	iso	0.526	0.48	-	0.394	0.25	-	0.384
	GW	1	0.72	iso	0.426	1.01	iso	0.525	0.62	-	0.448	0.20	-	0.136
	WWT	3	1.83	-	0.583	2.31	iso	0.538	1.17	-	0.408	0.39	-	0.21
TL	GL	1	0.61	-	0.607	0.66	-	0.794	0.72	-	0.767	0.38	-	0.55
	GW	1	0.61	-	0.513	0.70	-	0.767	0.71	-	0.658	0.20	-	0.132
	WWT	3	1.54	-	0.703	1.64	-	0.806	1.78	-	0.802	0.88	-	0.452
GL	GW	1	0.91	iso	0.77	0.99	iso	0.893	0.86	iso	0.750	0.98	iso	0.442
	WWT	3	2.02	-	0.923	2.39	-	0.966	2.21	-	0.936	1.82	-	0.646
GW	WWT	3	1.42	-	0.765	2.08	-	0.931	1.60	-	0.775	0.32	-	0.251

Legend:
WWt = Total Wet Weight, FL = Fin Length, EL = Eye Length, GL = Gladius Length
ML= (Dorsal) Mantle Length, FW = Fin Width, EH = Eye Height, GW = Gladius Width
MW = Mantle Width, HL = Head Length, TL = Tentacle Length, Ind.= Independent variable
Dep. = Dependent variable, Smrg = Semarang, Crbn = Cirebon, Iso & Allo. = Isometry & Allometry

Changes in the relative (allometric) growth of the brachial crown (tentacles) in squid are thought as an adaptation of pelagic biomass spectra [20]. In most squid as predators, arm size increases relatively rapidly in relation to overall body size especially in early life. It has probably evolved in response to the need to shift predation from one peak in the biomass spectrum to the next, accommodating the transition between diets differing in body size by at least one order of magnitude [23]. Discontinuities often occur in allometric growth of the brachial crown of juvenile squid [24, 25]. In this study, however, growth in tentacle length of *P. duvauceli* increased slower than fin and head length ($P < 0.01$), isometrically to the mantle width and positively allometric toward gladius length and width as well as body weight in those four juvenile's populations (Table 2; $P < 0.01$).

Recent studies in India waters (b= 2.368) [16], Red Sea (b=2.02) [17], Sabah, Malaysia for the congeneric *U. chinensis* (b= 2.579) [26], South China Sea and Beibu Gulf, China (b = 2.217 and 2.229, respectively) [27], Goa in the west coast of India (b= 1.613 to 1.672) [28], shown that all recorded 'b' values of length-weight relationship significantly differed from the ideal cube law of '3' or isometry (mostly $R \geq 0.900$) [29]. In line with those studies, wet weight proved negatively allometric to length (b= 2.05, 2.37, 2.13 and 2.30 for Semarang, Tuban, Kendal and Cirebon, R ranged from 0.813 to 0.969 respectively; Table 2) as well as to almost all any other part of the body (Table 2), suggesting that as a true swimmer *P. duvauceli* urges light body weight. Meanwhile, females at the same length were heavier than males; yet, males ultimately attained a larger size. These findings of weight and female body length conformed to that of Choi [14].

4. CONCLUSIONS

As a true swimmer *U. (P.) duvauceli* put forward the need of fin and lightweight body (perhaps to escape predation) more than for brachial crown and eye size, which supposed to be the main feature of squid as predator. Regardless the variability of squid caught in four localities along northern coast of Java island, for which the maximum attainable size in particular of *U. (P.) duvauceli* is the smallest among other countries, for example compared to various places in India, Malaysia, Thailand, Hong Kong, Red Sea and China, it is an urgency for the Government of the Republic of Indonesia to set up a regulation on squid fisheries, including open and close season scheduled throughout the country. This is due to the lack of current and accurate data on basic fisheries analyses for mollusks in general, including cephalopods, in the Fisheries Bureau.

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Research Article

State of the Art of Global Dimethyl Ether Production and It's Potentional Application in Indonesia

Bayu Prabowo[1,2]*, Mi Yan[2], Mochamad Syamsiro[3], Roy Hendroko Setyobudi[4, 5], and Muhammad Kunta Biddinika[6]

[1]Department of Renewable Energy Engineering, Universitas Prasetiya Mulya, Tangerang, Banten 15339, Indonesia
[2]Institute of Energy and Power Engineering, Zhejiang University of Technology, Hangzhou, Zhejiang 310014, China
[3]Department of Mechanical Engineering, Janabadra University, Jl. TR Mataram 57, Yogyakarta 55231, Indonesia
[4]Ma Chung Research Center for Photosynthetic Pigments, Villa Puncak Tidar N-01 Malang 65151, East Java, Indonesia
[5]Indonesian Association of Bioenergy Scientist and Technologist. BPPT Building II, 22nd floor Jl. MH. Thamrin No. 8 Jakarta 10340, Indonesia,
[6]Department of Environmental Science and Technology, Tokyo Institute of Technology, Yokohama, Kanagawa 226-8503, Japan

Abstract: Dimethyl ether (DME) is a flammable gas that changes to liquid phase when applied a relatively mild pressure to it, i.e., 0.5 MPa. Therefore, DME has a special advantage to be utilized as both the gas and liquid fuels. Moreover, DME can be produced from various feedstock, including natural gas, residual oil, coal and biomass. On account of these features, DME might be a suitable form of intermediate fuel for Indonesia, which has abundant and unutilized energy resource but in other hand still highly relies on the import of energy commodities, particularly liquefied petroleum gas (LPG) and diesel oil. This paper summarized the state of art of worldwide DME development and application, as well as discussed the potential application of DME in Indonesia. DME properties, production process and brief development history were first introduced. Then, state of the art of the global DME application and commercial market along with the available technology providers were summarized. Economic analysis and challenges for global DME application were subsequently discussed. Finally, the analysis of driving factor, potential benefit, and current state of DME application in Indonesia were presented. This paper could be an initial guide for the development of DME industry and application in Indonesia.

Keywords: Application, DME, Indonesia, LPG, review, energy, fuel

1. INTRODUCTION

1.1 Introduction of DME and it's Benefit

Dimethyl ether (DME) is an organic compound with the formula of CH_3OCH_3. It is a highly flammable gas at ambient conditions which forms liquid phase when it is pressurized above 0.5 MPa. Therefore, DME is commonly handled and stored as liquid. As shown in Table 1, the calorific value of DME in liquid form, 4 620 kcal · L^{-1}, is about 85 % of liquefied petroleum gas (LPG); while in gaseous form, 14 200 kcal · Nm^{-3}, is about 1.6 times of natural gas. Moreover, its cetane number, 55 to 60, is 1 to 1.5 times of diesel oil. Owing to these properties, DME has the special advantage to be penetrable to the gas and liquid fuel market [1]. The potential major uses of dimethyl ether are either as a propane substitute in LPG for residential cooking, or as a fuel in gas turbine power generator, and as a transportation fuel in diesel engines or petrol

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*Corresponding author: Bayu Prabowo; Email: 2847728511@qq.com

Table 1. Properties of DME and other fuels [1, 2].

Properties	DME	Propane (LPG)	Methane (Nat.gas)	Diesel fuel
Chemical formula	CH_3OCH_3	C_3H_8	CH_4	
Boiling Point (˚C)	-25.1	-42.0	-161.5	180 to 370
Liquid density (g cm^{-3} at 20 ˚C)	0.67	0.49	0.42	0.84
Liquid viscosity (kg m^{-1} s^{-1} at 25 ˚C)	0.12 to 0.15	0.2	-	2 to 4
Specific gravity of gas (vs. air)	1.59	1.52	0.55	-
Vapor pressure (MPa at 25 ˚C)	0.61	0.93	-	-
Explosion limit (%)	3.4 to 17	2.1 to 9.4	5 to15	0.6 to 6.5
Cetane number	55 to 60	5	0	40 to 55
Net calorific value (kcal Nm^{-3})	14 200	21 800	8 600	-
Net calorific value (kcal L^{-1})	4 620	5 440	5 040	8 400
Net calorific value (kcal kg^{-1})	6 900	11 100	12 000	10 000

engines (30 % DME / 70 % LPG) [1–3].

Furthermore, DME is known to be a clean and valuable alternative energy [2, 3] for several reasons:

- It can be safely stored and handled, as it does not produce explosive peroxides
- It's combustion products, such as carbon monoxide and unburned hydrocarbon emissions, are less than those of natural gas since DME only has C-H and C-O bond, but no C-C bond, and since it contains about 35 % oxygen
- Owing to its high cetane number, DME is considered to be an excellent alternative to the present transportation fuel with no emission of particulate matter and toxic gases such as NO_x at burning
- It has a similar vapor pressure to that of LPG, and hence can be used in the existing infrastructures for transportation and storage.
- DME is degradable in the atmosphere and is not a greenhouse gas

Another advantage of DME is that it can be produced from a variety of feedstock including natural gas, crude oil, residual oil, coal, biomass and waste products. Fig. 1 shows illustrate the beneficial "Multi-Source and Multi-Use" feature of DME. This feature is favorable for providing a flexibility and sustainability not only on resource supply but also subsequently on the product marketing.

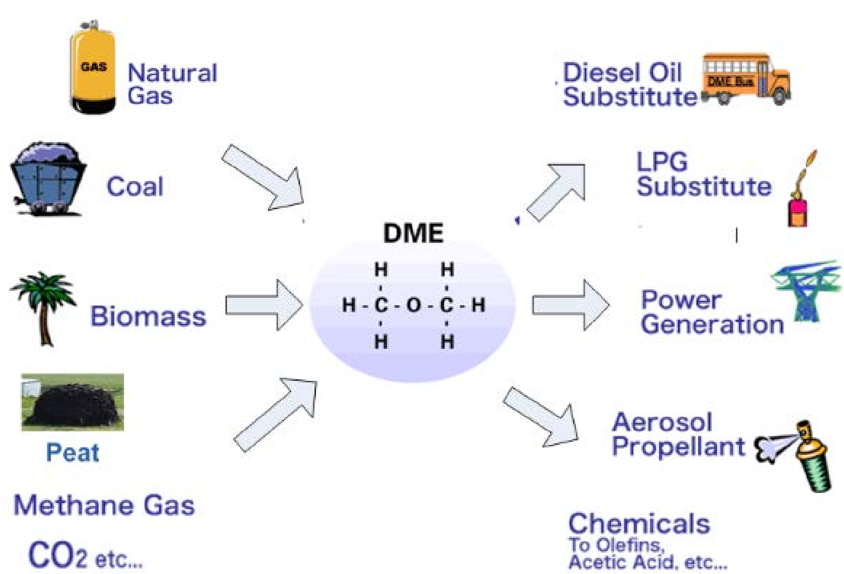

Fig. 1. "Multi-source and multi-use" feature of DME.

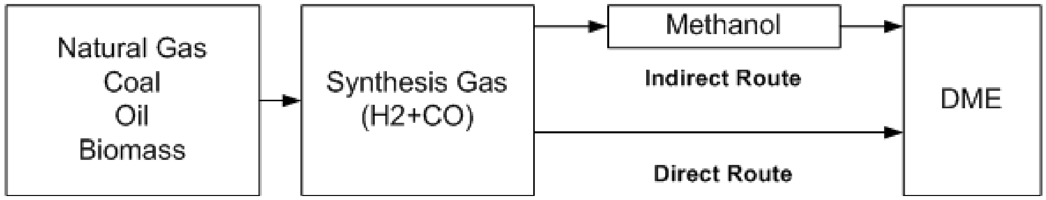

Fig. 2. DME production pathways.

Fig. 2 shows the routes of DME production in two distinct ways: The first way, called as the indirect route, is through the synthesis of methanol which then followed by its dehydration to DME (involving reaction shown in Eq.1 and Eq. 2); the second way, called as the direct route, is through a single stage of DME synthesis using bi-functional catalyst (shown in Eq. 3). As shown in Eq. 1 and Eq. 3, all of the process routes are involving synthesis gas (syngas: the mixture of H_2 and CO) as the intermediate feedstock. Meanwhile, syngas composition could be highly varied depend on the utilized feedstock and it will subsequently affect the selection of the suitable DME synthesis route. Coal or biomass derived syngas, obtained through a high temperature gasification process, has a composition of H_2/CO = 0.5 to 1. Therefore, the direct route through Eq. 3 is appropriate since the required H_2/CO = 1. In contrary, natural gas derived syngas, obtained through a methane reforming process, has a composition of H_2/CO = 1 to 3. Therefore, the indirect route through Eq. 1 is appropriate since the desired H_2/CO = 2. However, the adjustment of H_2/CO ratio for each syngas is possible by utilizing water gas shift reaction (Eq. 4) [2]. In addition, it is studied that the direct route is potentially more efficient than the indirect [4, 2]. That is related to a cost saving by process simplification and an occurrence of high CO conversion due to the prompt consumption of the "produced methanol [4, 5]."

Methanol synthesis:

$$2CO+4H_2 \leftrightarrow 2CH_3OH \quad (1)$$

Methanol dehydration:

$$2CH_3OH \leftrightarrow CH_3OCH_3+H_2O \quad (2)$$

DME Synthesis:

$$3CO+3H_2 \leftrightarrow CH_3OCH_3+CO_2 \quad (3)$$

Water gas shift reaction:

$$CO+H_2O \leftrightarrow H_2+CO_2 \quad (4)$$

Catalyst is utilized for obtaining a higher selectivity towards DME formation and a lower tendency on hydrocarbons and coke generations. In the indirect process, metallic catalyst is employed in the methanol synthesis then solid-acid catalyst is employed in the methanol dehydration to DME. While for the direct process, bi-functional catalysts, composed of a metallic synthesis and a solid-acid function, is employed. The metallic function is mainly composed of such oxides as CuO, ZnO, Al_2O_3, and Cr_2O_3 with the compound of CuO-ZnO-Al_2O_3 (CZA) being the mostly applied [6, 2]. The solid-acid function includes γ-Al_2O_3 and zeolites such as H-ZSM-5, HY, SAPO, MCM, and Ferrierite with H-ZSM-5 being the mostly applied [2, 5]. As methanol synthesis is the rate limiting step in DME synthesis, research works for improving metallic catalyst activity have been widely reported [2, 5, 6]. Those were aimed to maximize Cu active site on Cu-ZnO interface by altering blending ratio [2, 5] and preparation method [2, 6] of Cu and ZnO. Improvement of bi-functional catalyst activity and selectivity by altering the composition and blending method of metallic and acid function also become the emerging focus on DME catalyst development [2, 5, 6].

DME reactor typically operated at the temperature of (200 to 350) °C with the pressure of (30 to 50) bar [2]; (1 bar = 100 kPa). This resulted in the syngas conversion thermal efficiency of around 80 % to 90 % and DME purity of more than 99.5 %. Combined with the efficiency of the upstream process for syngas production (gasification or methane reforming), the overall DME production process thermal efficiency can be around 53 % to 66 % for coal/biomass based process [7, 8], and 70 % for natural gas based process [1]. These efficiencies are relatively high compared with other synthetic fuel production through Fischer-Tropsch reaction, e.g. syn-diesel, gasoline, etc., that produced thermal efficiency of around 30 % to 50 % for coal/biomass based process and 53 % to 63 % for natural gas based process [9]. Therefore, DME can be manufactured at a lower cost than Fischer-Tropsch fuel [8].

1.2 Brief History of DME Development

- ***1970s to 1980s***

The research and development of the novel fuels including DME was motivated by the increasing of oil prices due to the oil embargos. On the other hand, the remarkable growth in stranded gas also led to an effort to utilize these gas resources by converting them into some easily transportable liquid fuels. Amoco (now BP) was named as a pioneer in this novel fuels development [1]. In the 1980s preliminary engine tests were performed at the National Institute for Petroleum and Energy Research laboratories at Bartlesville in Oklahoma (US) addressing chances and challenges in the use of DME as a fuel in diesel vehicles [3].

- ***1990s***

In 1995, collaborative research on DME among Amoco, Haldor Topsoe and Navistar International Corp showed that DME could be a novel, low-emissions alternative fuel for diesel engines and could be manufactured at large-scale from a simple methanol with dehydration technology [1, 3]. However, the development of the DME diesel market was challenging because of the prerequisite adjustment of the fuel distribution infrastructure and the modifications to the engines themselves. Meanwhile, the obvious primary market of DME was the blending with LPG up to 20 volume % (similar to the blends in automotive applications). This blend worked well in standard cooking and heating applications without any significant adjustment to existing LPG infrastructure [1], except 20 % to 35 % increase in the storage capacity or more frequent delivery of the LPG container [3].

In the mid-to late 1990s, Amoco with the General Electric Co. and Electric Power Development Corporation of Japan (EPDC) tested DME as a gas turbine fuel. The results showed excellent performance with a significant improvement in efficiency and the same low emissions as natural gas [1]. DME as a new multi-purpose fuel was introduced to the world through press releases, press conferences and publications in 1995. About fifteen companies and agencies from around the world expressed interest in sharing in work aimed at the commercialization of DME.

In 1998, Amoco formed a joint venture with both the India Oil Company (IOC) and the Gas Authority of India (GAIL). The India joint venture identified the power market as the primary market because of its ease of entry and lower market risk. The LPG blending and diesel market were targeted for future DME trains after the appropriate technical demonstrations, regulatory work and development of standards were completed. Supply agreements were signed with several Indian power producers. For gas access, the joint venture held talks with several countries with large gas resources, particularly Qatar. However, in 2001, the DME project was terminated by BP (the merger company of British Petroleum and Amoco) due to other more favorable gas-related ventures.

- ***2000's***

Despite the project termination, a global interest in DME had been awakened. The International DME Association (IDA) was formed in 2001. In the next few years, The Japan DME Forum (JDF), the Korea DME Forum (KDF) and the China DME Association (CDA) were formed. Information was shared in both regional Asian meetings and in international DME meetings. The significant results on commercialization of DME were produced by CDA in the past decades due several reasons:

- The presence of a large demand for additional home cooking and heating fuel in China. DME positively contributed to this issue since their distribution was relatively simple through LPG blending using the existing infrastructure and marketing channels
- A clear government direction to use the domestic coal resource as a feedstock for chemicals and fuels. On the other hand, the established coal-to-methanol plants had resulting in a large oversupply of methanol. Therefore, the DME manufacturing was lead to near-term profit.
- History have shown that Chinese authorities and business are quick in adapting new technologies and products

2. STATE OF THE ART OF GLOBAL DME APPLICATION AND INDUSTRY

2.1 State of the Art of the Application and Market of DME

In 2011, the global DME demand is estimated about 3 000 000 metric t yr^{-1} and it is growing at 32 % year-over-year. The demand is expected to rise to over 7 000 000 metric t yr^{-1} by 2015 while the global capacity ranges between 10 000 000 and 12 000 000 metric t yr^{-1}. In summary, China, a late-comer in DME, has been the unmatched player in DME production and consumption. Nearly all of the commercial DME plants for fuel applications have been built in China and about 90 % of the global DME demand is in China [1].

2.1.1 LPG Blend Stock in Residential Cooking/ Heating Sector

The largest market for the use of DME is as a blend fuel with LPG for residential cooking and heating, particularly in China [1]. This is related to the fact that, the use of (15 to 20) volume % of DME in LPG/DME blends would not require any modification either in the existing distribution infrastructure or in the users' appliances. More than 90 % of DME produced in China is blended with LPG. Various regulations and industry standards for the use of DME in such applications have been or are being implemented in China. In South Korea, DME-LPG Blended Fuel Field Test was performed for household and commercial use since Aug 2010 to Oct 2011 (15 mo). In the U.S., the LPG industry still require additional experimental studies to determine the safe limits for DME in LPG/DME blends as a prerequisite to commercialization using existing appliances for residential and restaurant applications [1].

2.1.2 Transportation Fuel Alternatives

DME offers many other advantages such as significantly reduces engine noise, absence of cold-start problems and the likelihood of light-weight, low-cost DME diesel engines because of the very low ignition pressure of DME. Despite the promising properties of DME and significant progress of DME-engine development in many countries, the utilization of DME as an alternative transportation fuel is still in the early step of commercialization. This is because some adjustment of the fuel distribution infrastructure and the modifications to the engines are required to able to accommodate the DME feed.

- **Diesel Fuel Alternative**

The Amoco consortium work conducted around 1994 showed for the first time that DME is an ultra-clean alternative fuel for diesel engines with emissions levels that could meet the 1995 ULEV (the California Ultra Low Emissions Vehicle) regulations for medium duty vehicles [1].

In Europe, Haldor Topsoe (Denmark) developed the first DME-fueled vehicles in 1996. Volvo (Sweden) developed the first DME-fueled bus in 1999 which had a fuel consumption of 1.17 km L^{-1}. Then, a second generation of DME vehicle with a 224-kW engine launched in 2005 and, a third generation DME truck (displacement: 13 L) with 343 kW (max.) engine power and 2732.56 Nm of torque has been scheduled for release in 2015 [10]. Moreover, for biomass-derived DME (Bio-DME), ten Volvo Bio-DME trucks have been operated in Sweden and the first of these passed the 100 000 km [1].

In the USA between 1999 and 2001, a consortium under the Department of Energy (DOE) with some companies developed a project to convert diesel buses to DME-diesel blended fuels (14 % DME and 25 % DME) buses. They suggested optimization of the injection strategy and engine control logic for DME blended diesel fuels, and emphasized the need for an oxidation catalyst [10].

In Japan, between 1998 and 2001, a consortium led by the National Traffic Safety and Environment Laboratory (NTSEL) developed a heavy-duty DME bus operated by a mechanical injection device with Nissan diesel motors and Bosch Japan. Then the National Institute of Advanced Industrial Science and Technology (AIST) developed a medium-duty DME-fuelled truck with 7.1 L of displacement and showed that this truck had very low emission levels with its average fuel consumption was 2.61 km L^{-1} (diesel equivalent fuel consumption: 4.93 km L^{-1}). Nissan diesel motors developed an in-line, 6-cylinder truck operated by DME fuel with NTSEL. Isuzu Motors is also developing light- and medium-duty DME engines with a common-rail injection system. They reported based on field test that light-duty and medium-duty DME-fuelled vehicles had fuel consumption rates of 2.83 km L^{-1} and 3.81 km L^{-1}, respectively [10].

In China, 10 DME-fueled buses with a mechanical fuel supply system along with the filling station were developed by a consortium of the Shanghai Motor Company, Shanghai Jiao Tong University (SJTU), and Shanghai Coking & Chemical Corporation in 2005. These vehicles were successfully tested in a commercial operation for more than 220 000 km. Since then, second and third generation DME vehicles have been developed to satisfy the Euro-5 emission standards by a common-rail injection system and after-treatment devices. Shanghai has examined the extension of DME as vehicle fuel for trucks, taxis, and buses in order to reduce PM 2.5 pollution in the city (Park SH & Lee CS, 2014). As of 2009, there were three DME city buses with commercial license plates in use in Shanghai [1].

In Korea, the Korea Institute of Energy Research (KIER) manufactured a proto-type DME truck with 3.0 L of displacement in 2003. In addition, they undertook a project to convert diesel bus with 8.1 L of displacement to a DME-fuelled bus in 2005. KIER successfully developed a proto-type DME bus for 33 passengers which was successfully driven on the road in 2010. Hanyang University (HYU) developed a DME engine for passenger car with 1.6 L of displacement based on a common-rail injection system and it has been successfully driven on the road. Korea Automotive Technology Institute (KATEC) modified a sports utility vehicle (SUV) with 2.0 L of displacement to a DME-fuelled vehicle in 2009. They also studied a 2.9 L DME-fuelled light-duty truck with a common-rail injection system in 2012 which emission levels satisfy the Euro-5 emission standard [10].

- **LPG/DME Blends as Transportation Fuel Using Spark Ignition (SI) Engines**

In the U.S., LPG is the leading alternative transportation fuel, after gasoline and diesel, primarily for marine applications that use SI engines. There are over 10 000 000 LPG-fuelled vehicles in the world, including about 270 000 in the U.S. where LPG is facing severe competition and new stricter emission regulations [1]. For this market sector, DME, especially, bio-DME produced via biomass gasification, may find a niche application in the future as a blend fuel, at about (15 to 20) vol. %. Preliminary research by Amoco in 1997 indicated that, a DME-Propane blend of about (10 to 25) wt. % DME can be used in specific SI engines due to the relatively high Octane Numbers of LPG [1].

2.1.3 Gas Turbine Fuel

In 2001, Amoco conducted a pressurized combustion tests to evaluate the suitability of DME as a gas turbine fuel. The test results demonstrated that the fuel-grade DME, can be successfully used in all modes of turbine operation with emission properties, specifically, NOx, CO and UHC-Unburned hydrocarbons, comparable to natural gas [1]. Based on those tests results, General Electric (GE) was to pursue commercial offers of DME-fired E class and F class heavy duty gas turbines. Amoco had also worked with GE and Fluor to estimate the comparative performance of nominal 1000-MWe 50-cycle grid Combined Cycle Power Plants fuelled with natural gas and Amoco's fuel-grade DME using GE 9FA gas turbines. Around 1.5 % higher power generation efficiency than that of the natural gas fuelled power plant can be expected from the DME fuelled power plants (55.9 % with DME compared to 55.4 % with natural gas) [1]. Various DME-fuelled gas turbine related test programs have also been carried out in Japan (e.g., TEPCO/JFE and Mitsubishi Chemicals GT tests) and South Korea.

2.1.4 Chemical Intermediate for Producing Olefins

China is significantly interested to produce propylene and ethylene from coal, primarily via various methanol-to-olefins (MTO) processes, such as Honeywell UOP's Advanced MTO process and Lurgi's methanol-to-propylene (MTP) process. In 2010, more than 20 such coal-to-olefins projects are currently at the planning stage in China while three such demonstration plants, owned by Shenhua Baotou, Datang (Duolun) and Shenghua Ningxia Coal Inc, have been operated. UOP announced that they would license their MTO process technology to China's Wison (Nanjing Clean Energy Co.) to produce about 295 000 metric t yr^{-1} of ethylene plus propylene from coal-derived methanol. Dow Chemical and the Shenhua Group also announced their plans to build a USD 10 000 000 000 coal-to-olefins complex, which is scheduled to start operating around 2016, in China. Key Chinese and Japanese groups have also developed similar technologies: (i) Dalian Institute of Chemicals Physics, under the Chinese Academy of Sciences, has demonstrated technology to convert a mixture of methanol/DME to olefins with very high selectivity to ethylene and propylene, and (ii) Japan Gas Chemical Company has also pursued a process to produce olefins from DME [1].

2.2 DME Industry

2.2.1 Technology Provider

The first-generation processes for the synthesis of DME are based on dehydration of methanol. For this relatively mature indirect route technology there are several licensors including Haldor Tospoe, Linde/Lurgi, Toyo Engineering, Uhde, Mitsubishi Gas Chemical Company, China Southwestern Research Institute of Chemical Industry and China Energy (Jiutai Group). Meanwhile for the relatively new but arguably more efficient direct route technology, several companies: Haldor Topsoe, Japan JFE Holdings Company, and Korea Gas Corporation, have developed their DME synthesis technologies.

2.2.2 Established and Projected DME Plants

- ***China***

Nearly all of the commercial DME plants for fuel applications have been built in China. During 2011, there were 60 DME producers in 18 provinces, of which 17 have capacities of at least 200 000 metric t yr^{-1} [1]. Five companies represent 60 % of total capacity; namely, Jiutai Energy Group (aka China Energy), XinAo Group (aka ENN Group), Lutianhua, Tianmao and Lanhua Kechuan. The Jiutai Group and SWI (China Southwestern Research & Design Institute of Chemical Industry) of China have also developed their own DME technologies [2].

- ***Japan***

Three groups have been studying DME production; namely, Japan DME, Ltd. Group, DME International Ltd. Group and Mitsui &Co., Ltd. Group. These studies focused on the feasibility of commercial DME production in gas-producing regions such as the Middle East, Southeast Asia and Oceania. In 2009, the Japan DME Ltd. Group build an 80 000 metric t · yr^{-1} DME plant using imported methanol, in the Mitsubishi Gas Chemical Factory in Niigata, Japan [1].

- ***Korea***

Some important step of development and commercialization of DME are actively conducted by KOGAS especially for catalyst invention, reactor design and process intensification. 50 kg · d^{-1} pilot plant was built in 2003 and 3 000 metric t · yr^{-1} demo plant in operation since 2008. DME-LPG Blended Fuel Field Test was performed for 15 mo for household and commercial use since Aug 2010 to Oct 2011. KOGAS also provides 300 000 metric t · yr^{-1} basic engineering package.

- **Other Countries** [1]

i. Saudi Arabia: 300 000 metric t yr^{-1} planned by KOGAS, with Unitel Technologies chosen to do frontend engineering design for the plant

ii. Sweden: world's first 4 metric t · d^{-1} bio-DME plant operational by Chemrec. Chemrec is planning a 300 metric t d^{-1} commercial-scale bio-DME plant

iii. Egypt: 200 000 metric t yr^{-1} planned

iv. Indonesia: 800 000 metric t yr^{-1} planned

v. India: 265 000 metric t yr^{-1} planned

vi. Vietnam: project announced in 2010

vii. Uzbekistan: 100 000 metric t yr^{-1} planned

viii. KOGAS has announced plans to form joint ventures to build commercial DME plants in Oman, Mongolia, Myanmar and Australia.

3. ECONOMIC ANALYSIS AND CHALLENGE FOR GLOBAL DME APPLICATION

3.1 Economic Analysis of General DME Production

The business prospect of DME as a new fuel is determined by the economics for the supply chain including the feedstock resource, production cost, and transportation to the destined market. An economic analysis for DME supply chain to China has been conducted previously. For the case of DME produced from integrated plants in the Middle East from natural gas; that cost USD 1.50 per MMBtu (LHV) (1 MMBtu = 28.263 682 m^3) it is estimated that DME to be deliverable to China at USD 368 per MT. The bases for these estimates are shown in Table 2. These economics show that DME can be competitive with conventional petroleum-derived products, on an energy equivalent basis, when crude oil costs at least (USD 70 to USD 80) bbl^{-1} (1 bbl = 159 L), depending if DME is valued as LPG or as higher-valued diesel, on an energy equivalent basis.

Coal-derived DME is more expensive than natural gas-derived DME due to: (i) higher capital investment, (ii) higher feedstock cost on an energy equivalent basis, and (iii) lower plant thermal efficiency. Therefore, the key assumption for coal-to-DME plant is that the capital investment costs is 50 % more than plants using natural gas per annual metric ton produced. For the case of DME produced from integrated plants in China from coal, costing USD 100 per MT, it is estimated that

Table 2. Bases for DME cost analysis [1]

Cost component	DME resource	
	Natural gas	Coal
Plant capacity, MT/day	3 520	35 20
Plant capital cost/CAPEX, ($ million)	1 110	1 508
Feedstock consumption, ((MMBtu or MT)/ MT-DME)	38 MMBtu	2.05 MT
Feedstock cost (USD/MMBtu or USD/MT)	1.5/MMBtu	22.7/MT
Plant overall thermal efficiency (% LHV basis)	71	58
Operating expenses: non-feedstock (% Capex/yr)	6	6
Capital costs @ 12 % DCF ROR (% Capex/yr)	20	20
Plant availability (%)	94	85
Shipping distance	6 200 nautical miles	1 000 km inland
Shipping cost (USD/MT-DME)	47	53

DME to be deliverable to East China at USD 644 per MT. The bases for these estimates are shown in Table 2. This economic analysis show that coal-derived DME can be competitive with conventional petroleum-derived products, on an energy equivalent basis, when crude oil costs at least (USD 30 to USD 140) bbl^{-1}., depending if DME is valued as LPG or as higher-valued diesel, on an energy equivalent basis.

However, it should be noted that these economics analyses are for illustrative purposes only, since the DME economic are strongly case sensitive to some factors. Those factors are, among others, government policy, incentives, capacity, construction cost, environment, availability of supporting infrastructure, technology, feedstock cost, the degree of process integration, and product specifications and pricing. These analysis is conducted based on the two-step/indirect synthesis that has been commercialized. The commercial one-step/direct synthesis plant, that is theoretically more efficient than the two-step plant, is still not presence to best of our knowledge.

The case sensitivity can be seen from the successful application of DME in China which is produced from the expensive coal-derived methanol at around 2009. Some supporting specifics are [1]:

- DME is sold at a higher price than its energy equivalent basis. IDA reported that DME sold as an LPG blend stock was sold at 75 % to 90 % of LPG price whereas the DME energy value is about 62 % of LPG on a weight basis.
- LPG prices in China are higher than parity with crude oil due to increased demand in winter and when domestic refineries, which produce LPG, were shut down for maintenance (Oct and Nov 2009, and in Jul and Aug 2009)
- Plants once-built are yielding a lower DCF ROR on the capital investment than 12 %,
- Government support and incentives to encourage production from domestic resources for strategic reasons.

3.2 Challenge and Opportunity for DME Application

The commercialization of DME were challenging, primarily due to competition from not only established conventional fuels with distribution and marketing infrastructures but also other alternative fuels. A significant challenge involves the risks in building commercial-scale production and distribution facilities before the market is fully developed. However, in some extent as shown in the case of China, DME has been able to overcome this challenge due the relative simplicity of (i) making DME from methanol, which already has an established market, and (ii) marketing DME as an LPG blend stock, using the established local LPG distribution infrastructure: the cylinder filling facility, the cylinder, and household combustion appliance, with minor modifications.

The development of the DME diesel market was more challenging because of the prerequisite adjustment of the fuel distribution infrastructure and the modifications to the engines themselves. However, DME-fuelled engines and vehicles is necessary due to soot-free combustion and the potential of a high EGR to reduce NOx emissions. In addition, development and application of DME for transportation vehicles can address the shortage of resources including fossil fuel because DME is a synthetic fuel. Therefore, institutes and car-making companies around the world are extending their research from bench-level engine testing to modification and development of DME-fuelled vehicles.

Process intensification is likely required to make DME economically competitive to the conventional fossil fuel. In this case, one-step/direct synthesis shows a potential since it is theoretically produced higher efficiency than the mature two-step process. However, research work encompassing optimization and scaling up of the one-step synthesis of DME and furthermore its economic aspects of the process was hardly been reported. This should be the focus of DME development in the near future.

Finally, government support is still be the most important factor for the massive implementation of DME as energy source. This is since some relatively significant adjustment on the conventional energy infrastructure and management is required. Direction, regulation, and incentives need to be issued in order to get the optimum benefit of DME as flexible (considering the "multi-source and multi-use" feature), clean and efficient fuel.

4. DRIVING FACTOR, POTENTIONAL BENEFIT AND STATE THE ART OF DME APPLICATION IN INDONESIA

Similar to the case of China, Indonesia could be the specific place where domestic DME production and consumption can be beneficial. This is related to following driving factors and potential benefits that might specifically belong Indonesia.

- ***Driving Factors***:

i. Plenteous availability of any kind of DME resource, namely: natural gas, coal, peat, and biomass

ii. Huge demands on DME target market, namely: LPG, diesel oil, and power generation fuel, where the most of those demands are fulfilled by import.

iii. The overall cost of DME production in Indonesia can be lower from the general case shown in section 3.1 since the distance of the DME resource and demand could be much closer. Sea transportation, that is cheaper than land transportation, also could be optimized since Indonesia is highly inter-connected by ocean.

iv. The above mentioned factors have been attracting some technology providers to build DME production facility and infrastructure in Indonesia.

- ***Potential Benefits***:

i. Domestic DME production from local resource can reduce the dependence of Indonesia's energy supply from other countries.

ii. The easily-transportable feature of DME can lead to the better exploitation of the unutilized resources in Indonesia, particularly which is problematic due to its distant location from the energy demand.

iii. The transportability of DME also can lead to its wider and deeper distribution, especially to the remote area which is difficult to serve.

iv. DME synthesis has a potential to increase the added value of the low rank and low commercial value energy resource, e.g. peat, lignite, wasted biomass, etc. However a deeper feasibility study must be performed with this feedstock since the utilization of low rank resource will result in the lower process efficiency.

Despite the described driving factors and potential benefits of DME application, only limited case of commercial utilization can be found in Indonesia. To the best of our knowledge, only PT. Bumi Tangerang Industry can be tracked as a commercial DME supplier. The company is a relatively small-scale DME distributor (65 kg to 700 kg pack unit) which supply DME for aerosol-related purposes. Research works on DME recently performed by Anggarani et al., [11, 12] from the Research and Development Center for Oil and Gas Technology (Lemigas). This research group examined the DME application as replacement of LPG for household stove [11] and as a gasoline alternative for SI engine [12]. Some important findings were produced from their research and worth for further trail for the smooth application of DME in current commercial appliances. From the production side, Priyanto [13] from the Agency for Assessment and Application of Technology (BPPT) presented the proposed DME project in West Java (840 000 TPA) and in West Papua (200 000 TPA) in 2011. Information about the recent status of the West Java project, which was scheduled operated in 2012, is hardly accessible and the projects might possibly had been cancelled. The West Papua project, targeted to monetize LNG from Tangguh Plant, was likely shifted for the production of other chemicals such as methanol, propylene and polypropylene [14].

5. CONCLUSIONS

DME has the special advantage to penetrate the gas and liquid fuel market. DME can be produced from a wide range feedstock grade including natural gas, crude oil, residual oil, coal and waste products. The development of DME was started in USA and Europe since the late 1970's but the significant results on commercialization of DME were done by China in the past decades. In 2011, the global DME demand is estimated about 3 000 000 metric $t \cdot yr^{-1}$, 90 % of which is in China. The utilization of DME is mostly for a fuel blend with LPG for residential cooking and heating. Research and development of DME has been conducted by technology providers in some specific countries namely USA, Sweden, Denmark, Japan, Korea, and China. However, almost all of the commercial DME plants have been built in China. A clear government direction to utilize the domestic coal resource and the presence of a large demand for additional home cooking and heating fuel were among the determinant of the success application of DME in China. Similar to the case of China, Indonesia could be the specific place where domestic DME production can be advantageous. Widespread availability of DME resource and huge demands on DME target market which are still fulfilled by import were among the driving factors for DME application. However, at the current state, commercial utilization as well as the R&D activity for DME application hardly can be found in Indonesia.

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Research Article

Organic Acid and Nutrient Composition of Lactic Acid Bacteria Inoculated Total Mixed Ration Silage under Tropical Condition

Ahmad Wahyudi[1*], Dicky Pamungkas[2], Roy Hendroko Setyobudi[3, 4], Listriari Hendraningsih[1], and Zane Vincēviča-Gaile[5]

[1]Faculty of Agricultural and Animal Science, Muhammadiyah Malang University, Malang 65144, Indonesia
[2]Institute of Beef Cattle Research, Jl. Pahlawan, Grati, Pasuruan 67184, Indonesia
[3]Ma Chung Research Center for Photosynthetic Pigments, Vila Puncak Tidar N-01, Malang 65151, Indonesia
[4]Indonesia Association of Bioenergy Scientist and Technologist, BPPT Building II, 22nd Floor, Jl. MH. Thamrin No. 8 Jakarta 10340
[5]Department of Environmental Science, University of Latvia, Raina Blvd. 19, Riga, LV-1586, Latvia, European Union

Abstract: Organic acids and nutrients composition of total mixed ration (TMR) silage inoculated with local Lactic acid bacteria (LAB) derived from paddy rice was studied. This study was conducted to prepare TMR silage in tropical climate. LAB was isolated from local paddy rice that was planted around Muhammadiyah University of Malang, East Java, Indonesia. The local LAB ability was compared with commercial strain *L. plantarum* FCC 123 for organic acids and nutrients composition on TMR silage fermentation. The study was designed by completely randomized with five treatments (T0: TMR silage without LAB, T1: TMR silage + *L. plantarum* FCC 123, T2: TMR silage + local LAB Ciherang 1, T3: TMR silage + local LAB Membramo 1, T4: TMR silage + local LAB Rajalele 1), each treatment was replicated four times. This study showed the inoculation of local LAB in TMR silages preparation significantly ($P \leq 0.01$) increased lactic acids production and tended to reduce acetic acid and pH value compare to control. There were not different all treatment and control on nutrients composition. It could be concluded that the local LAB inoculant had ability similar to *L. plantarum* FCC 123 on improving quality of TMR silage.

Keywords: Lactic acid bacteria, nutrient composition, organic acid, silage, total mixed ration

1. INTRODUCTION

Silage is a chopped, fresh plant material that has been preserved by a process of anaerobic fermentation (ensiling) in which organic acids are formed, particularly lactic acid. The ensilage technique was consistently applied until now, even in a number of countries have developed to generate as a functional feed, for supporting livestock productivity and health. The ensilage technique has proven to ensure availability of feed in hard season (winter and long dry season) without reduction of nutrients value. Ensilage changes carbohydrates into lactic acid by lactic acids bacteria (LAB) resulted in a decline of pH to protect the growth of any dangerous microorganisms. The low pH silage can be stored for a long time without any decay.

Development technique on ensilage continued until now, even not only use of forage but also to availability of raw materials including food and beverage industrial waste for example dregs of beer, potatoes and sweet potatoes by-product, molasses, waste tea, palm oil by-product, etc. That needs different methods for making silage. Ensilage technique even has developed on complete feed or total mixed ration (TMR silage). Based on consideration of economic efficiency some improvement methods of ensilage have been done in beef cattle fattening and dairy industry. Some new methods of ensilage preparation have been studied to increase quality of silage. Currently, silage

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*Corresponding author: Ahmad Wahyudi; Email: wahyudi_biotek@yahoo.co.id

preparation is not only done in cold temperate regions but also begins to develop in tropics area. Conserving feed as silage is a strategy to alleviate feed constraints and maintain animal productivity during dry spells in the tropics [1]; however the TMR ensilage technique is not yet commonly applied. In this study, organic acids and nutrients composition would be determined in order to understand effect of local LAB inoculants in TMR silage preparation under tropical condition.

Trend the use of industrial waste as a fodder in tropics like; palm oil by-product, copra by-product, rice bran, rice straw, *tumpi* corn, molasses, shoots of sugar cane, coffee by product, shell of a nut, and another industrial waste is increased. It needs TMR formulation to prepare nutritious feed to cater the needs of cattle for 24 h. This is a real challenge in Indonesia constraining productivity of ruminants. Besides TMR formulation, preservation methods also need to consider of feed availability, quality, and its security. In addition [2] stated that TMR silage has a better aerobic stability in comparison with ensiled feedstuff alone.

Sugar and water contents of silage are important determinants in attaining good quality silage. The raw material of silage must contain more than 2 % of sugar (glucose, sucrose, or fructose) and 35 % to 40 % dry matter [3]. If the sugar content less than 0.5 %, addition of molasses or glucose is necessary. The addition of a cellulose enzyme to produce sugar from crude fiber is also an effective technique in process of silage making [4]. Crude fiber will hydrolysis into glucose as energy source of LAB and will be changed into lactic acid. If the moisture content more than 60 %, silage materials should be dried to reduce of excessive butyric acid and increases lactic acid production, in contrast if the moisture content less than 20 %, the moisture needs to be increased [5].

2. MATERIALS AND METHODS

2.1 TMR Silage Preparation

TMR silages were prepared in a small-scale system of silage fermentation 1 [6]. Ingredients composition of TMR were: 16 % rice straw, 20 % rice bran, 34 % cassava chip, 6 % molasses, 17.5 % soybean meal, 4.5 % fish meal, and 3 % minerals. The ingredients composition was arranged based on contents 14 % of crude protein (CP), 65 % to 70 % total digestible nutrients (TDN), and metabolism energy (ME) 2000 kcal. The TMR were treated with 1 % of inoculants *L. plantarum* FCC 123, local LAB *Ciherang* 1, local LAB *Membramo* 1, local LAB *Rajalele* 1 respectively and un-treatment control. *Ciherang*, *Membramo*, and *Rajalele* were varieties name of local paddy rice respectively. LAB was isolated from local that paddy rice varieties, respectively [6, 7]. Approximately 100 g portion of TMR materials were adjusted at 55 % moisture and packed into plastic film bags (KRIS BR 2205 type, 22 cm × 500 cm) and then the bags sealed with a vacuum sealer machine (KRIS VS200). The bag silos were stored in room temperature (average 25 $^{\circ}$C) for 30 d of incubation.

2.2 Organic Acids Analysis

Fermentation products of TMR silages were determined from cold-water extracts. Wet material (10 g) was homogenized with 90 mL of sterile distillated water [8, 7]. The pH was measured with a glass electrode pH meter (Echem E-512 ex GR Scientific) the organic acid contents were measured by gas chromatography (GC) according to the methods describes by reference [9]. The analytical condition was as follows: cold-water extracts (1 μL) was injected into column RTX-5, 30 mm × 0.25 mm, injector temperatute was 220 $^{\circ}$C, split 70 Kpa and flame ionize detector (FID) temperature was 250 $^{\circ}$C, and retention time 40 min.

2.3 Nutrients Analysis

Samples were dried in forced-air oven at 65 $^{\circ}$C for 48 h and ground to pass a 1 mm screen with a Willey mill (ZM200, Retsch GmbH & Co.). Contents of DM, OM, CP, and EE were analyzed according to methods 934.01, 942.05, 976.05, and 920.39 respectively, of AOAC by reference [10].

2.4 Research Design

The study was designed by one way completely randomized design (CRD) with five treatments (N0: TMR silage without LAB, LP: TMR silage + *L. plantarum* FCC 123, CH: TMR silage + local LAB *Ciherang* 1, MR: TMR silage + local LAB *Membramo* 1, RL: TMR silage + local LAB *Rajalele* 1), each treatment replicate four times. Organic acid contents and nutrients composition were measured as experiment parameters.

Table 1. Effect of tropical inoculants on organic acids composition⁺.

Inoculant*)	Organic Acids (g kg^{-1})			
	Lactic acid**)	Acetic acid**)	Propionic acid	Butyric acid
N0	40.87±0.78^{a}	29.90 ± 0.46 ^{a}	nd	nd
LP	94.81±0.78^{b}	17.82 ± 0,41 ^{b}	nd	nd
CH	113.91±0.29^{b}	25.07 ± 0.45 ^{a}	nd	nd
MR	91.30±0,52^{b}	17.88 ± 0.13 ^{b}	nd	nd
RL	113.23±0.44^{b}	16.95 ± 0.18 ^{b}	nd	nd

*) N0: no-LAB inoculant, LP: *L. plantarum* FCC 123, CH: *Ciherang* LAB1, MR: *Membramo* LAB1, RL: *Rajalele* LAB1,
**) ab mean ± SD value on the same column show the significant difference.
+) value are mean of four samples.

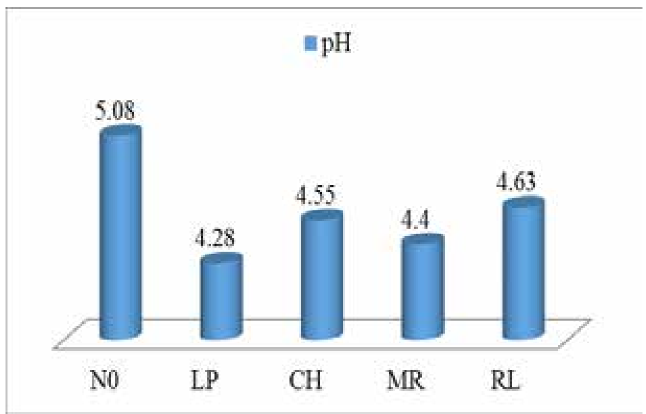

Fig. 1. pH values.

3. RESULTS AND DISCUSSION

3.1 Organic Acids Composition

LAB inoculants gave highly significant ($P \leq 0.01$) effect on increasing lactic acids as described by reference [11, 12]. The lowest lactic acid content was observed in the TMR prepared without inoculants (Table 1). In contras, LAB additions tend to depress acetic acids concentration except for CH that has non significant value with the N0, acetic acid is one of indicator of less than desirable silage fermentation [13], eventhough Driehuis et al. [14] stated that a concentration of acetic acid that ranges from (36 to 50) g kg^{-1} DM is suitable to control yeasts during aerobic exposure of silage. Acetic acid in the products was 16.95 to 29.90 g kg^{-1} DM; Nkosi et al. [15] reported that *L. buchneri* and *P. acidilactici* in TMR silage, after 56 d of ensiling, also play an important role in the aerobic stability of TMR silages.

In this study, there were no significant difference of propionic and butyric acid in all treatments. Propionic acids and butyric acids content were not detected (nd) in all treatments as describe in Table 1, It mean that all TMR silages have prepared in a good condition.

The provision of LAB inoculants showed depress acetic acid production, this may be due to the inoculants type has been used is LAB homo-fermentative where almost 90 % of fermentation product is lactic acid.

The pH value of TMR silage with inoculants were lower than control and ranged pH 4.28 to pH 4.63. This result was in accordance with (16) study for TMR silage. A rather unexpected finding from this study that the pH value was not correlated with lactic acid productions as found in earlier studies. In some case this tendency also found in (17) and (16).

Table 2. Effect of tropical inoculants on nutrients composition+.

Inoculant*)	Nutrient Contents (%)							Energy (kcal)**)
	Moisture**)	Ash**)	OM**)	CP**)	EE**)	CF	NFE**)	
N0	56.66 ± 2.3	17.12 ± 2.0	82.88 ± 2.0	14.74 ± 0.5	2.05 ± 0.1	12.35 ± 0.4***)	65.57 ± 5.1	2 290.2
LP	57.69 ± 1.0	16.47 ± 3.3	83.53 ± 3.3	14.96 ± 1.0	1.82 ± 0.4	14.91 ± 3.9***)	63.84 ± 6.8	2 173.5
CH	54.07 ± 2.3	15.37 ± 1.0	84.64 ± 1.0	14.23 ± 0.7	2.13 ± 0.2	14.18 ± 2.0***)	64.55 ± 6.2	2 238.2
MR	53.47 ± 4.4	16.29 ± 1.3	83.71 ± 1.3	15.40 ± 1.7	1.94 ± 0.5	14.06 ± 3.5***)	64.33 ± 6.3	2 240.9
RL	53.13 ± 1.2	16.84 ± 3.2	80.16 ± 3.2	15.45 ± 1.5	2.04 ± 0.8	14.35 ± 3.0***)	64.53 ± 10.5	2 027.9

*) N0: no-LAB inoculant, LP: *L. plantarum* FCC 123, CH: Ciherang LAB1, MR: Membramo LAB1, RL: Rajalele LAB1, OM: organic matter, CP: crude protein, EE; extract ether, CF: crude Fiber, NFE: nitrogen free extract.
**) not significant.
***) mean ± SD value in the same column show the significant different.
+) value are mean of four samples

Acetic acids content 1.5 % to 3.0 % of dry matter could reduce growth of fungi when silage was opened and given to livestock. The acetic acids concentration in this study tended to decline by the addition of LAB inoculants, eventhough *Ciherang* LAB1 yielded relatively same content with no LAB inoculants. Propionic acid on silage could increased if lactic acid would be converted to 1,2-propanediol and further converted to be propionic acid by *L. Diolivorans* [18]. The provision of inoculants in this research has not caused significant differences on propionic acid contents indicate that *L. diolivorans* didn't effect on this TMR silages preparation. On a limited number propionic acid would play a role as acetic acid to reduce growth of fungi at the time when silo were opened and condition became aerobic.

Butyric acid in silage is one indication of contamination clostridia. *Costridia* degrade proteins into butyric acid, caused a decline of protein silage and palatability. *Clostridia* could be prevented by declining pH under 4.5 quickly. Butyric acid contents in this study was not detected on TMR silage with and no LAB inoculants. This condition could be explained that pH was low enough to prevent the growth of *Clostridia* on all treatments, as describe in Table 1 and Fig. 1.

3.2 Nutrients Composition

There were no significant differences on nutrients composition of TMR, both with and without LAB inoculants, except for crude fiber content. Crude fiber decline in TMR without LAB inoculant indicated the silage was fermented by hetero-fermentative microorganisms that has ability to convert some fiber into acetic acid. Generally the nutrients content: moisture, dry matter, organic matter, ash, crude protein, extract ether, nitrogen free extract, and energy were relatively constant. The similar results has been reported by reference [19] that except a little decreasing of water soluble carbohydrate (WSC) and maintain crude fiber, BAL inoculants not affected nutrients composition of silage on mini silo. The results was also strengtened by reference [20] that nutrients composition of silage is not different with or without inoculation of *L. buchneri*. The TMR nutrients composition of this study is given Table 2.

Ensilage could not able to improve the quality of nutrients, but could reduce growth of pathogenic microorganisms (like clostridia), so feed could be preserved for a long period.

4. CONCLUSIONS

This research revealed that local LAB inoculants have the ability similar to the commercially available LAB *L. plantarum* FCC123 inoculants to produce organic acids and preparing the silage with nutrients composition suitable for tropical conditions.

5. ACKNOWLEDGEMENTS

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Research Article

Ballast Weight Review of Capsule Husk *Jatropha curcas* Linn. on Acid Fermentation First Stage in Two Phase Anaerobic Digestion

Praptiningsih Gamawati Adinurani[1], Roy Hendroko Setyobudi[2, 3]*, Satriyo Krido Wahono[4, 5], Maizirwan Mel[6], Anggi Nindita[7], Endang Purbajanti[8], Soni Sisbudi Harsono[9], Andoniana Rakoto Malala[10, 11], Leopold Oscar Nelwan[12], and Andi Sasmito[13]

[1]Faculty of Agrotechnology, Merdeka University, Jl. Serayu,
PO. Box 12, Madiun 63131, Indonesia
[2]Indonesian Association of Bioenergy, BPPT Building II, 22nd Floor Jl. MH. Thamrin
No. 8 Jakarta 10340, Indonesia
[3]Ma Chung Research Center for Photosynthetic Pigments, Villa Puncak Tidar N-01
Malang 65151, East Java, Indonesia
[4]Future Industry Institute and School of Engineering, University of South Australia,
Mawson Lakes SA 5095, Adelaide, Australia
[5]Technical Implementation Unit for Development of Chemical Engineering Processes, Indonesian Institute of Sciences, Yogyakarta, 55861, Indonesia
[6]Department of Biotechnology & Engineering, Faculty of Engineering, International Islamic University Malaysia, Gombak, Kuala Lumpur, 50728, Malaysia
[7]Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University, Jl. Meranti, Kampus IPB Darmaga, Bogor 16680, Indonesia
[8]Agricultural and Animal Science Faculty, Diponegoro University, Jln. Prof Sudharto SH, Tembalang Semarang, 50275, Indonesia
[9]Faculty of Agricultural Technology, University of Jember (UNEJ), Jl. Kalimantan 23,Jember 68121 Indonesia
[10]Centre de Formation et d`Application du Machinisme Agricole (CFAMA) Route Betafo Ambaniandrefana – B.p. 109, Antsirabe, Madagascar
[11]Agricultural Science, Kobe University 1-1 Rokkodai, Nada-ku, Kobe, 657-8501, Japan
[12]Department of Mechanical and Engineering Biosystem, Faculty of Agricultural Engineeringand Technology, Bogor Agricultural University, Kampus IPB Darmaga,
Bogor 16002, Indonesia
[13]Alumnus Department of Biology, Faculty of Mathematics and Natural Sciences
Bogor Agricultural University, Bogor 16680, Indonesia

Abstract: Increasing biogas production through the use of Dried Capsule Husk - *Jatropha curcas* Linn. (DH-JcL) as raw materials study was conducted at PT Bumimas Ekapersada experiment field, in Bekasi, West Java, Indonesia from September 2013 to March 2014. Four HDPE pipes with 5 L volume used as first stage-hydrolysis digester, while an HDPE (high-density polyethylene) drum with working volume 80 L was used as second phase - methanogenesis digester. DH-JcL at the first stage was pressed by a 1 800 g ballast, which was to be compared to a 900 g ballast pressed, diluted in water with volume ratio of 1:8. One third of the second stage digester was filled by immobilized growth of random packing system on special plastic design. Organic Loading Rate was predetermied at 3 000 cc $\cdot$ d^{-1} with Hydrolic Retention Time of 4 wk. This study was repeated three times and conclusion was made using t test as inferential statistics. The test concluded that 900 g ballast produced the best result. DH-JcL biogas productivity is increased, by two phase system, as much as 163 % when compared to semi continuous single phase system. Furthermore, the set up produces methane content higher (90 % to 91 %) than semi continuous single phase system (83.15 %). VFA average decrease of 16 %, and average alkalinity decreased by 11 %. Average ratio of VFA/ alkalinity is 0.5 similar with semi continuous single phase system.

Keywords: Acid fermetation, biogas, capsule husk, *Jatropha curcas* Linn., two phase anaerobic digestion

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*Corresponding author: Roy Hendroko Setyobudi; Email: roy_hendroko@hotmail.com

1. INTRODUCTION

Praptiningsih et. al. [1] stated that DH-JcL (Dried Husk *Jatropha curcas* Linn.) used as raw material for methane fermentation of biogas digester produced unsatisfactory result. This statement was supported also by several other studies [2, 3]. However, it must be understood that those unsatisfatory results of DH-JcL biogas was conducted due to zero waste principle reason. A reference [4] stated that DH-JcL produced biogas quantity similar with rice husk on semi-continuous single phase digester. Moreover, it produced higher methane content than others biogas raw materials, such as cowdung, solid waste of tapioca production process, cassava skin, fruits and vegetables wastes. However, Praptiningsih et al. concerned about VA/Alk ratio showing 0.5 value [4]. This ratio exceeded or approached the threshold which was recommended by some researchers [5–10].

Several researchers suggested to apply two phase digester, particularly for solid form feeding, which is not easily degraded, having high carbohydrate content, highly toxic, unbalanced C:N:P content, having hydrolysis and liquefaction problems, and unstable process [11–16]. Some study of DH-JcL as raw material concluded that two phase digester was better than single phase digester [17–22]. This research was conducted to review comparison results between laboratory scale and pilot plant scale on two phase biogas digester of DH-JcL as raw material. The research also studies improvement of DH-JcL biogas productivity and VA/Alk ratio on two phase digester

2. MATERIALS AND METHOD

The study was conducted at PT Bumimas Ekapersada's experiment field, in Bekasi, West Java, Indonesia, from September 2013 to March 2014. JcL husk was collected from JatroMas toxic cultivar which was sun dried, to reduce moisture content up to 5 %. An HDPE (high-density polyethylene) drum with a total volume of 90 L and working volume of 80 L was used as methanogenesis digester/ second digester (Fig. 1). There are three holes on methanogenesis digester. Two holes were closed by drum, the first was used for flowing biogas into the holder and the second hole was used for feeding slurry DH-JCL from hydrolysis digester. The feeding pipe's end was submerged into slurry at about 10 cm to prevent O_2 from entering into digester. The third hole was located under the drum for slurry dispensing and for analysis sample taking. One third volume of methanogenesis digester was filled by organic artificial immobilized growth of random packing system on special plastic design. Methanogenesis digester was filled by rain water and 10 % v/v [23] of semi-artificial starter from DB-JcL digester. From 1 d until 10 d operation, methanogenesis digester was provided by synthetic feeding of 25 g $\cdot$ L^{-1} brown sugar for 3 000 cc [4]. At the 11 d, if biogas bubbles are produced in the water holder, then synthetic feeding was replaced by slurry from hydrolysis digester. Organic Loading Rate (OLR) was calclated at 3 000 cc $\cdot$ d^{-1} with Hydrolic Retention Time (HRT) of 4 wk [4].

Fig. 1. The first and second digester.

Hydrolysis digester/ first digester used 5 L volume of HDPE pipe which was closed tightly (Fig. 1). On the bottom part, there is a slurry discharge hole to move the slurry to methanogenesis digester periodically. On the top part, there was another discharge hole to release gas production and a covered hole to pour the water diluent consisting of 375 g sun-dried added with 4 000 cc of rain water as diluent. The DH-JcL was pressed by ballast, so it submerged in water diluent [18]. There were four hydrolysis digesters for feeding a methanogenesis digester, because the slurry was harvested every 4 d based on the previous studies [17, 19, 22]. 3 000 cc d^{-1} of slurry from hydrolysis digester was transferred to methanogenesis digester. Hereafter, 3 000 cc of 1:8 concentration rain water [21] was filled to soak

DH-Jcl d^{-1}. The soaking was conducted in hydrolysis digester for 4 wk [19].

pH and temperature reading was conducted every day during experiment by digital measurement tools. Biogas volume of hydrolysis and methanogenesis digester was determined by water displacement method on the holder [25], and methane determination was conducted using orsat apparatus. Volatile fatty acid (VFA) content and alkalinity was analyzed by distillation and titration based on APHA 2320 [25]. This study was repeated three times and conclusion was obtained by using test as inferential statistics

3. RESULTS AND DISCUSSION

This paper is written to elaborate a study on the ballast weight on DH-JcL in hydrolysis digester. As reported by reference [18], the ballast application on DH-JcL bundle increased biogas productivity because the bundle did not flow. However, it can be seen that DH-JcL did not degrade perfectly, particularly on the inside part of bundle. It was so happened because the ballast weight was too heavy, so diluent water could not enter into the bundle. This study compared between maximum (1800 g) and minimum (900 g) ballast.

3.1 Review on Temperature and pH

This research was conducted on the temperature ranges which shown on Fig. 2 and 3. Fig. 2 shows that research condition in hydrolysis digester which is conducted at average temperature of 29.71 °C (27.0 °C to 32.3 °C range) for maximum ballast, and average temperature of 29.2 °C (26.6 °C to 31.50 °C range) for minimum ballast. This research was conducted at ideal temperature of 30 °C to 35 °C [26] and/or 30 °C to 38 °C [27]. The average temperature was relatively similar for two treatments, but the minimum ballast has lower temperature because of the lower temperature effect on the 1 d to 11 d. Fig. 3 shows research condition in methanogenesis digester which is conducted at average temperature of 29.89 °C (27.8 °C to 32.1 °C range) for maximum ballast and average temperature of 29.95 °C (27.7 °C to 31.9 °C range) for minimum ballast. Methanogenesis digester temperature on this study was conducted at ideal temperature of 30 °C to 35 °C [26] and/or 30 °C to 38 °C [27]. The average temperature was relatively similar for the two treatments, although the maximum ballast has lower temperature.

Based on the varied temperature results, the minimum ballast was more suitable, which was shown by lower relative temperature on the 1 d to 11 d. Hereafter, the temperature increased until the end of study period. This increasing temperature had positive impact to arachea methanogen [28]. pH observation of this research is shown on Fig. 4 and Fig. 5.

Fig. 4 shown that this study was conducted on average pH of 6.26 (range pH 5.60 to pH 6.80) for maximum ballast and average pH of 5.93 (range pH 5.00 to pH 7.00) for minimum ballast. This research was conducted on ideal pH because some references reported the ideal pH value of 5.00 to 7.00 [20]. The average pH of the minimum ballast was lower, which mean it produced more acid. The lower pH was expected to increase biogas production. pH curve of hydrolysis digester decreased from day to day, particularly on the minimum ballast which was reported also by reference [29, 30]. The decreasing pH will have negative impact to arachea methanogen. To minimize this impact, Lopez [30] on his research of single phase digester, conducted NaOH addition. However, Fig. 4 shows that pH of two phase digester is still have a normal pH range. The lower pH of the minimum ballast was happened because water diluent and DH-JcL was functioning well, therefore acidity process conducted optimally.

Fig. 5 shows that this study was conducted on average pH of 6.79 (pH range 6.0 to 7.3) for maximum ballast and average pH of 7.13 (pH range 6.80 to 7.50) for minimum ballast. This study was conducted on ideal pH because some references reported the ideal pH value of pH 6.0 to pH 8.5 for methanogenesis digester [20]. The average pH of the minimum ballast was higher than the maximum ballast. Because of arachea methanogen is appropriate with neutral-base pH [32], so this condition showed that the minimum ballast treatment is better producing biogas. However, the maximum ballast also shows the possibility of optimum biogas production. This possibility is shown by low pH on the early and then it's likelihood to increase. Increasing pH value happened because of better the balancing process between acetogen microbe growth and methanogen. Acid compounds, produced by acid-producing bacteria, were consumed by arachea

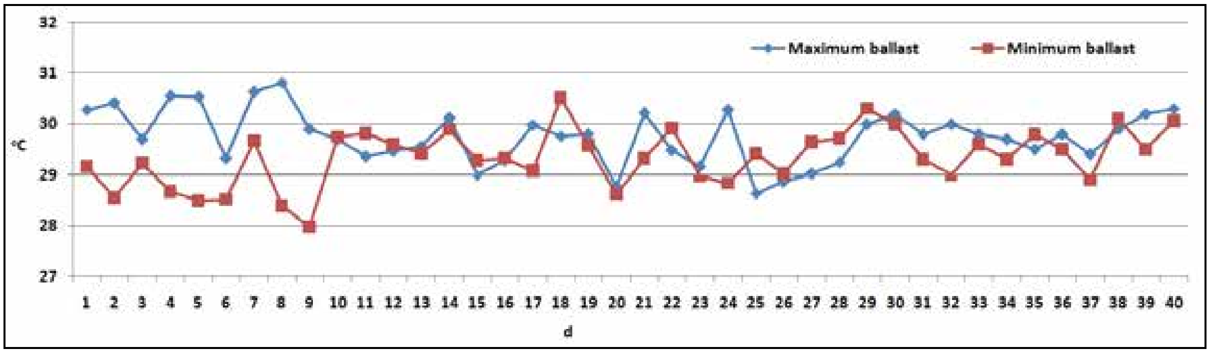

Fig. 2. Slurry outlet temperature of two phase DH-JcL hydrolysis digester system with maximum and minimum ballasts.

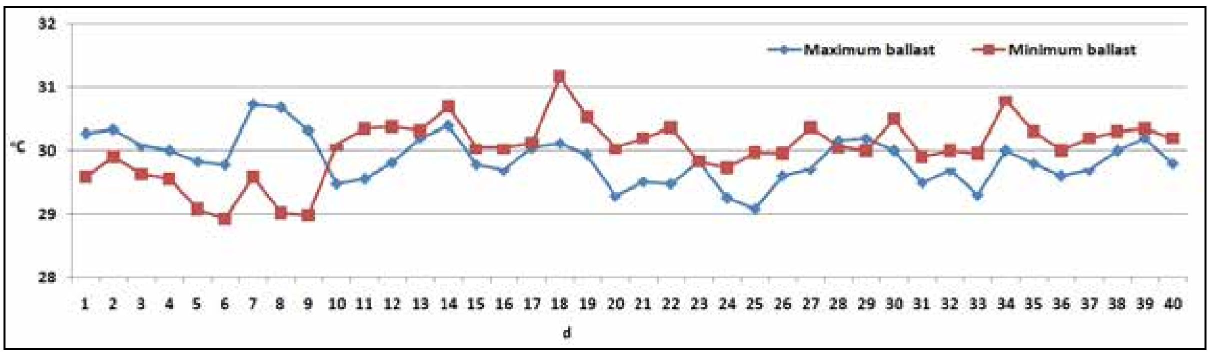

Fig. 3. Slurry outlet temperature of two phase DH-JcL methanogenesis digester system with maximum and minimum ballasts.

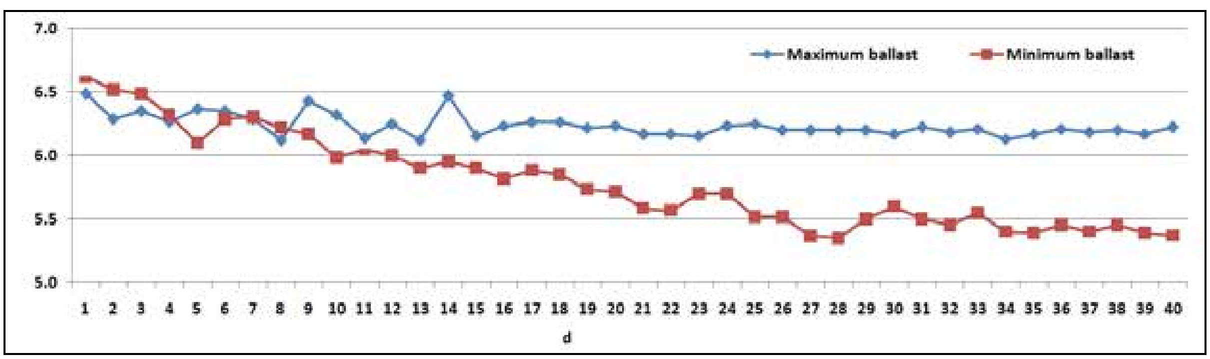

Fig. 4. Slurry outlet pH of two phase DH-JcL hydrolysis digester system with maximum and minimum ballasts

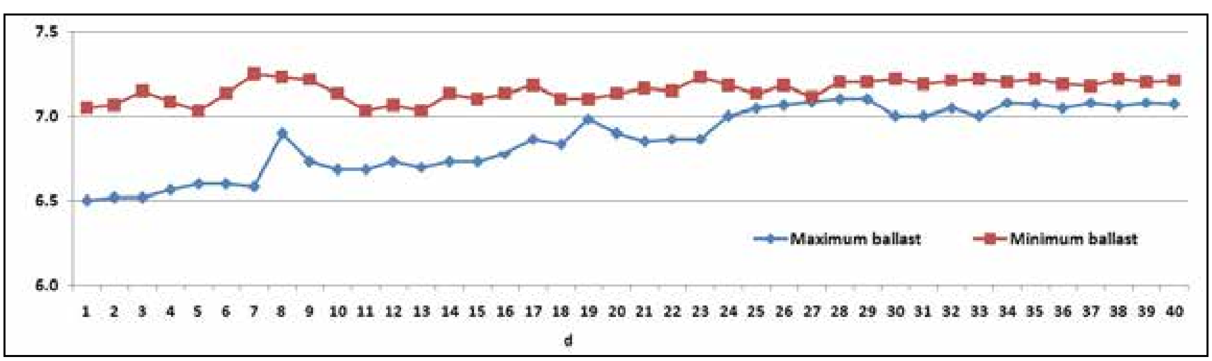

Fig. 5. Slurry outlet pH of two phase DH-JcL hydrolysis digester system with maximum and minimum ballasts.

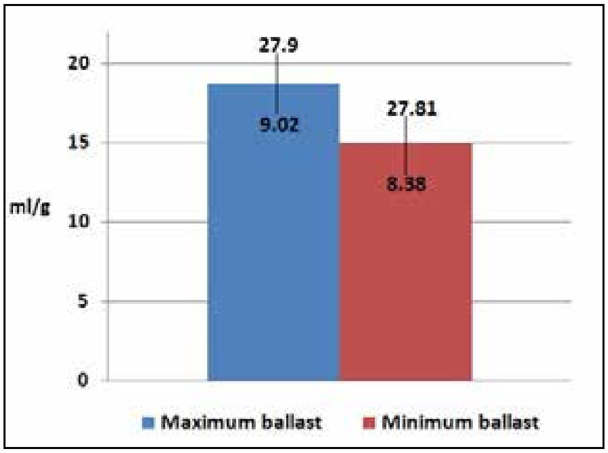

Fig. 6. DH-JcL biogas production in hydrolysis digester with HRT of 40 d for two type ballasts.

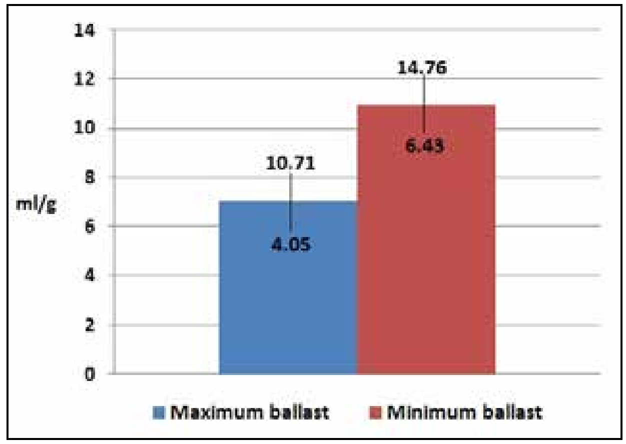

Fig. 7. DH-JcL biogas production in methanogenesis digester with HRT of 40 d for two type ballast.

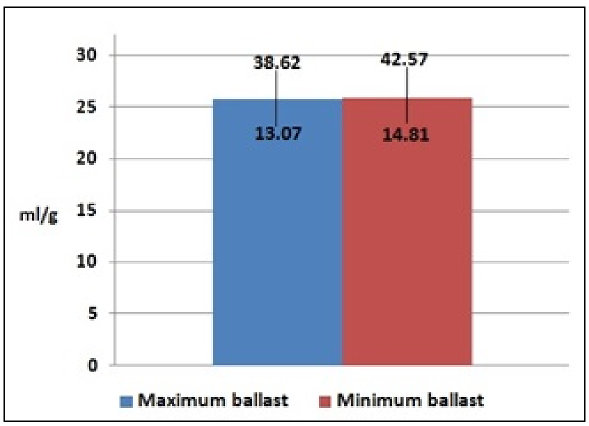

Fig. 8. Total biogas production of DH-JcL as raw material with HRT of 40 d.

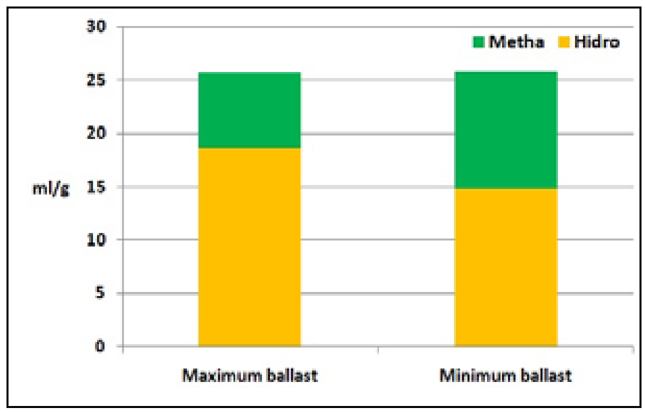

Fig. 9. Total biogas production of DH-JcL as raw material with HRT of 40 d on separation of hydrolysis and

methanogen quickly, so it produced much CO_2 which dissolved in water. It produced more bicarbonate ion (HCO_3^-) which caused solvent more alkaline and system changed from neutral to base [33, 34].

3.2 Review on Biogas Production

Fig. 6 and Fig. 7 show biogas production from hydrolysis and methanogenesis digester. Fig. 6 showed biogas production of hydrolysis digester on the minimum ballast is lower than the maximum ballast. This data, showing that the minimum ballast produce lower biogas, indicated that degradation occured slowly. This condition was expected to optimize hydrolysis process because DH-JcL contain carbohydrate particularly high cellulose which degrade slowly [12, 35]. This condition is one of benefits resulted from two phase system. Increasing HRT on hydrolysis process of single phase system was hard to apply [36] because acidity process impacted negatively to arachea methanogen [37]. Whereas, increasing HRT was able to enhance degradation efficiency [38].

The maximum ballast on hydrolysis digester produced more biogas because degradation process was conducted only on solid feeding partially. On this treatment, there was small part of solid feeding which contacted with diluent water, so degradation process was conducted faster [39] and biogas production was produced also faster. Whereas, Fig. 7 shows that methanogenesis digester of the minimum ballast produces biogas higher of 157.14 %. This data elucidated that methanogenesis digester is better for arachea methanogen growth which is shown by Fig. 3 about temperature observation and Fig. 5 about pH observation.

Fig. 8 and Fig. 9 show total production of hydrolysis and methanogenesis digester with HRT of 40 d. Fig. 7 shows total biogas production of hydrolysis and methanogenesis digester. Furthermore, Fig. 9 is separation biogas production of Fig. 8 which shows biogas production of hydrolysis and methanogenesis digester as shown by Fig. 6 and Fig. 7. Fig. 8 shows that production of two treatments is similar relatively of 0.026 $m^3 \cdot kg^{-1}$ DH-JcL. This production data represents an increase of 163 % than DH-JcL in single phase digester which produce of 0.016 $m^3 \cdot kg^{-1}$ DH-JcL [4]. The production data of DH-JcL biogas on this research shows cow dung biogas productivity range of (0.023 to 0.04) $m^3 \cdot kg^{-1}$ [40, 41], which is higher compared to rice husk biogas production (0.014 to 0.018) $m^3 \cdot kg^{-1}$ DM [42].

The increased biogas production of 163 % was relevant because some of previous researches reported the increasing productivity of two phase digester than single phase digester. Demirer and Chen [43] noted the increase of 150 % to 167 %; Hagesawa et al. [44] reported increasing of 150 %; Sarada and Joseph [45] stated 124 % to 144 %. However, this result was lower than the results of Sirirote et al. [46].

Statistical inference by t test on biogas production at methanogenesis digester and total biogas production of hydrolysis and methanogenesis digester was shown by Table 1. Table 1 supports Fig. 7 that biogas production of methanogenesis digester on minimum ballast produces biogas more than maximum ballast and it is very significant different of statistical. It also supports Fig. 8 that every treatment of ballasts produces similar total biogas relatively and it is not significant different of statistical. Determination of methane content with orsat apparatus is shown by Table 2.

Table 2 shows that biogas content average of minimum ballast is higher than maximum ballast. Table 2 also shows that methane content of two phase digester is higher than single phase digester [4] which supports Parawira [11] and Paranjpe et al. [47]. Table 2 shows that “the weakness” of this study because Deublein and Steinhauser [28]; Quang [48] said that hydrolysis digester produced CO_2 and H_2 only. Whereas, Table 2 shows that there is high methane content. Moreover, Fig. 9 shows that biogas production percentage of hydrolysis digester is higher than methanogenesis digester which is showed also by Table 3. This was related with the delay time of hydrolysis digester which was suspected too long. This was suspected also that this research was conducted on rain season, so DH-JcL had been degraded on harvesting which was different with previous references [17, 19, 22].

However, this study was stated “wrong”, if it referred to system of Hutnan et al. only [49] which suggested capturing and flowing biogas from methanogenesis digester only. Some other researchers [50, 51] suggested about collaboration system of biogas production from hydrolysis and methanogenesis digester. Furthermore, there was also another system, namely capturing and flowing biogas production separately between hydrolysis and methanogenesis digester [12, 52].

3.3 Review on VFA/ Alk. Ratio

Table 4 VFA average, Alkalinity, and VFA/alkalinity data in two phase digester of methanogenensis was compared to single phase digester with DH-JcL as raw material

Total volatile acid (acetic acid) ratio to total alkali (calcium carbonate) – VFA/Alk is an important indicator to check acid and base balancing or process stability of digester [23, 53, 54]. This data is shown by Table 4. Table 4 shows average of VFA/Alk on two phase digester of 0.50 which is similar with previous research on single phase digester [4]. This average ratio is higher than recommendation of reference [9] of < 0.25 or ratio recommendation reference [35] of 0.1 to 0.25. However, Bolzonella [55] said that high carbohydrate material, such as DH-JcL which shown by reference [1], was recommended by ratio of > 0.3. Drosg [56] supported that ratio parameter of VFA/Alk was not generalized because every type of digester has different ratio standar value which was affected by processed raw material.

The review shows that there were some researches who reported ratio of > 0.5. Kaosol and Sohgrathok [57] said that ideal ratio was 0.4 to 0.8; Powar et al. [58] stated that ideal ratio was 0.5 to 0.8; Schon [59] and Acton [60] stated that ideal ratio was > 0.9 to < 1.0. Therefore, based on reference [56–60], ratio average of 0.5 which was resulted by this research and previous research [4] was able to deem as “fair”. Moreover, average ratio on two phase digester with minimum ballast

Table 1. t test for biogas production d^{-1} in two phase digester of DH-JcL with two ballast treatments.

Treatment	Biogas Production			
	Methanogenensis Digester		Total	
	Average	Sig	Average	Sig
Maximum ballast	0.1768		0.6436	
Minimum ballast	0.2739*	0.000	0.6464**	0.962

*) Significant different
**) Not significant different on trust level of 95 %

Table 2. Methane content of hydrolysis and methanogenesis digester.

Treatments	Biogas Content	
	Hydrolysis	Methanogenesis
Maximum ballast	79.73	89.55
Minimum ballast	81.81	91.13

Table 3. Biogas production percentage of hydrolysis and methanogenesis digester compared to total.

Treatment	Hydrolysis	Methanogenesis	Total
Maximum ballast	73.08 %	26.92 %	100 %
Minimum ballast	57.69 %	42.31 %	100 %

Table 4. VFA average, Alkalinity, and VFA/alkalinity data in two phase digester of methanogenensis was compared to single phase digester with DH-JcL as raw material.

No.	Content	Two phase metanogenesis digester					One phase *)	+/- One/ Two Phase
		1st wk	2nd wk	3th wk	4th wk	Average		
1	VFA**)							
a	Maximum ballast	1 239	1 293	1 486	1 297	1 329		-13 %
b	Minimum ballast	1 521	1 253	1 213	1 015	1 251		-18 %
	Average					1 290	1 532	-16 %
2	Alkalinity***)							
a	Maximum ballast	3 572	4 408	3 420	3 534	3 734		+16 %
b	Minimum ballast	1 860	1 820	2 064	2 290	2009		-37 %
	Average					2 872	3 211	-11 %
3	VFA/Alk Ratio							
a	Maximum ballast	0.35	0.30	0.40	0.40	0.40		-20 %
b	Minimum ballast	0.80	0.70	0.60	0.40	0.60		+ 20 %
	Average					0.50	0.50	0 %

*) Praptiningsih et al, [4]. (one phase = single phase digester)
**) mg Acetic Acid L^{-1}
***) mg $CaCO_3$ L^{-1}

of 0.6 was not able to conclude that minimum ballast was worse than maximum ballast. Table 4 shows that ratio value of minimum ballast decrease from 1 wk to 4 wk (0.80 to 0.40). It indicated that performance of hydrolysis and methanogenesis digester on minimum ballast works optimally. Table 4 also shows that two phase digester is better than single phase because decreasing VFA average of 16 % with minimum ballast treatment of 18 %. For alkalinity average, it is decreased of 11 % for minimum ballast of 37 %.

4. CONCLUSIONS

DH-JcL biogas productivity is increased in two phase system, with resulted increment of 163 % compared to semi continuous single phase system. Furthermore, it produces methane content (90 % to 91 %), higher than semi continuous single phase system (83.15 %). VFA average decrease of 16 %, alkalinity average decrease of 11 %, and average ratio of VFA/ alk is 0.5.

The two-phase treatment is hydrolysis digester with DH-Jcl and diluent water of 1:8 as feeding. Ballast pressure placed on the DH-JcL bundle with 50 % weight of original one produced the best result. Methanogenesis digester is attached to growth system with immobilized growth which is produced by special plastic design and placed on random packing.

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Research Article

Different Oxidation Treatments on Polystyrene (PS) Microspheres by using an Ultraviolet/Ozone (UVO_3) System

Mohd Azmir Arifin[1, 2], Maizirwan Mel[1,]*, Nurhusna Samsudin[1], Yumi Zuhanis Has-Yun Hashim[1, 3], Hamzah Mohd. Salleh[1,3], and Iis Sopyan[4]

[1]Department of Biotechnology Engineering, Faculty of Engineering, International Islamic University Malaysia, Jalan Gombak, 50728, Kuala Lumpur, Malaysia
[2]Faculty of Engineering Technology, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300, Gambang, Kuantan, Pahang, Malaysia
[3]International Institute for Halal Research and Training, International Islamic University Malaysia, Jalan Gombak, 50728, Kuala Lumpur, Malaysia
[4]Department of Manufacturing and Materials Engineering, Faculty of Engineering, International Islamic University Malaysia, Jalan Gombak, 50728, Kuala Lumpur, Malaysia

Abstract: Oxidation is a common physical treatment used to render the surface of polystyrene suitable for cell adhesion and spreading. In this work, the effects of oxidation of polystyrene (PS) microspheres by three different treatments; oxidation by ozone aeration only, UV irradiation only or by the combination of both (UVO_3), by using a self-fabricated UVO_3 system were investigated. Results expressed in carboxylic acid (COOH) concentrations on the surface of microspheres revealed that, the treatment by UVO_3 yielded the highest concentration. Presence of atomic oxygen species in UVO_3 treatment has contributed to higher COOH concentration.

Keywords: Microspheres; oxidation; polystyrene; ultraviolet/ozone

1. INTRODUCTION

Polystyrene (PS) is an inexpensive synthetic polymer made from the monomer styrene, a liquid hydrocarbon derived from petroleum. PS is used in an extremely wide range of applications as it has many interesting properties such as low specific weight (~1.04 g/cm^3), good optical properties, high chemical resistance, mechanical flexibility and biocompatibility [1]. In cell culture, PS has been used as early as in the 1960s for producing tissue culture flasks, roller bottles, vacuum canisters and culture medium filters. However, unmodified PS is unsuitable for cell attachment due to its hydrophobic surface chemistry. PS must undergo surface treatment to render it suitable for cell attachment [2].

Ultraviolet/ozone (UVO_3) treatment has been reported as able to improve the hydrophilicity of PS by introducing polar oxygen functional groups such as hydroxyl, carbonyl and carboxyl on its surface [3]. Hydroxyl and carboxyl functional groups are known to have positive influences on cell attachment on polymer surfaces. Numerous studies have demonstrated that, carboxylic acid enriched PS surfaces allow good adhesion and growth of multiple cell lines [4-6]. The surface of PS may also be derivatized by the incorporation of extracellular matrix and recombinant proteins to facilitate cell attachment. These proteins can be conjugated on PS surfaces by using covalent bonding. This method generally requires the surface of PS to be activated prior to conjugation. One possible way to accomplish that requirement is by introducing carboxyl functional groups on its surface.

In this work, we have fabricated a UVO_3 treatment system that allows oxidation to be done by three different modes; oxidation by ozone aeration only, UV irradiation only or by the combination of both, UVO_3. PS microspheres

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*Corresponding author: Maizirwan Mel; Email: maizirwan@iium.edu.my

were treated with all three modes, and their effects on carboxyl functional group deposition on their surfaces were investigated.

2. MATERIALS AND METHODS

2.1 Production of PS Microspheres

PS microspheres were prepared by an oil-in-water (O/W) emulsion solvent-evaporation method. Four grams of PS powder (250 µm, homopolymer) were dissolved in 20 mL chloroform by magnetic stirring to form the oil phase. The organic solution was then emulsified in 100 mL of 0.25 % PVA (partially hydrolyzed, MW = 30 000) solution (water phase). Resulting o/w emulsion was later stirred at 300 rpm (1 rpm = 1/60 Hz) and 80 °C for 6 h to extract chloroform. Next, microspheres were retrieved by vacuum filtration, washed vigorously with distilled water and dried at 50 °C overnight. Finally, dried PS microspheres were stored in vacuum desiccator at room temperature until further use.

2.2 Treatment on PS Microspheres

Surface modification of PS microspheres was carried out in the self-fabricated UVO_3 system. A 250 mL conical flask for containing PS microspheres was fixed on a shaker and shaken at

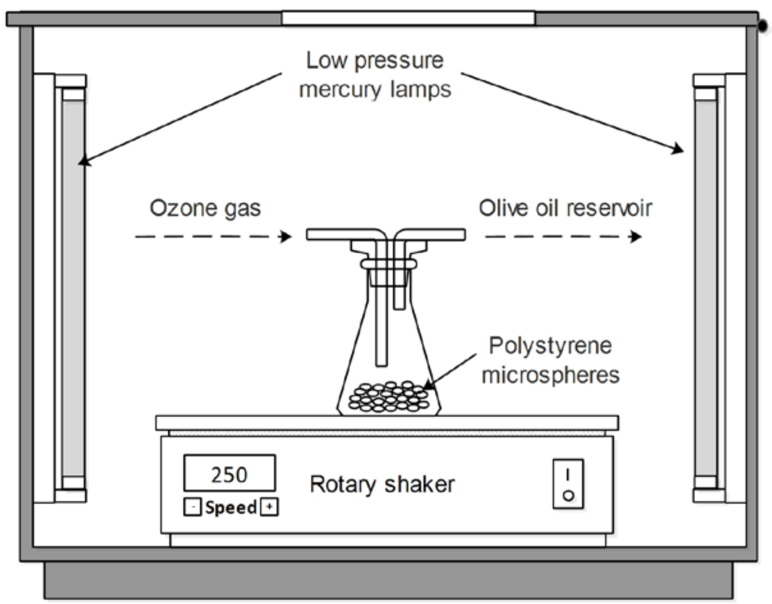

Fig. 1. Treatment of PS microspheres in UVO_3 system.

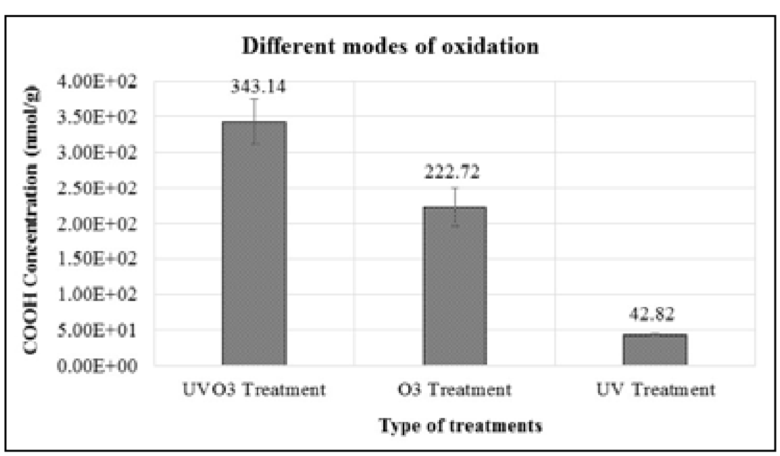

Fig. 2. Effect of different treatments on COOH deposition on PS microspheres.

250 rpm for homogenization during treatment. For treatment using both UV and ozone, 1 g of PS microspheres were aerated with ozone concentration of 42 494.57 mg kg^{-1} (in O_2 flow rate of 2 rpm) and irradiated with UV lights from two oppositely located low pressure mercury lamps with wavelengths of 254 nm. Ozone was produced by silent discharge of oxygen (99.9 %) while irradiation from both UV lamps to the sample was measured to be at 0.343 mW cm^{-2}. Samples were treated by the UVO_3 system for 10 min. For treatment by using ozone only, all parameters were set identical as the previous UVO_3 treatment but the UV lamps were not turned on and for treatment by using UV only, the ozone was not supplied but UV lamps were turned on. Setup of the treatment is illustrated in Fig. 1.

2.3 Measurement of Carboxylic (COOH) Functional Group Concentration

Toluidine blue O (TBO) assay was used to determine the amount of carboxyl (COOH) functional group introduced on the surface of PS microspheres. The assay was adapted from the article published by Rodiger [7] with various modifications. One gram of microspheres was incubated in 10 mL of toluidine blue O (TBO) solution (1 mM NaOH, 0.1 % TBO) for 30 min at 40 °C and was shaken at 400 rpm. After that, the microspheres were washed with 1 mM NaOH solution until the rinsing solution become colourless. Dyed microspheres were later recovered by using vacuum filtration. Next, TBO was desorbed by incubation of microspheres in 10 mL of 20 % sodium dodecyl sulfate (SDS) solution (30 min, 40 °C, shaken at 1 300 rpm). Microspheres were then pelleted by centrifugation. Finally, TBO absorption of SDS supernatants was measured for absorbance at a wavelength of 625 nm using a spectrophotometer. Amount of COOH surface concentration on PS microspheres was determined by comparison to the standard curve.

3. RESULTS AND DISCUSSION

Fig. 2 shows the concentration of carboxyl (COOH) functional group per gram of PS microspheres after different treatments as measured using TBO assay. It can be observed that UVO_3 treatment has introduced the highest concentration of COOH functional group on the surface of PS microspheres with 343.16 nmol g^{-1}. This is followed by the treatment of PS microspheres with ozone only which is 222.72 nmol g^{-1} and the least concentration of COOH was achieved when PS microspheres were treated with UV only with concentration of 42.82 nmol g^{-1}. This result is in accordance with the result reported in reference [8] in which treatment of polystyrene by both UV and ozone have yielded higher COOH concentration on the surface of polystyrene as compared to treatment by using ozone or UV only.

Fig. 3 shows one of the possible reactions when PS is treated with ozone alone as reported by Kefeli [9]. C-H bonds available on polystyrene polymer chain are accessible to ozone as reactive sites to form peroxides and its derivatives [10]. Kefeli [9] has demonstrated that, the C-H bond that is linked to the tertiary carbon as the most reactive site while ozone interaction with aromatic rings of PS represents no more than 1 % to 2 % in the total interactions. The treatment of polystyrene with ozone give rise to the formation of polymer peroxy radicals (POO•) which occurs in a rapid reaction. In the presence of ambient oxygen molecules, POO• radicals subsequently form carbonyls, carboxylic acids or hydroperoxides in slow reactions which may involve chain-scissions [11].

In the treatment of PS with UV radiation (λ = 254 nm) only, absorption of UV by the aromatic ring provides the energy for the formation of alkyl radicals by the dissociation of aromatic ring from the tertiary carbon or from the scission of the C-C or C-H bonds. These radicals later crosslinked to each other, disproportionate or may form polymer peroxy radicals (POO•) by reacting with ambient oxygen molecules [12]. Similar to the treatment of PS with ozone alone, POO• that resulted from the interaction of PS and UV radiation may also undergoes slow reactions to form carbonyls, carboxylic acids or hydroperoxides, in the presence of oxygen molecules. Apart from this mechanism, there are still many possible reactions that may occur when PS is exposed to UV radiation. In Fig. 4, possible reaction mechanisms that may occur when PS is treated with UV are presented.

PS treated with UVO_3 was modified to a greater extent when compared to the treatment that uses ozone aeration or UV radiation only. Apart from modifications mentioned in the treatment of PS with ozone or UV only, polymer chains of PS

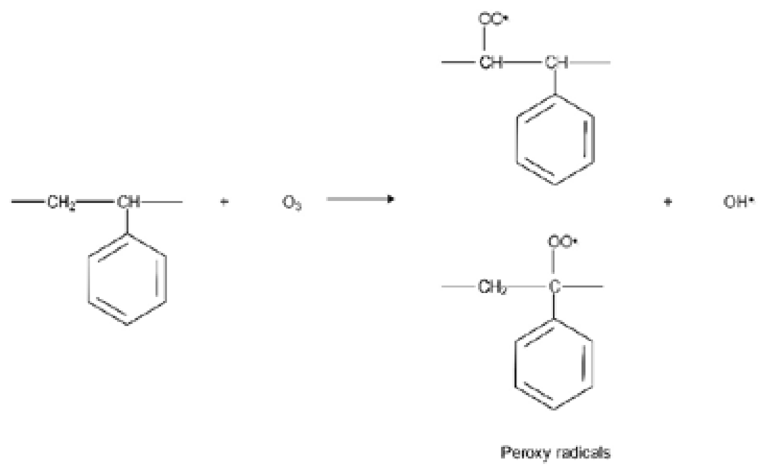

Fig. 3. Formation of POO• from the interaction of polystyrene with ozone.

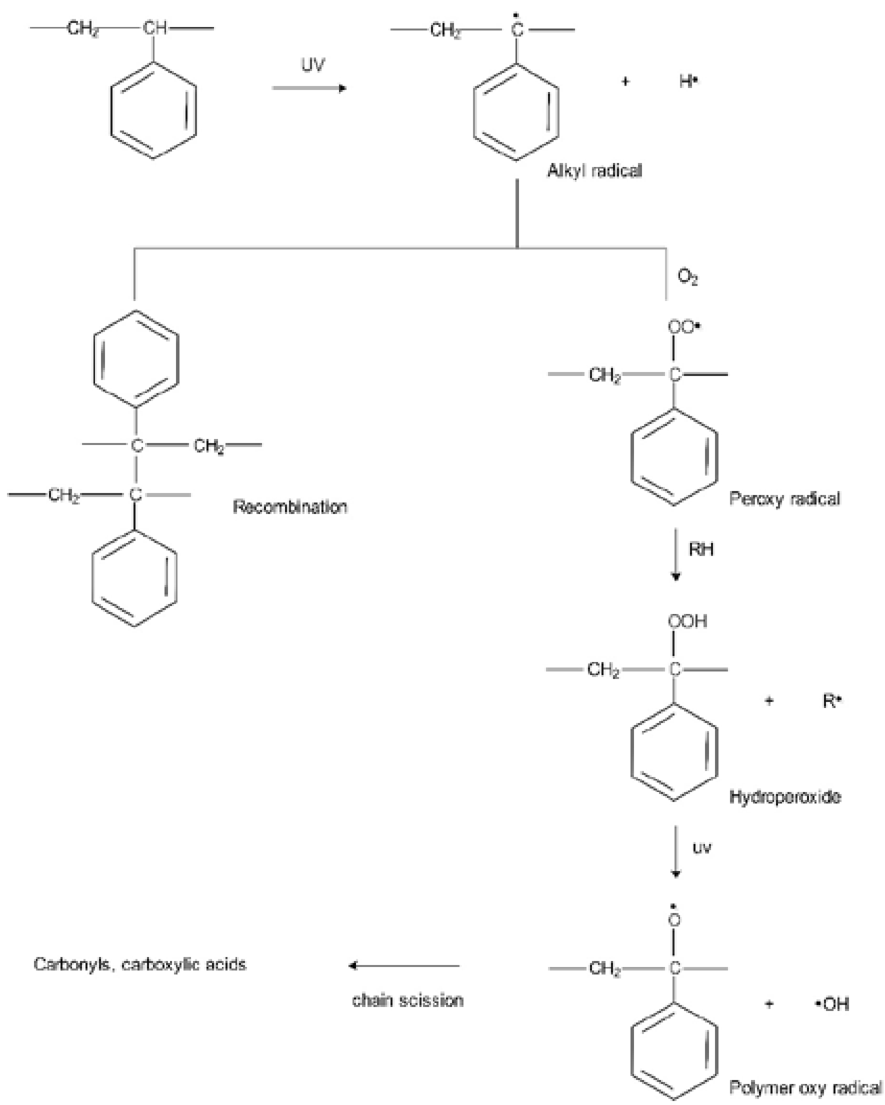

Fig. 4. General interaction mechanism between PS and UV radiation; R represents carbon containing substituents [12].

were also modified by atomic oxygen, O(^{1}D) which arises as one of the co-products alongside molecular oxygen, O_2 ($^1\Delta_g$ or $^1\Sigma_g^+$) from the decomposition of ozone after absorbing UV radiation (λ=254 nm). Walzak et al. [11] has reported that, in the presence of ozone, the interaction between polymer chains with UV radiation reduced significantly, since most of the radiation has been absorbed by ozone. O(^{1}D) is a very reactive oxygen species and may react with the polymer chain, ozone, ambient oxygen and water vapor in many possible reactions resulting in various oxygen functional groups. Interactions of O(^{1}D) with ozone, oxygen and water vapor present during the treatment are presented in the following equations [13]:

$$O(^1D)+O_2 \rightarrow O(^3P)+O_2(^1\Sigma_g^+) \quad (1)$$

$$O(^1D)+O_3 \rightarrow O_2+2O(^3P) \quad (2)$$

$$O(^1D)+H_2O \rightarrow 2\bullet OH \quad (3)$$

The interaction of ground state atomic oxygen atoms, O(^{3}P) with the polymer chain may produce alkyl radicals and subsequently form hydroxyl groups when combined with hydroxyl radicals. While for molecular oxygen, interactions with alkyl radicals may produce polymer peroxy radicals (POO•) which eventually can turn into carbonyls, carboxylic acids or hydroperoxides in slow reactions in the presence of oxygen. The interaction of O(^{1}D) with the polymer chain may also produce hydroxyl groups from mass insertion of the reactive groups into C-H bonds or forms ether when inserted into C-C bonds. Further oxidation of these groups may result in the formation of higher oxidized functional groups, ketones and ester [13].

4. CONCLUSIONS

As the conclusion, among three different oxidation treatments applied on PS microspheres, the combination of both UV and ozone (UVO_3) has yielded the highest COOH concentration. The result obtained by UVO_3 was 54 % higher than that achieved by the treatment by ozone only and 701 % higher when compared to the treatment by UV only. High surface COOH concentrations on UVO_3 treated PS microspheres may render them suitable as cell microcarriers for suspension culture of adherent cells.

5. ACKNOWLEDGEMENTS

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