UNIVERSITI PUTRA MALAYSIA

EFFECTS OF CULTIVATION SYSTEM AND TEMPERATURE ON METHANE EMISSIONS, WATER CONSUMPTION AND METHANE-OXIDIZER BACTERIA COMMUNITIES IN PADDY SOIL

PARDIS FAZLI

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By

PARDIS FAZLI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

September 2014

DEDICATION

It is my great pleasure to dedicate this dissertation work to my family, especially...

to my dear parents **Khalil Fazli** and **Zohreh Mahmoudi Nejad Dezfouli** for dedication of all their lives to support me in all aspects of life;

to my dear brother and sisters **Payam**, **Parnian** and **Paramis** for their kind and sincere cooperation, their patience and generosity;

and to my dear daughters **Bita** and **Anahita** for their love that was my powerful encouragement and inspiring to accomplish my education.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

EFFECTS OF CULTIVATION SYSTEM AND TEMPERATURE ON METHANE EMISSIONS, WATER CONSUMPTION AND METHANE-OXIDIZER BACTERIA COMMUNITIES IN PADDY SOIL

By

PARDIS FAZLI

September 2014

Chair: Associate Professor Hasfalina Che Man, PhD

Faculty: Engineering

Methane (CH_4) is a potent greenhouse gas with a global warming potential of 25 times more than CO_2. Paddy fields are important sources of methane and contribute in approximately 15–20% of the annual global methane efflux. Methanogens and methanotrophs are two microbial communities contribute in the biogeochemical methane cycle in soil by producing and oxidizing the methane respectively. In fact, the total methane emission from rice soil is the balance between methanogens and methanotrophs activities. Methane emission rate could be affected by several factors such as irrigation pattern, fertilizer type, soil organic matter and soil temperature. Between them, soil temperature is a determining factor which deserves to be investigated. Also, cultivation systems can affect the methane emission by their different water management and practices. One of the cultivation methods is the System of Rice Intensification (SRI) which includes Original SRI and Oblong-Triangular SRI. Therefore, this study was performed with the main aim of introducing a sustainable rice production system with less contribution to global warming and more productivity. Therefore, the effect of soil temperature and different cultivation systems on the methane emission rate from rice soil was studied. In addition, identification of methane-oxidizer bacteria (MOBs) was conducted in two rounds. Static chamber method has been applied to evaluate the influence of two SRI methods and conventional method on methane emission. As a result, conventional method showed the highest total methane flux with emitting of 26.4 g CH_4 m^{-2} compared to original SRI treatments and triangular pattern with 7.7 g CH_4 m^{-2} and 8.9 g CH_4 m^{-2} respectively. The pattern of water management was the most influencing factor led to lower methane emission in SRI treatments. SRI treatments produced higher yield than the conventional method so that original SRI produced 6.98 and 7.00 ton ha^{-1}, oblong-triangular SRI yielded 7.08 and 7.01 ton ha^{-1} at first and second round respectively. On the other hand, conventional method presented

6.74 and 6.80 ton ha^{-1} grain yield at first and second round respectively. Besides, more than 40% water saving was observed in original and oblong-triangular SRIs while higher water productivity including 7.93 and 7.86 kg ha^{-1} mm^{-1} were recorded in these cultivation systems compared to conventional method with 4.49 kg ha^{-1} mm^{-1}. This could be a promising result toward a sustainable rice production. Moreover, soil temperature showed positive correlation with methane emission both in daily measurements (0.768) and during rice growth season (0.528). However, factors such as water management and plant age decreased this correlation during growth season. Furthermore, this experiment applied PCR-DGGE to detect MOBs within the rice soil from two depths between 0 to 5 and 5 to10 cm in different rice growth stages and cultivation systems. Consequently, several MOBs from type I and type II have been identified. However, type I was detected at 0-5 cm depth and drained condition rather than 5-10 cm depth and flooding condition. Both systems of rice intensification (SRI treatments) were revealed to provide stimulating condition for MOBs (Esp. Type I) compared to conventional method because of aerating the soil alternatively. Besides, SRIs showed higher diversity of MOBs in comparison with Conventional method. SRIs reduced the methane emission by affecting the MOBs' communities. In this regard, SRIs provided favourable condition for type I MOBs to be active and oxidize more portion of produced methane in the soil before being released into the atmosphere. Consequently, SRIs could succeed in methane emission mitigation from paddy soil while they produced more grain yield and represented less water usage. As a conclusion, this study can introduce SRIs as green cultivation systems for a sustainable rice production.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia Sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN SISTEM PENANAMAN DAN SUHU KE ATAS PENGELUARAN METANA, PENGGUNAAN AIR, DAN KOMUNITI BAKTERIA PENGOKSIDA-METANA DI DALAM TANAH SAWAH

Oleh

PARDIS FAZLI

September 2014

Pengerusi: Profesor Madya Hasfalina Che Man, PhD

Fakulti: Kejuruteraan

Metana (CH_4) adalah gas rumah hijau dengan potensi pemanasan global sebanyak 25 kali ganda lebih kuat berbanding karbon dioksida (CO_2). Sawah padi merupakan sumber penghasilan metana yang utama dan menyumbang kira-kira 15–20% daripada penghasilan metana setahun. Methanogen dan methanotroph adalah dua komuniti mikrob yang menyumbang kepada kitaran biogeokimia metana dalam tanah dengan menghasilkan, dan mengoksidaan metana masing-masing. Jumlah pelepasan metana daripada tanah di kawasan penanaman padi terhasil daripada keseimbangan di antara aktiviti-aktiviti methanogen dan methanotroph. Kadar pelepasan metana boleh dipengaruhi oleh beberapa faktor seperti pola saliran, jenis baja, kandungan organik dalam tanah dan suhu tanah. Suhu tanah adalah faktor penentu utama yang perlu dikaji selain daripada itu sistem penanaman dengan pengurusan air dan kaedah yang berbeza juga boleh menjejaskan pelepasan metana. Salah satu kaedah penanaman adalah menggunakan “System of Rice Intensification” (SRI) yang terdiri daripada SRI Asli dan SRI Segitiga Oblong. Oleh itu, kajian ini telah dijalankan dengan tujuan utama untuk memahami kesan suhu tanah dan sistem penanaman yang berbeza pada kadar pelepasan metana daripada tanah, dan pengenalpastian bakteria pengoksida metana (MOBs) dalam dua pusingan tanaman. Suhu didalam tanah menunjukkan korelasi positif dengan pelepasan metana berdasarkan bacaan harian (0.768) dan ketika musim penanaman padi (0.528). Walau bagaimanapun, korelasi diantara suhu tanah dan pelepasan metana menurun disebabkan oleh faktor-faktor seperti umur pokok padi dan pengurusan air semasa musim penanaman padi. Di samping itu, kaedah ruangan statik (Static Chamber) telah digunakan untuk menilai pengaruh dua kaedah SRI dan kaedah secara konvensional terhadap kadar pelepasan metana. Kaedah konvensional menunjukkan jumlah metana fluks yang tinggi dengan menghasilkan 26.4 g CH_4 m^{-2} berbanding SRI Asli dan SRI Segitiga Oblong dengan 7.7 g CH_4 m^{-2} dan 8.9 g CH_4 m^{-2} masing-masing. Corak pengurusan air merupakan

faktor yang mempengaruhi pengurangan pelepasan metana melalui kaedah SRI. Kaedah SRI mengeluarkan hasil yang lebih tinggi berbanding kaedah konvensional yang mana kaedah SRI Asli menghasilkan 6.98 dan 7.00 tan ha^{-1}, SRI Segitiga Oblong menghasilkan 7.08 dan 7.01 tan ha^{-1} pada penanaman pusingan pertama dan kedua. Sebaliknya, kaedah konvensional hanya menghasilkan 6.74 dan 6.80 tan ha^{-1} padi pada pusingan pertama dan kedua. Di samping itu, lebih daripada 40% air telah dapat dijimatkan dengan menggunakan kaedah SRI manakala produktiviti air termasuk 7.93 kg dan 7.86 kg ha^{-1} mm^{-1} telah direkodkan dengan menggunakan kaedah ini berbanding kaedah konvensional dengan hanya menghasilkan 4.49 kg ha^{-1} mm^{-1}. Ini menunjukkan kebarangkalian yang memberangsangkan ke arah pengeluaran padi lestari. Selain itu, kajian ini juga menggunakan PCR-DGGE untuk mengesan MOBs daripada tanah padi di dua kedalaman antara 0 hingga 5 cm; dan 5 hingga 10 cm dalam sistem penanaman dan peringkat pertumbuhan padi yang berbeza. Beberapa MOBs dari jenis I atau jenis II telah dikenal pasti. Walau bagaimanapun, pada kedalaman 0-5 cm dan dalam keadaan kering, MOB jenis I telah dapat dikesan; dan tidak pada kedalaman 5-10 cm dan keadaan berair. Kedua-dua sistem SRI telah terbukti dapat merangsang pertumbuhan MOBs berbanding kaedah konvensional, dengan pengudaraan tanah sebagai alternatif. Selain itu, SRI menggalakkan kepelbagaian MOBs yang lebih, berbanding dengan kaedah konvensional. SRI mengurangkan pelepasan gas methane dengan memberi kesan terhadap masyarakat MOBs. Dalam hal ini, SRI menggalakkan MOBs Jenis I supaya aktif mengoksidakan sebahagian besar metana yang dihasilkan di dalam tanah sebelum dilepaskan ke dalam atmosfera. Oleh yang demikian, SRI berjaya mengurangkan pelepasan metana dari tanah padi seterusnya memberi hasil bijirin yang lebih dengan penggunaan air yang kurang. Kesimpulannya, kajian ini boleh memperkenalkan SRI sebagai sistem penanaman hijau untuk pengeluaran padi yang lestari.

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Especially, I am always extremely thankful and grateful to my supervisor **Associate Professor Dr. Hasfalina Che Man** for all her precious help and kind support.

I certify that a Thesis Examination Committee has met on 12 September 2014 to conduct the final examination of PARDIS FAZLI on her degree thesis entitled "Effects of Cultivation System and Temperature on Methane Emissions, Water Consumption and Methane-Oxidizer Bacteria Communities in Paddy Soil" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Desa bin Ahmad, PhD
Professor Ir.
Faculty of Engineering
Universiti Putra Malaysia
(Chairman)

Mohd Amin bin Mohd Soom, PhD
Professor Ir.
Faculty of Engineering
Universiti Putra Malaysia
(Internal Examiner)

Luqman Chuah Abdullah, PhD
Professor
Faculty of Engineering
Universiti Putra Malaysia
(Internal Examiner)

Zhengqin Xiong, PhD
Professor
Nanjing Agricultural University
China
(External Examiner)

NORITAH OMAR, PhD
Associate Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Hasfalina Che Man, PhD
Associate Professor
Faculty of Engineering
Universiti Putra Malaysia
(Chairman)

Azni Idris, PhD
Professor
Faculty of Engineering
Universiti Putra Malaysia
(Member)

Umi Kalsom Md Shah, PhD
Associate Professor
Faculty of Biotechnology and Bimolecular Sciences
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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I hereby confirm that:

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- the research conducted and the writing of this thesis was under our supervision;
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Signature : ___________________

Name of
Chairman of
Supervisory
Committee: Hasfalina Che Man, PhD

Signature : ___________________

Name of
Member of
Supervisory
Committee: Azni Idris, PhD

Signature: ___________________

Name of
Member of
Supervisory
Committee: Umi Kalsom Md Shah, PhD

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LIST OF ABBREVIATIONS

ARDRA	Amplified ribosomal DNA restriction analysis technique
AWD	Alternative Wetting and drying water the soil
C	Conventional rice cultivation system
C-D	Methane emission from subsoil of conventional method
C-S	Methane emission from the soil surface of conventional method
DAT	Days after transplanting
DGGE	Denaturing gradient gel electrophoresis
DNA	Deoxyribonucleic acid
E_h	Soil redox potential
FISH	Fluorescence in situ hybridization
GHGs	Greenhouse gases
GWP	Global Warming Potential
Hs	Heading stage
MOBs	Methane-oxidizer bacteria
PCI	Phenol: chloroform: isoamyl alcohol
PCR	Polymerase chain reaction
PIs	Panicle initiation stage
PLFA	Phospholipid-derived fatty acids
R1	First round of experiment
R2	Second round of experiment
RT-PCR	Real-time polymerase chain reaction
SME	Seasonal methane emission
SRI-O	Original system of rice intensification

SRI-O-D	Methane emission from subsoil of original system of rice intensification
SRI-O-S	Methane emission from the soil surface of original system of rice intensification
SRI-T	Oblong-triangular system of rice intensification
SRI-T-D	Methane emission from subsoil of Oblong-triangular system of rice intensification
SRI-T-S	Methane emission from the soil surface of Oblong-triangular system of rice intensification
Ts	Soil temperature
T-RFs	Terminal restriction fragments
T-RFLP	Terminal restriction fragment length polymorphism
Vs	Vegetative stage
WM	Water management

CHAPTER 1

INTRODUCTION

1.1. Introduction

Methane (CH_4) is an effective greenhouse gas with global warming potential (GWP) of 21 (Yang et al., 2010). It can be produced through the final step of biodegradation process of organic matter in flooded soils (Mitra et al., 2012). In fact, this greenhouse gas is a bio-product of methanogenic activity in anaerobic condition of soil. Methanogens and methanotrophs are two microbial communities, both involved in the biogeochemical methane cycle in soil. Methanogens are responsible for producing methane (Kumar and Viyol, 2009). These microorganisms are obligate anaerobic and thus, they are active in flooding and high-reduced environment with lower soil redox potential (Pazinato et al., 2010). In wetlands such as paddy fields, flooding is an obstacle for entering the oxygen from the atmosphere into the soil, which results in anaerobic fermentation of soil organic matter (Kumar and Viyol, 2009) and thus, some substances such as H_2/CO_2 and formate will be produced. Thereafter, methanogens utilize these substances as carbon source. Methane gas is a byproduct of this process (Figure 1.1).

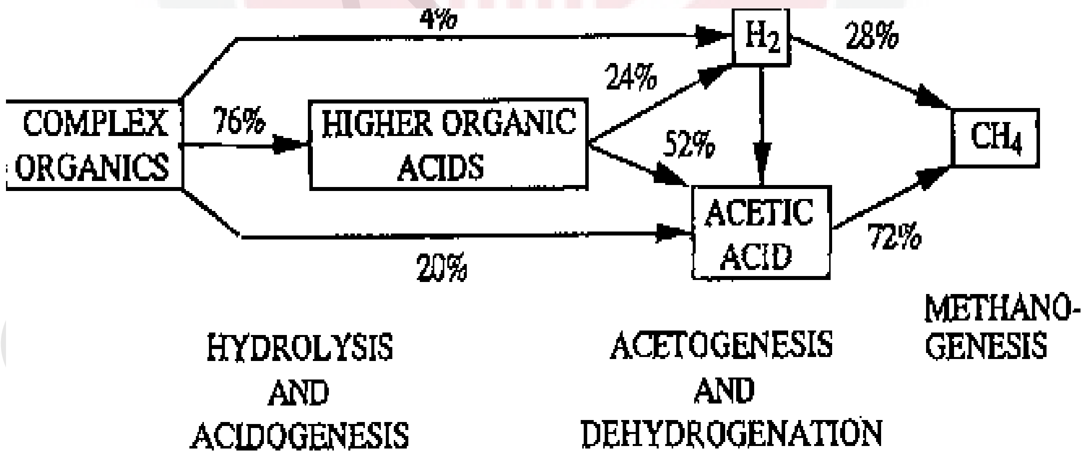

Figure 1.1. Schematic of methane production by methanogens process. (Source: FAO,1997)

On the other hand, methanotrophs including methane-oxidizer bacteria (MOBs) account for methane oxidization before releasing methane from the soil into the atmosphere. In contrast with methanogens, methanotrophs are mostly aerobic unicellular microorganisms which are active in oxic area of soil. Methanogens and methanotrophs have been investigated frequently in different ecosystems and conditions such as, sludge digestors (Hwang et al., 2008), peatland (Godin et al., 2012), fresh water and marine sediments (Newby et al., 2004), lakes (Carini et al., 2005; Antony et al., 2012) and rice soil (Vishwakarma et al., 2010; Wang et al., 2010). However, different methods have been applied for this purpose. These methods include fluorescence in situ hybridization (FISH), phospholipid-derived fatty acids (PLFA), real-time polymerase chain reaction (PCR), and fingerprinting techniques (e.g. denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP) and temperature gradient gel electrophoresis) (Fey and Conrad, 2000; Lu and Conrad, 2005; Conrad et al., 2006; Wu et al., 2009 a, b; Surakasi et al., 2010; Bodelier, 2011; Kumar et al., 2011; Ma and Lu, 2011).

Furthermore, rice (*Oryza sativa* L.) is a main food for about one third of the world's population (Fusi et al., 2014). The demand for this crop is anticipated to rise with growing population of the world as it is anticipated to be increased to about 9.2 billion by 2050 (Wang et al., 2012a). Increasing the rice cultivation area can be a fulfillment to hit adequate rice production. Currently, rice crop mostly produces by conventional method with flooding the soil. Thus, a remarkable amount of methane (about 112 Tg CH_4 Yr^{-1}) emits during rice production (IPCC, 2007). As methane global warming potential is 21 times more than carbon dioxide, increasing the rice cultivation area means more contributing of rice fields to climate change. This fact emphasizes the necessity of finding a low-methane emitting cultivation system concerning a sustainable future.

There are some cultivation systems for rice including, conventional method and system of rice intensification (SRI). In conventional rice cultivation system, flooding continuously is used as water management. As it mentioned before, likewise natural wetlands, flooding rice field cuts off oxygen from the atmosphere entering to the soil (Kumar and Viyol, 2009). Therefore, it could induce an anaerobic condition within the soil. Anaerobic condition is favorable for methanogens (methane producers). Activation of these microorganisms can be influenced widely by water regime type. Consequently, type of water management appliedby different cultivation systems can be considered as an effective factor for reducing methane emission from paddy soils (Singh et al., 2003; Tyagi et al., 2010).

Of rice cultivation systems, SRI was developed during the early 1980's in Madagascar (Stoop et al., 2002; Tsujimoto et al., 2009). Water management in SRI method is done by wetting and drying the soil alternately (AWD) in a day cycles of 3-6 (Farooq et al., 2009; Krupnik et al., 2012). This way less water is needed for growing rice plants (Suryavanshi et al., 2013). Furthermore, drying the soil can lead to induce aerobic condition in the soil. Therefore, methane production is expected to be reduced (Anand et al., 2005). This method has been studied in several regions such as India (Sinha and Talati, 2007), Bangladesh (Latif et al., 2005), Africa (Krupnik et al., 2012). In this method chemical herbicides are not used for weeding. On the contrary, weeding is being done manually or mechanically (e.g., with a rotary hoe or con-weeder due to active soil aeration) every 10-12 days starting almost 10-12 days after transplanting (Dobermann, 2004). Other principle of SRI method is planting only one seedling per spot at two-leaf stage (Dobermann, 2004). Planting one seedling per hill, results in about 80–90% reducing in plant populations (Barison and Uphoff, 2011). In addition, rice plant is the most effective pathway for methane transport from soil to the atmosphere due to its well-developed aerenchyma (Neue et al., 1996). Thus, less plant density leads to less methane transferring from soil to the atmosphere. Consequently, SRI method practices along with providing better condition for rice growing (Chapagain et al., 2011), could decrease methane emission from rice soil. Although, there are some reports on no significant modification of grain yield by SRI (Latif et al., 2005; McDonald et al., 2008; Chapagain et al., 2011) other reports confirmed higher yield from SRI compared to conventional cultivation (Sinha and Talati, 2007; Glover, 2011). In other words, benefits of this cultivation method include saving the water about 15-20%, producing stronger rice plants with resistance to pests and diseases (Menete et al., 2008; Glover, 2011; Watanabe et al., 2013) and its high potential to suppress the methane emission due to the AWD water management. Synergies among the practices of SRI have been reported as the reason (Sinha and Talati, 2007; Glover, 2011).

SRI is not a restricted method so that any useful creativity can be applied (Uphoff, 2008). For example, SRI oblong-triangular (SRI-T) (3 seedlings per hill, separated by 7 cm distance with 40×45 cm regular distances) is a modified SRI which follows all original SRI's principles while, it has different cultivation pattern (Zheng et al., 2004). SRI-T provides a plant density of 24 plants per m^2 instead of 18 plants per m^2 in original SRI (SRI-O). Increasing plant density while inducing low competition between plants by a 7 cm separation might lead to a higher yield.

1.2. Problem statement

Methane as one of the main greenhouse gases (GHGs) contributes to global warming and climate change (Rodhe, 1990; Kumar and Viyol, 2009). This GHG has the effect 25 times more powerful than carbon dioxide (IPCC, 2007). It is responsible for 15 to 20% of global warming (IPCC, 1996; Wang et al., 2012b; Ali et al., 2013). In fact, the molecule of methane is like a trap for infrared radiation emitted from the earth toward the atmosphere after receiving and absorption of a portion of solar radiation by the earth. Otherwise, the infrared could escape to the space. Methane molecules would be energized and then will begin to emit heat in all directions including toward the earth (Bouwman, 1991; Khosa et al., 2010; Nema et al., 2012). Unfortunately, the concentration of methane in the atmosphere has increased by 1070 part per billion by volume (ppbv) in 2008 compared to pre-industrial time (Singh and

Dubey, 2012). Moreover, it has been stated that methane emission will increase 60% by 2030 (Pittelkow et al., 2013).

Agricultural and forestry activities have been considered as the main sources of greenhouse gases especially for methane emissions; in a way that, agricultural activities contribute to about 50-52% of anthropogenic CH_4 emissions (IPCC, 2007; Smith et al., 2007; Jia et al., 2012). From the agriculture sector, rice cultivation is a significant contributor to methane emissions (Wataru et al., 2001; Datta et al., 2009; Tyagi et al., 2010; Li et al., 2011; Dubey et al., 2012; Suryavanshi et al., 2013; Das and Adhya, 2014; Fusi et al., 2014; Sander et al., 2014) while, wetland rice was a major anthropogenic methane source (10%) in 2005 (Kemfert and Schill, 2009). In fact, rice fields are responsible for about 20% of global anthropogenic methane emission (Misra and Verma, 2013). Moreover, according to Feng et al., (2012) and Singh and Dubey, (2012; Hang et al., 2014), flooded rice fields account for emission of 25-54 Tg methane CH_4 annually. However, this amount was indicated about 25–100 Tg by Xu et al., (2007) and Li et al., (2011).

Pathways of methane emissions from paddy fields are molecular diffusion, ebullition and plant transport (Figure 1.2) (Dubey, 2005). Between these pathways, rice plant account for about 90% of methane release into the atmosphere (Holzapfel-Pschorn et al., 1985; Schutz et al., 1989; Wang et al., 1997; Anand et al., 2005; Khosa et al., 2010).

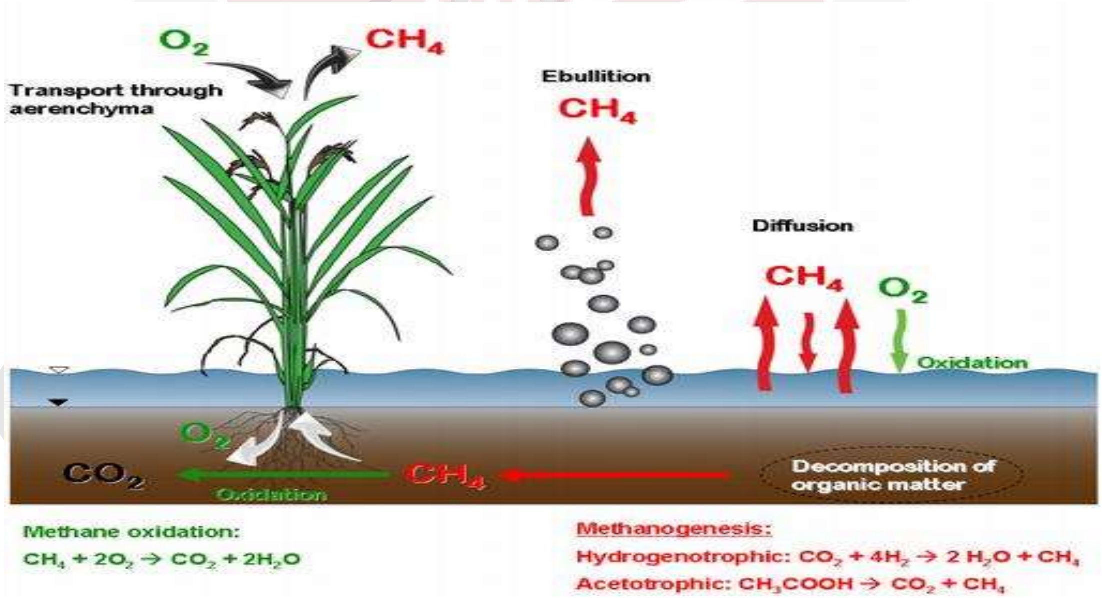

Figure 1.2. Pathways of methane emissions from paddy fields (Source: IBP ETH)

Since, rice is a staple food for about 90% of world's population, its production is anticipated to increase about 50% by the year 2030 (Pramanik et al., 2013; Misra and Verma, 2013). As a result, methane emissions from flooded paddies will rise to about 150 million tonnes in 2025 (IPCC, 2007). Therefore, there is a need of introducing a sustainable rice production system which is capable to produce acceptable yield while it can decrease the emission of methane from paddies. Thus, strategies leading to mitigate the methane emission along with producing higher rice yield are required (Misra and Verma, 2013). Towards achieving this goal, the influencing factors on methane production and emissions in rice soils need to be studied. Subsequently, it has been a major concern of researches in recent decades (Watanabe et al., 1995; Neue et al., 1996; Wang et al., 1997; Mitra et al., 1999; Khosa et al., 2010; Tyagi et al., 2010; Itoh et al., 2011; Zhao et al., 2011; Bhatia et al., 2012; Bhattacharyya et al., 2012; Misra and Verma, 2013). In this regard, some affecting factors on the CH_4 production and emissions from rice soil include soil temperature, water regime, rice cultivars, fertilizers, soil properties, phosphate and sulphate content of soil and organic matter (Parashar et al., 1993; Neue et al., 1996; Anand et al., 2005; Kumar and Viyol, 2009; Khosa et al., 2010; Yang et al., 2010; Itoh et al., 2011; Mitra et al., 2012).

Of contributing factors for methane emission, water management can play a main role as it is a key factor for methane emissions (Zhao et al., 2011; Sander et al., 2014). Comparing current irrigation methods and different irrigation patterns, some water regimes leading to less methane emissions have been suggested by these studies. A main common policy was applying drainage, but in different pattern and quantity (such as different times of drinage). Accordingly, different cultivation systems might influence methane emission mostly by implementation of different water regimes. However, assessing the effectiveness of rice cultivation methods on decreasing the methane emission from rice fields along with production of acceptable yield seems necessary. In addition, it might result in introducingan environmental promising rice cultivation system. System of rice intensification (SRI) is a cultivation system with a special water management which avoids flooding the soil continuously. Subsequently it might be beneficial for lowering methane emission from paddies. The modified version of SRI was introduced by Zheng et al., (2004) with a different cultivation pattern. However, this cultivation system has not been examined yet comprehensively regarding methane emission.

In addition, methane oxidization is a microbial process which is done within the soil by Methane-Oxidizer bacteria. These microorganisms can be affected by environmental factors. Thus, any regulation of methane emission by different cultivation systems is done through influencing MOBs. Nevertheless, still there is a gap for study the diversity of MOBs in paddy soil under different cultivation systems including SRIs in Malaysia.

Moreover, except for some works on original SRI (Jayadeva et al., 2009, Suryavanshi et al., 2013) different SRI cultivation systems has not been studied yet properly about methane emission and rice water productivity. As a result, the aim of current study is to assess the effectiveness of SRIs -as water conservation rice cultivation methods- in decreasing the methane emission from rice fields and about water saving. Thus, this study can lead to introduce an environmental promising rice cultivation system.

1.3. Aims of the research

The main aim of current study was to introduce a sustainable rice production system with less contribution to global warming and more productivity. Therefore, this study was done with the aims of determining the efficiency of SRIs especially SRI-T inreducing methane emission from paddies. In this regard, water consumption of rice and its grain yield were evaluatedin order to indicate the most environmental friendly rice cultivation system. Furthermore, the influence of different rice cultivation systems on diversity of methane-oxidizer bacteria was studied to indicate the pattern of changes in MOBs' communities during rice growing season under different cultivation systems. Also, it provides a clear vision of the regulating process of methane emission from paddies by SRIs and might be an insight to future studies regarding stimulating these microorganisms in paddy fields.

1.4. Objectives of the research

The objectives of this study included:

1. To determine an effective cultivation system (Original system of rice intensification, Oblong-triangular system of rice intensification and Conventional method) in order to decrease methane emission from paddy field in Malaysia and thus, reducing the Malaysia contribution to global warming as much as possible.

2. To evaluate the water consumption and yield of rice plants under different cultivation systems to indicate the most sustainable cultivation system for supplying food for the fast growing world population in future using less natural resources.

3. To assess the role of soil temperature in regulating the methane emission from paddy soil in a daily basis and during rice growing season in Malaysia.

4. To identify the microbial diversity ofmethane-oxidizer bacteria in Malaysia rice soil under different cultivation systems by culture independent microbial detection technique (PCR-DGGE) to achieve a clear vision of methane emission regulation by different cultivation systems.

1.5. Scope of the research

In order to fulfil the aims and objectives, the scopes of this study were determined as follows.

This experiment was performed in lab scale using a high yield Malaysian rice cultivar MR 219 which is cultivating in a large area in Malaysia. Rice seedlings were transplanted in three tanks so that each tank allocated to one cultivation system including original system of rice intensification, oblong-triangular system of rice intensification and conventional method. A comprehensive monitoring of methane emission was performed. For this purpose, daily and hourly methane emission from rice soil was measured under different cultivation systems in order to indicate the most effective cultivation system for suppressing the methane emission from paddies. Tetra gas detector was utilized for detection of methane in Tedlar® bags. The amounts of methane in Tedlar® bags for the emission from 8 pm to 7 am was zero or below detection limit ($\leq$ 1 ppm). Therefore, hourly methane emission was measured between 7 am and 8 pm every day. Study of methane emission was done considering rice yield and water usage in all cultivation systems. These factors were used as criteria for evaluation of cultivation systems regarding introducing a sustainable rice cultivation system.

Water consumption of rice plants was determined under different cultivation systems. In this regard, all input water into the cultivation systems including irrigation and rainfall was measured and subjected to calculation of water consumption and water saving. In addition, grain yield in hectare was estimated to calculate the water productivity and also to have a criterion for indicating the most productive cultivation system.

Moreover, soil temperature was measured hourly at the same time with gathering data of hourly methane emission every day. This study helped to achieve a pattern for relation between methane emission and soil temperature in paddy field.

Another scope was to identify methane-oxidizer bacteria communities and study their changes in different rice growing stages under different cultivation systems. For this purpose, detection was done during four main rice growth stages and in two different depth ranges 0-5 cm and 5-10 cm. These ranges were located in the methane production zone of soil which was determined applying the E_h of -200 mV as an indicator (Chen and Avnimelech, 1986). Besides, a modified protocol was applied for DNA extraction because the soil type was hard to lysis and regular DNA extraction protocol was unable to succeed.

1.6. Output/benefits of the research

The outcomes of current study can be beneficial in some aspects including, indicating a promising low emitting cultivation system for rice which could be a reasonable replacement for conventional method in Malaysia. As a result, this study's findings can be helpful to reduce the portion of Malaysia in global warming. Moreover, for determining the most beneficial cultivation system in current research, grain yield and water productivity were considered which can help toachieve asustainable rice production.

Furthermore, study of Methane-Oxidizer Bacteria in relation to the trend and pattern of methane emission during the rice growing season can bring about a clear vision of the mechanism of regulating the CH_4 emission by SRIs. Also, it can be an inspiration for future studies regarding stimulating favourable condition for MOBs in paddies through different strategies.

In addition, the methane emission alteration was studied in relation to soil temperature to establish a correlation between them. This was done with the purpose of generating an idea for further studies. In fact, after determining the favourable temperature for lowering the emission, there would be a need to perform further researches for finding a way to adjust the soil temperature to its optimum in order to regulation the methane emission from paddy fields.

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