CHAPTER I
INTRODUCTION

1.1 Research rationale

The public is seriously concerned with the huge petroleum-based plastics waste accumulated in environment. The increasing of non-biodegradable plastics are not only negative impacts on life quality of human but also animals as well as single cell lives because of their persistence in environment. Several communities are now more supposing to the impact of discarded plastic on the environments and searching for new resources capable of degradable polymers instead of petroleum-base plastics. Nowadays, a wide variety of bio-based polymers are produced in worldwide including polylactic acid (PLA), polybutylene succinate (PBS) and polyhydroxyalkanoates (PHAs). Among them, PHAs is drawing the mostly considerable attention to be a new biodegradable plastic. It was produced as an intracellular storage compounds that are accumulated as discrete granules in cytoplasm of cells as carbon and energy reserve in several kinds of microorganisms but mostly found in bacteria such as *Alcaligenes* spp., *Pseudomonas* spp., *Hydrogenophaga* spp. *Azotobacter vinelandii*, *Bacillus* spp., etc. PHAs are biodegradable thermoplastic polyester (Anderson & Dawes, 1990), compatible compound with mammalian cell (Philip, Keshavarz, & Roy, 2007), and have the widely physical properties similar to synthetic polypropylene. The applications of PHAs as biodegradable plastics are widely used in multi purposes such as veterinary practice, food packaging, soil cover, medicines, tissue engineering as implant materials, etc. However, their use is currently limited due to the cost of manufacturing process of this biodegradable plastic which are attributed to the cost of carbon source, fermentation process and also the downstream process (Choi & Lee, 1999). Considerately, only the carbon source costs up to 50% of the overall production costs of the PHAs (Halami, 2007). Thus, the use of inexpensive carbon sources such as agro-industrial raw materials, by-product, waste and even other cheap renewable sources could contribute as much as 40-50% reduction in the overall

production cost (Ramadas, Singh, Soccol, & Pandey, 2009; Santimano, Prabhu & Garg, 2009).

Previously various cheaper renewable substrates were used for PHAs production such as carbon dioxide (Ishizaki, Tanaka, & Taga, 2001), molasses (Gouda, Swellam, & Omar, 2001; Kulpreecha, Boonruangthavorn, Meksiriporn, & Thongchul, 2009), methanol (Kim, Kim, & Oh, 2003; Mokhtari-Hosseini, Vasheghani-Farahani, Heidarzadeh-Vazifekhoran, Shojaosadati, Karimzadeh, & Darani, 2009), maple sap (Yezza, Halasz, Levadoux, & Hawari, 2007) sucrose (Tanamool, Imai, Danvirutai, & Kaewkannetra, 2011) and recently, sugar cane (Suwannasing, Mooamart, & Keawkannetra, 2011). However, another sugar plant, viz. sweet sorghum (*Sorghum bicolor*) could appear to be an alternative substrate for PHAs production due to several advantages including low price, grow in short time approximately 3-4 months, well adapted to sub-tropical and temperate regions of the world, water efficient (Almodares & Hadi, 2009) and high total sugar content in their stem. With these properties, it would be attractive for using as an alternative carbon source medium for microbial growth to produce added value products such as ethanol (Mamma, Christakopoulos, Koullas, Kekos, Macris, & Koukios, 1995; Sipos, Reczey, Somorai, Kadar, Dienes, & Reczey, 2009; Laopaiboon, Nuanpeng, Srinophakun, Klanrit, & Laopaiboon, 2009), hydrogen, (Antonopoulou, Gavala, Skiadas, Angelopoulos, & Lyberatos, 2008), lactic acid (Hetényi, Gál, Németh, & Sevella, 2010) and unsaturated fatty acids like docosahexaenic acid (DHA) (Liang, Sarkany, Cui, Yesuf, Trushenski, & Blackburn, 2010). While, Kaewkannetra, Tanonkeo, Tanamool, and Imai (2008) has played attention and previously reported the production of PHAs by Gram-negative bacteria of *Alcaligenes eutrophus* TISTR 1095 from sweet sorghum juice (SSJ) although the production yield was still low (Kaewkannetra et al, 2008). Thus, we have tried to screen novel bacterial strains able to grow in SSJ. The medium optimisation using Response Surface Methodology (RSM) was studied in batch fermentation. Fed-batch fermentation strategy was used to improve the yield of PHAs. Furthermore, the properties of the PHAs produced from the SSJ were also analysed in comparison with the standard PHA. Moreover, feasibility of PHAs production from the SSJ was evaluated.

1.2 Objectives

1.2.1 Main objective

To investigate and evaluate the potential of PHAs production from sweet sorghum juice via bath and fed-batch fermentation.

1.2.2 Sub-objectives

1) To evaluate the potential of sweet sorghum juice as raw material for biopolymer production.

2) To screen the PHAs producing bacteria from sugar cane plantation soil.

3) To compare the efficiency of PHAs production from sweet sorghum juice by isolated and referenced PHAs producing strains.

4) To optimise the medium formation via Response Surface Methodology (RSM) for batch fermentation of sweet sorghum juice for PHAs production.

5) To investigate the PHAs production from sweet sorghum juice under fed-batch fermentation.

6) To study the PHAs properties obtained from fermentative sweet sorghum juice production.

1.3 Scope and limitation of the study

In the present study, we explore the feasibility of the bioconversion of sweet sorghum juice to produce PHAs through fermentation process by Gram-positive bacteria of *B. aryabhattai* PKV01. The medium optimisation of PHAs production by using response surface methodology (RSM) was examined prior to apply to upscale batch and fed-batch bioreactor. Furthermore, the properties of the PHAs produced from sweet sorghum juice were also analysed.

1.4 Expected outputs

The expected output of this study consists of the following;

1.4.1 Understanding the potential of usability of sweet sorghum juice (SSJ) for the production of biopolymer as polyhydroxyalkanoates (PHAs).

1.4.2 Obtaining the optimisation formulation of media for PHAs production from SSJ.

1.4.3 Understanding the structure and thermal properties of PHAs produced from SSJ.

1.4.4 Obtaining basic information about the added agricultural product value of SSJ for PHAs production.

1.4.5 Attending oral and poster presentations of research results in international conferences.

1.4.6 Demonstrating the results of this research for publication in established scientific journals.

1.5 Organisation of the thesis

This thesis contains mainly 6 chapters. The details are summarised as follows;

1.5.1 Chapter I consists of research rationale, objectives of the study, scope and limitation of the study, expected outputs and the organisation of the thesis.

1.5.2 Chapter II illustrates the research work focusing on biosynthesis of polyhydroxyalkanoate (PHA) by *Hydrogenophaga* sp. isolated from soil environment in batch fermentation.

1.5.3 Chapter III represents the research work emphasizing on biopolymer generation from sweet sorghum juice (SSJ): screening, isolation, identification and fermentative polyhydroxyalkanoates (PHAs) production by *Bacillus aryabhattai.*

1.5.4 Chapter IV exhibits the research work concentrating on an alternative approach to the fermentation of sweet sorghum juice into biopolymer of poly-β-hydroxyalkanoates (PHAs) by newly isolated *Bacillus aryabhattai* PKV01.

1.5.5 Chapter V illustrates the research work target for the improvement of polyhydroxybutyrate (PHB) production from sweet sorghum juice (SSJ) via fed-batch fermentation under different C/N ratios.

1.5.6 Chapter VI demonstrates the conclusions and discussion of this research.