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Production of PHA from Cassava Starch Wastewater in Sequencing Batch Reactor Treatment System

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Abstract

The project attempt to investigate the potential of cassava starch wastewater for producing polyhydroxyalkanoate (PHA) from sequencing batch reactor (SBR) treatment system seeded with *Bacillus tequilensis* MSU 112, a PHA-producing bacterial strain. A total sequence of 24 h with a reaction phase (22 h) comprised anoxic/aerobic steps of 4/18 h was employed. The effect of varies chemical oxygen demand (COD) concentration of the synthetic cassava starch wastewater (CSW) (3,000 4,000 and 5,000 mg/L) on PHA production and treatment efficiency was determined. The CSW with COD at a concentration of 4,000 mg/L produced the highest PHA concentration, PHA yield, and total kjeldahl nitrogen and total phosphate removal efficiencies at 3,346 mg/L, 79.2% as dry sludge weight, 20.6% and 27.7%, respectively, while the CSW with COD concentration of 5,000 mg/L provided the highest COD removal efficiency at 94.8%. In addition, the activated sludges obtained from an anoxic phase provided higher PHA production than an aerobic phase. The results revealed that the SBR system treating CSW with the presence of *B. tequilensis* MSU 112 offers a promising approach for PHA production.

Keywords: Polyhydroxyalkanoate, PHA production, sequencing batch reactor, cassava starch wastewater

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1. Introduction

Synthetic plastic plays an important role in human daily life but causes widespread environmental pollution as a consequence of recalcitrant properties [1]. Bioplastic, a biodegradable plastic, has been developed to replace synthetic plastic. One of the most well-known bioplastics is PHA. PHA is mostly synthesized by PHA-producing bacteria as an energy source material when the bacteria are grown under unbalanced conditions, such as a shortage of nitrogen phosphate and oxygen but the content of the carbon source is excessive [2]. PHA is the only 100% biodegradable polymer [3]. However, it is less admissible than the other bioplastics due to its high cost which is raised by the carbon source for bacteria [4]. Therefore, various attempts have been devoted to the development of economical processes or the search for suitable wastes as carbon sources in order to lower the production cost. Presently, PHA production from activated sludge produced from wastewater treatment system has been noted. PHA accumulation in activated sludge in a SBR system fed with several types of wastewater has been studied [5]-[7]. However, little information is available on the use of CSW and especially the use of PHA-producing bacteria as seed. Cassava starch is one of the major industries of Thailand. Thus, this study aims to investigate the potential of CSW for PHA production from a SBR system which was seeded with *Bacillus tequilensis* MSU 112, a PHA-producing bacterial strain. The treatment efficiency of the SBR system was also determined.

2. Methods

2.1 Microorganisms and Preparation of Inoculums

The *Bacillus tequilensis* MSU 112 used in this study was isolated from cassava pulp from the cassava starch industry in Kalasin Province, Thailand, on the basis of the ability to produce PHA from cassava starch. The culture medium was composed of 17.0 g/L, tryptone; 3 g/L, phytone peptic digest of soya meal; 5 g/L, sodium chloride; 2.5 g/L, di-potassium hydrogen phosphate; and 2.5 g/L, glucose. For inoculums preparation, the *Bacillus tequilensis* MSU 112 was grown on the culture medium for 24 h at 30°C with an agitation rate of 200 rpm. Cells were harvested by centrifugation at 8,000*g* for 15 min and used as inoculum culture.

2.2 Experimental Set-Up

A schematic diagram of a SBR is illustrated in Fig. 1. The reactor had a total volume of 12 L and a working volume of 5 L. It was operated under non-sterile condition and at room temperature. Agitation was performed with the speed varying from 200 to 300 rpm using a stirrer motor (Model OST 20 digital, IKA works, Inc, Germany). When needed, operation was provided by using an air pump.

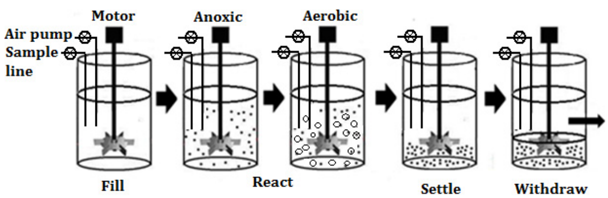

Fig. 1. Configuration of sequencing batch reactor treatment system

2.3 Experimental Procedure

A synthetic cassava starch wastewater (CSW) was used throughout the study. It was composed of cassava starch, $FeCl_3$, $CaCl_2$, $MgSO_4.7H_2O$, KH_2PO_4, Urea, and $NaHCO_3$. The amount of cassava starch added was varied to obtain COD concentration at 3,000, 4,000, and 5,000 mg/L. The initial pH of the CSW was adjusted to 7.00±0.2. The reactor was started up with the CSW having COD concentration at 3,000 mg/L. After the inoculum culture (2% inoculums size) was seeded, the reactor was then operated batchwise with aeration and mixing for several days to obtain a dense activated sludge. After that, the reactor was operated in sequencing batch mode with a total cycle of 24 h (20 min, filling phase; 22 h, reaction phase; 1 h, settling phase; 40 min, withdrawal phase). At reaction phase, the two-step SBR operation comprised anoxic/aerobic steps of 4/18 h was used at all COD concentrations. The dissolved oxygen (DO) during the anoxic and aerobic steps was maintained at 1 and 2 mg/L respectively. At the end of each cycle, the activated sludge was allowed to settle and 4 L of effluent was withdrawn. The settled activated sludge of 1 L volume was used for the next cycle with the addition of 4 L fresh CSW. Attainment of steady state was considered to have been achieved when reactor performance, as measured by COD remained constant for at least 7 consecutive SBR cycles, and had a good settling property of sludge. The reactor was continued to operate with COD concentration at 4,000 and 5,000 mg/L, respectively, until the steady state of each COD concentration was obtained. The mixed liquor suspended solids (MLSS) was maintained between 1,500 and 5,000 mg/L.

2.4 Analytical Procedures

At the end of the anoxic and aerobic steps, the activated sludges were sampled for the determination of MLSS [8] and PHA accumulation. PHA in activated sludge was extracted using a solvent extraction method [9] and the PHA content was measured using spectrophotometer method [10]. For determination of treatment efficiency, samples were taken from the wastewater at the beginning and the end of the anoxic/aerobic steps and were allowed to settle for 1 h. Clear supernatants were analyzed for COD, total kjeldahl nitrogen (TKN) and total phosphate (TP) according to the standard methods as described by [8]. The MLSS was analyzed by using the standard methods. DO was measured using a DO meter (Model YSI 200, YSI Inc., USA). The pH level was measured using a pH meter (Beckman Coulter Inc., USA).

3. Results and Discussion

3.1. Evaluation of SBR Start-Up

The *B. tequilensis* MSU 112, which was seeded into the SBR system, showed a good adaptation to the CSW. The system reached the steady state within 14 days in which an average COD removal efficiency and MLSS concentration at 94.6% and 4,161 mg/L were obtained (Data not shown).

3.2. Evaluation of SBR Performance

The SBR operation was continued at various COD concentrations (3,000, 4,000, and 5,000 mg/L) in order to evaluate the effects of COD concentration on PHA production and treatment efficiency. Consolidated results of SBR performance at various COD concentrations are presented in Fig. 2 and Fig. 3. It is evident that, the PHA production increased when the COD concentration increased from 3,000 to 4,000 mg/L (Fig. 2). But increasing COD concentration to 5,000 mg/L led to a decrease of PHA production. The reason could be due to a high C/N ratio of the CSW which caused nitrogen deficiency of the system and reduced bacterial growth as a consequence [11]. The anoxic phase showed a trend in promoting higher PHA accumulation in activated sludge than the aerobic phase. These results coincided with the findings of [12]. The highest PHA concentration and PHA yield at 3,346 mg/L and 79.2% as dry sludge weight, respectively, were obtained from the COD concentration at 4,000 mg/L. These results were comparable to the study of [13] which produced PHA from organic acids (derived from anaerobic treatment of starchy wastewater) via a fermentation process. These results were also higher than the study of [14] which observed PHA production from tapioca industrial wastewater via a SBR system without seeding PHA-producing bacteria.

The performance of the SBR system showed that an increase in COD concentration from 3,000 to 5,000 mg/L resulted in an increase in treatment efficiencies. A slight increase in COD removal efficiencies from 93.2% to 94.9% was found when the system was fed with COD concentration from 3,000 to 5,000 mg/L (Fig. 3).

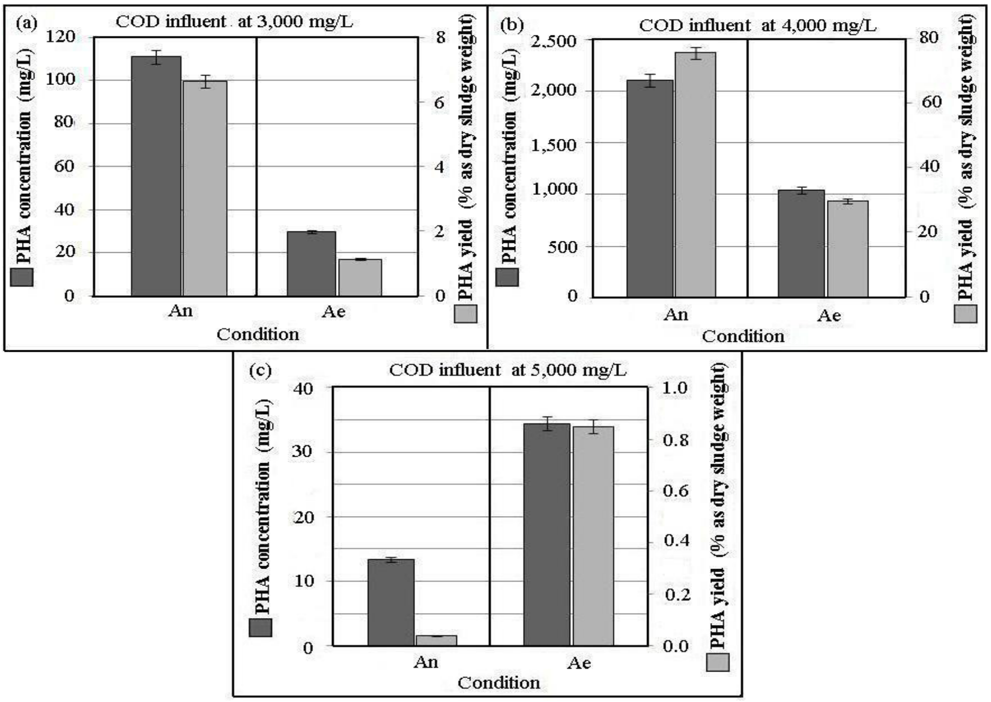

Fig. 2. Performances of the SBR system in PHA production from the CSW having COD concentrations at (a) 3,000 mg/L; (b) 4,000 mg/L; and (c) 5,000 mg/L. The data were collected for the performance of anoxic condition (An) and aerobic condition (Ae).

Trends in nutrient removal showed that at a COD concentration of 5,000 mg/L, the highest TKN removal efficiency approached 44.9%, while the highest TP removal efficiency reached 69.9%. These findings were due to the fact that a higher organic content in wastewater enabled the bacterial biomass to continue cell growth and anabolic metabolism [11]. The results also showed that the TKN and TP removal efficiencies under the aerobic phase were higher than the anoxic phase, although both parameters are generally removed under the anoxic phase. These results could be due to a sophisticated reaction of nitrate and phosphate. If nitrate in the system is decreased to a certain threshold value, phosphorus release will take place [15]. Since an amount of nitrogen and phosphorus is a key factor for PHA synthesis, therefore, further studies on the effects of nitrogen and phosphorus on PHA production are required.

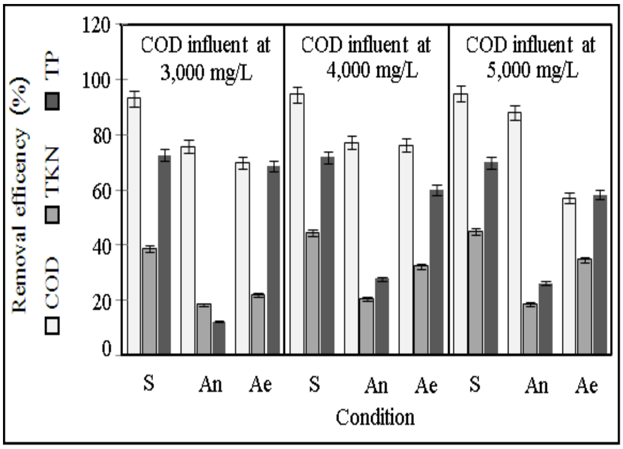

Fig. 3. Performances of the SBR system in al efficiency of COD; TKN; and TP from the CSW which having COD concentrations at 3,000; 4,000; and 5,000 mg/L. The data were collected for the performances of the overall system (S), anoxic condition (An) and aerobic condition (Ae).

4. Conclusion

The present study revealed a high potential of cassava starch wastewater towards PHA production via SBR treatment system. A seeding of *Bacillus tequilensis* MSU 112 to the system provided a great benefit in enhancing of the production of PHA.

Acknowledgements

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