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POLYHYDROXYBUTYRATE ACCUMULATION OF *Bacillus* sp. STRAINS USING WATER HYACINTH HYDROLYSATE AS A CARBON SOURCE

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ABSTRACT

The microbial cells can store energy in the form of polyhydroxyalkanoates (PHAs) while the nutrient sources are exhausted but have an excess of carbon sources. Poly(3-hydroxybutyrate) (PHB) was known as one of the most common PHA. Currently, PHB is assessed as a potential alternative to petroleum-based plastics such as HDPE, PP. In addition, PHB can be obtained from different microbes through the fermentation of renewable and sustainable materials such as waste from food or cassava starch industry, and other agricultural by-products. Although the spread of water hyacinth *(Eichhornia crassipes)* is becoming a problem in many provinces, it still is considered an opulent biomass source. Besides the application in the removal of heavy metals from wastewater, or making animal feed and fertilizer, water hyacinth can be converted into a carbon source used in microbial fermentation. This paper indicates the PHA synthesis ability of 28 bacterial strains which were isolated from soybean-growing soil samples and the Cau Dien Waste-treatment Plant's mud samples. Based on their PHA accumulation capability while using C5 and C6 sugars as carbon sources, *Bacillus* sp. AI 10 and *Bacillus* sp. CRCXL 2.2 were chosen to synthesize PHA using the water hyacinth hydrolysate as a carbon source. Pretreated water hyacinth biomass using $Ca(OH)_2$ was subjected to enzymatic hydrolysis with a suitable ratio of Cellic→CTec2 and Cellic→HTec2, which resulted in a 409.5 mg total reducing sugars/g pretreated biomass. After 48 hours of fermentation, the dry biomass and accumulated PHA amount from *Bacillus* sp. AI 10 and *Bacillus* sp. CRCXL 2.2 were 4.79 g/L, 51.2% and 3.84 g/L, 34.7%, respectively. The Fourier transform infrared spectroscopy (FTIR) spectra of both strains' PHA structure showed that they can accumulate the homopolymer of PHB. From these results, it is possible to produce PHB by microorganisms from water hyacinth biomass, and participate in the circular bio-economic chain.

Keywords: Polyhydroxybutyrate (PHB), *Bacillus*, water hyacinth, lignocellulose hydrolysis, bioplastic.

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INTRODUCTION

Global demand for polymer plastics is constantly increasing which has doubled in volume since 2000. Depletion of non-renewable resources, the greenhouse effect, as well as lack of cyclic technology for treating non-biodegradable materials are threatening the global environment. Biodegradable biopolymers such as Polyhydroxyalkanoate (PHA) and its homopolymer, polyhydroxybutyrate (PHB), have the potential to replace petroleum-based plastics due to their advantage of plastic recirculation (McAdam et al., 2020).

Polyhydroxyalkanoates (PHAs) are a class of polyester-based plastic that can be derived from microorganisms. They are synthesized by a variety of prokaryotes in unbalanced nutritional conditions as carbon and energy storage materials (Steinbüchel, 2001; Verlinden et al., 2007). Their metabolism activities were controlled by microorganisms in which acetyl-CoA is re-directed towards PHA synthesis instead of following the tricarboxylic acid cycle (TCA cycle) (Ushani et al., 2020). Poly (3-hydroxybutyrate) (P3HB), the common PHA was first isolated in the 1920s (Belgacem & Gandini, 2008). It has many advantages over synthetic polymers used for packaging, namely (i) higher permeability than polyethylene (PE) and polypropylene (PP), (ii) stiffer and less flexible than PP, and (iii) PHB presents better barrier properties in comparison with polyethylene terephthalate (PET) and polyvinylchloride (PVC). P3HB can be found as a homopolymer or as a copolymer in microbial cells (Rao et al., 2010). PHB materials can be generated in intracellular granules by more than 75 bacterial genera, including Gram-negative and Gram-positive bacteria (Grigore et al., 2019; Sindhu et al., 2011). Some of the extensively studied PHB-producing strains are *Ralstonia eutropha*, *Alcaligenes* sp., *Azotobacter* sp., *Bacillus* sp., *Nocardia* sp., *Pseudomonas* sp., and *Rhizobium* sp. We can take advantage of byproduct sources from agriculture, forestry, and fishery as materials to produce biopolymer PHB. In Vietnam, some research groups have been studied in receiving PHB from cassava starch (Pham, 2010; Doan et al. 2020), fish fat (Doan et al., 2019; Doan & Vu, 2021; Nguyen et al., 2020; Nguyen, 2021), and rice straw hydrolysates (Doan et al., 2021). The benefits in PHA-producing processes combined with the biocompatibility and biodegradation of PHB make it a leading candidate to replace synthetic polymers such as PP and PE.

Water hyacinth (*Eichhornia crassipes*) is an aquatic plant, native to South America which was brought to Vietnam in the early 20^{th} century. They are used for making animal feed and composting. The hyacinth fibers can be braided into yarn to craft various items including mats and handicrafts. Moreover, they can treat environmental pollution due to their ability to absorb heavy metals. However, the water hyacinth overgrowth has occupied rivers and canals in many provinces, becoming a hazard to waterway transport safety and the aquaculture industry. To solve this problem multiple solutions have been done, mainly by skimming, burning, or turning them into agricultural products or handicrafts. Nevertheless, it is still a challenge to get rid of these completely (Phan Dinh Tuan, 2014). There is a novel, effective method to use it as a lignocellulosic biomass source for microbial fermentation to create high-value chemical building blocks in industrial production processes. With this research orientation, the pretreatment and hydrolysis of water hyacinth biomass were performed to obtain materials used in PHA production by *Bacillus* strains. Out of 28 PHA accumulating strains, two strains *Bacillus* sp. AI 10 and *Bacillus* sp. CRCXL 2.2 were selected which can utilize C5 and C6 sugars. After fermenting in water hyacinth hydrolysate at a laboratory scale, the dry biomass received from both strains was 4.79 g/L and 3.84 g/L, respectively. In this

condition, *Bacillus* sp. AI 10 strain accumulated 51.2% of PHA while *Bacillus* sp. CRCXL 2.2 strain was recorded at 34.7%. These results showed a potential to progress into a larger-scale and step by step approach to the development trend of a circular economy in our country.

MATERIALS AND METHODS

Materials

A collection of 28 bacterial strains was kept in the Lab of Biomaterial technology, Institute of Biotechnology, which were isolated from soybean-growing soil samples of Ha Noi and Cau Dien Waste-treatment Plant's mud samples.

Water hyacinth was collected in several suburban communes of Hanoi, containing 33% cellulose, 25% hemicellulose, 10% lignin, 14.3% organic extractives, 5.6% ash, and 12.1% other components.

Cellic®CTec2 cellulase and Cellic®HTec2 xylanase were obtained from Novozyme (Denmark). The average cellulase activity was determined to be 162.3 FPU/mL and the total protein following the Bradford assay was 134.1 mg/L. Xylanase activity and Cellic®HTec2 protein content were measured as 1004 U/ mL and 151.6 mg/L, respectively.

Methods

Bacterial culture: The glycerol-stored strains were activated in liquid LB medium [g/L]: Bacto Tryptone 10, Yeast extract 5, NaCl 5, pH 7.2. These were inoculated overnight at 37 ^{o}C, 200 rpm. The inoculum was streaked on the LB agar surface to obtain the pure colonies.

Fermentation of PHA-producing bacteria: A loop of the pure culture was inoculated into a tube containing 5 mL LB medium and shaken at 200 rpm, 37 ^{o}C for 16−18 hours. Precultures were then transferred into the 500 mL conical flask containing 95 mL mineral medium, which composed of [g/L] sugar 30, Peptone 5, Yeast extract 1 $Na_2MoO_4.2H_2O$ 0.0005; $MnSO_4$ 0.0005, $FeSO_4.7H_2O$ 0.01, $CaCl_2.2H_2O$ 0.02, NaCl 0.1, $MgSO_4.7H_2O$ 0.2, $(NH_4)_2SO_4$ 1, $KH_2PO_4.2H_2O$ 0.2, $K_2HPO_4.3H_2O$ 1.6. The fermentation was carried out at 37 ^{o}C, 200 rpm in 48 hours.

The effect of different sugar sources on growth and PHA production of all strains were investigated, including glucose, xylose, arabinose, galactose, and mannose at a concentration of 30 g/L.

Pretreatment of water hyacinth was performed according to Doan et al. (2021). Freshwater hyacinth with long stem was thoroughly washed with tap water to remove adhering dirt, chopped into small pieces (~ 4−5 cm), dried in a hot air oven at 100 ^{o}C for 5−6 hours. Dried materials were powdered and stored at room temperature until used. The pretreatment process was carried out in Regmed Autoclave AU/ E 20 (Sweden), which consisted of four 2L-reactors. Water hyacinth powder was soaked with $Ca(OH)_2$ (12 g/L) solution at a ratio of 1:10 (w/v) before loading into the reactors. The cooking program was set at 110 ^{o}C for 1 hour. The reactors were cooled rapidly and the pretreated water hyacinth was filtered, washed several times by tap water to neutral pH. The pretreated water hyacinth was crushed by a mill to separate the microfiber bundles, facilitating the hydrolysis process subsequently. These were dried at 40±5 ^{o}C in an air-circulating oven to about 50% humidity.

Water hyacinth hydrolysis: A total of 5 g of pretreated water hyacinth was immersed in the desired volume of 50 mM sodium citrate buffer, pH 4.8 in the 1L-conical flask. The combination of Cellic®CTec2 cellulase and Cellic®HTec2 xylanase was used. Cellic®CTec2 cellulase was loaded from 0-40 FPU/g pretreated biomass, combined with an excessive Cellic®HTec2 xylanase concentration of 50 FXU/g pretreated biomass. In the next experiment, Cellic®HTec2 xylanase was loaded with 0, 25, 50, and 75 FXU/g pretreated biomass,

mixed with optimal Cellic®CTec2 cellulase loading. The 50 mM sodium citrate buffer was added to reach a total volume of 150 mL. The conical flask was shaken at 55 ^{o}C, 100 rpm for 72 hours. At the end of the hydrolysis process, the liquid was centrifugated at 8,000 rpm for 15 minutes before diluting to determine the reducing sugars according to the dinitrosalicylic acid (DNS) method (Miller, 1959). A 30 g/L reducing sugar of water hyacinth hydrolysis was used as a carbon source for PHA fermentation as described above.

PHA recovery: After fermentation, the cells were obtained by centrifuging at 6000 rpm for 10 minutes. The pellets were washed with 0.89% NaCl solution and dried at 60 ^{o}C to extract PHA.

Observation of intracellular PHA using Nile blue A staining has followed the method of Ostle & Holt (1982). The PHA granules were observed using Nikon confocal C1plus microscope (Tokyo, Japan) with an excitation filter of 460 nm.

PHA extraction: After drying at 60 ^{o}C, the pellets were dissolved in 15 mL of deionized water, heated by an oven at 140 W for 15 minutes, and then microwaved in 10-second intervals. Two milliliters of the ethanol: acetone mixture at a ratio of 1:1 (v/v) was added and incubated for 2 hours. This mixture was transferred into a screw-capped test tube before adding 5 mL of chloroform and shaking for 5–6 hours. The chloroform phase was pipetted to a new tube and mixed with 20 mL of cold methanol. The tube was kept at -20 ^{o}C for 30 minutes. The PHA residue was collected by centrifugation at 12.000 rpm for 20 min and rinsed with 70% ethanol. The residue was dried at 50 ^{o}C and stored for further studies.

PHA quantification by the crotonic acid method: PHA amount was quantified following the method of Law & Slepecky (1961). The principle of the method is based on the conversion of PHA into crotonic acid by sulfuric treatment, which has UV-Vis absorbance at 235 m.

PHA structural analysis: FTIR spectroscopy was conducted to analyze the functional group of PHA. IR spectra were recorded using a single beam spectrometer (Shimazu IRAffinity-1S, Kyoto, Japan) within the scanning range 4,000–400 cm^{1}.

RESULTS

Screening of PHA producing bacteria used C5 and C6 sugars as carbon sources

Twenty-eight strains isolated from soybean-growing soil samples and the Cau Dien Waste-treatment Plant's mud samples are all Gram-positive bacteria, that produced endospores and catalases. According to Bergey's Manual of Systematic Bacteriology, they were classified into the *Bacillus* genus (Bergey et al., 2009). Screening of their ability to accumulate PHA was performed by Nile blue A staining and observed under confocal microscopy (data not shown). Towards the goal of using water hyacinth as the carbon source to produce PHA, major sugars of biomass hydrolysate, including two C6 sugars (glucose, galactose) and three C5 sugars (xylose, arabinose, mannose), were selected as substrates for fermentation by the isolated strains. Glucose and galactose were the substrates on which the highest dry cell weight (DCW) (3.32–4.01 g/L), and maximum PHA production (42.7–60.1% (w/w)) were obtained (Table 1). Nearly all of the tested strains could not utilize C5 sugar, except for the isolates AI 10 and NRCXL 2.2. The isolate AI 10's dry cell weight was yielded at 2.87, 2.54, and 2.66 g/L after consuming xylose, arabinose, and mannose, with 50.2, 48.5, and 49.8% (w/w) of intracellular PHA accumulation, respectively. In the case of the isolate NRCXL 2.2, the total dry cell weight reached 3.03 and 2.71 g/L when xylose and arabinose were used as carbon sources. The PHA production achieved was 34.7 and 28.2%, respectively. The isolates AI 10 and NRCXL 2.2 were chosen for further investigation.

Table 1. Growth and PHA production of the *Bacillus* sp. strains on various sugar sources (30 g/L)

Strain	Glucose		Galactose		Xylose		Arabinose		Mannose	
	DCW (g/L)	PHA (%)	DCW (g/L)	PHA (%)	DCW (g/L)	PHA (%)	DCW (g/L)	PHA (%)	DCW (g/L)	PHA (%)
BTNĐV 4.2.1	3.64	43.4	3.32	42.9	-	-	-	-	-	-
BTNĐV 3.4	3.81	44.7	3.74	42.7	-	-	-	-	-	-
BTNĐV 4.6	4.01	48.2	3.87	46.4	-	-	-	-	-	-
BKCCXL 6.5	3.44	44.8	3.46	43.0	-	-	-	-	-	-
BKCCXL 6.6	3.62	53.7	3.16	52.8	-	-	-	-	-	-
BKCCXL 6.8.3	3.56	57.3	3.68	55.7	-	-	-	-	-	-
MA 2.10	3.33	43.8	3.47	43.4	-	-	-	-	-	-
BC 7 20	3.77	58.6	3.51	57.6	-	-	-	-	-	-
MA 2.2	3.74	48.4	3.78	44.8	-	-	-	-	-	-
MA 2.8	3.53	46.5	3.50	42.8	-	-	-	-	-	-
MA 2.1	3.97	60.1	3.89	52.6	-	-	-	-	-	-
MA 2.5	3.82	57.2	3.97	54.3	-	-	-	-	-	-
NRCXL 2.2	3.75	45.7	3.63	44.3	3.03	34.7	2.71	28.2	-	-
NRCXL 2.4	3.44	44.7	3.39	43.5	-	-	-	-	-	-
NRCXL 2.6	3.32	46.4	4.01	59.2	-	-	-	-	-	-
CPFC QN 2.2	3.52	48.8	3.54	48.2	-	-	-	-	-	-
BTNĐV 4.2.3	3.99	54.3	3.74	52.4	-	-	-	-	-	-
BC 7.14	3.36	42.9	3.33	43.1	-	-	-	-	-	-
M4 HD4	3.40	52.8	3.46	50.4	-	-	-	-	-	-
M4 HD3	3.32	42.7	3.34	42.9	-	-	-	-	-	-
M 1.6	3.69	55.6	3.52	51.5	-	-	-	-	-	-
M4.HD5	3.73	54.3	3.68	50.7	-	-	-	-	-	-
M4.HD8	4.00	57.4	3.83	55.8	-	-	-	-	-	-
AI 5.1	3.56	47.1	3.48	46.3	-	-	-	-	-	-
AI 9	3.66	50.2	3.54	49.6	-	-	-	-	-	-
AI 10	3.85	54.7	3.87	52.1	2.87	50.2	2.54	48.5	2.66	49.8
AI 13	3.88	52.5	3.87	51.7	-	-	-	-	-	-
VKSKT5	3.43	54.9	3.35	52.8	-	-	-	-	-	-

Water hyacinth hydrolysis

At the first set, Cellic®CTec2 cellulase loading of various concentrations was mixed with an excessive dose of 50 FXU/g Cellic®HTec2 xylanase. The highest amount of reducing sugars was 409 mg/g of pretreated biomass when using 20 FPU/g of Cellic®CTec2 cellulase (Fig. 1).

The concentration of Cellic®HTec2 xylanase was altered at 0, 25, 50, and 75 FXU/g, combined with the optimal dose of Cellic®CTec2 cellulase (20 FPU/g) to find the best enzyme cocktail for water hyacinth hydrolysis. The total reducing sugars at the combination between 20 FPU/g Cellic®CTec2 cellulases and 25 FXU/g Cellic®HTec2 xylanase was 409.5 mg/g of pretreated biomass (Fig. 2). The reducing sugars at higher concentrations of Cellic® HTec2 xylanase were not significantly higher than that at 25 FXU/g, while the hydrolysis cost was much more expensive.

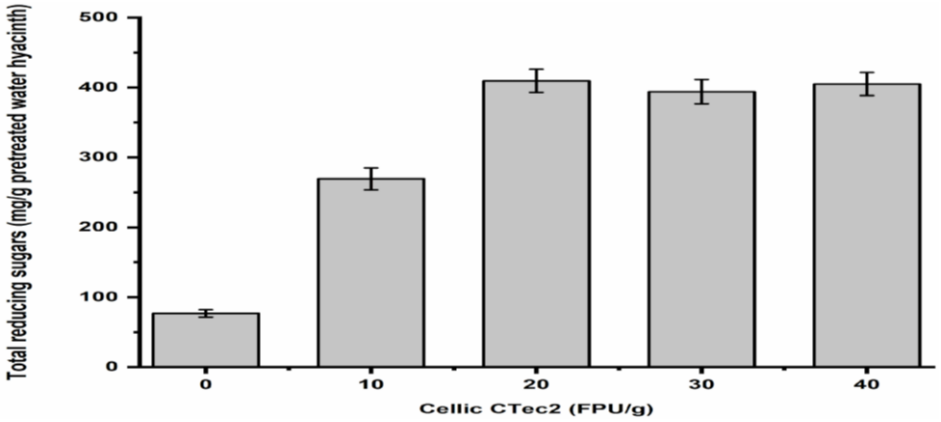

Figure 1. Total reducing sugars of water hyacinth using a combination of Cellic®CTec2 cellulase and Cellic®HTec2 xylanase. Cellic®HTec2 xylanase was fixed at 50 FXU/g, whereas Cellic®CTec2 cellulase concentration varied from 0-40 FPU/g. The hydrolysis process was carried out at 55 ^{o}C, pH 4.8 for 72 hours

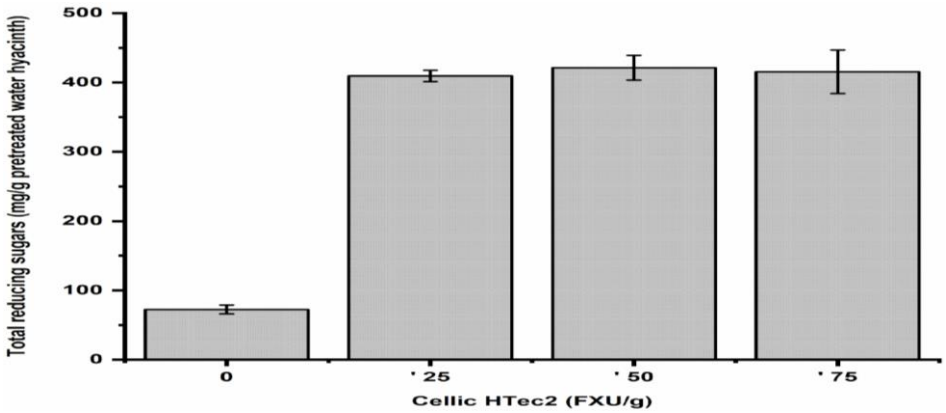

Figure 2. Total reducing sugars of water hyacinth using a combination of Cellic®CTec2 cellulase and Cellic®HTec2 xylanase. Cellic®CTec2 cellulase was fixed at 20 FPU/g, whereas Cellic®HTec2 xylanase concentration varied from 0-75 FXU/g. The hydrolysis process was carried out at 55 ^{o}C, pH 4.8 for 72 hours

PHA production using water hyacinth as substrate

Table 2. Growth and PHA production of the *Bacillus* sp. AI 10 and the *Bacillus* sp. NRCXL 2.2 obtained from water hyacinth fermentation at 200 rpm, 37 ^{o}C for 48 hours

Strain	DCW (g/L)	PHA (%)
AI 10	4.79	51.2
NCRXL 2.2	3.84	34.7

Two isolates AI 10 and NRCXL 2.2 were used for fermentation with water hyacinth hydrolysate as a carbon source at a final concentration of 3%. After 48 hours, harvested cells were stained with Nile blue A, showing a large orange area in the cells was observed with a strong signal by confocal microscopy at 460 nm (Fig. 3). The stained cells fluoresced bright orange indicated the accumulation of intracellular PHA granules (Kitamura & Doi, 1994). Moreover, dry cell weight was calculated at 4.79 g/L and 3.84 g/L, corresponding to PHA accumulation of 51.2% and 34.7%, respectively.

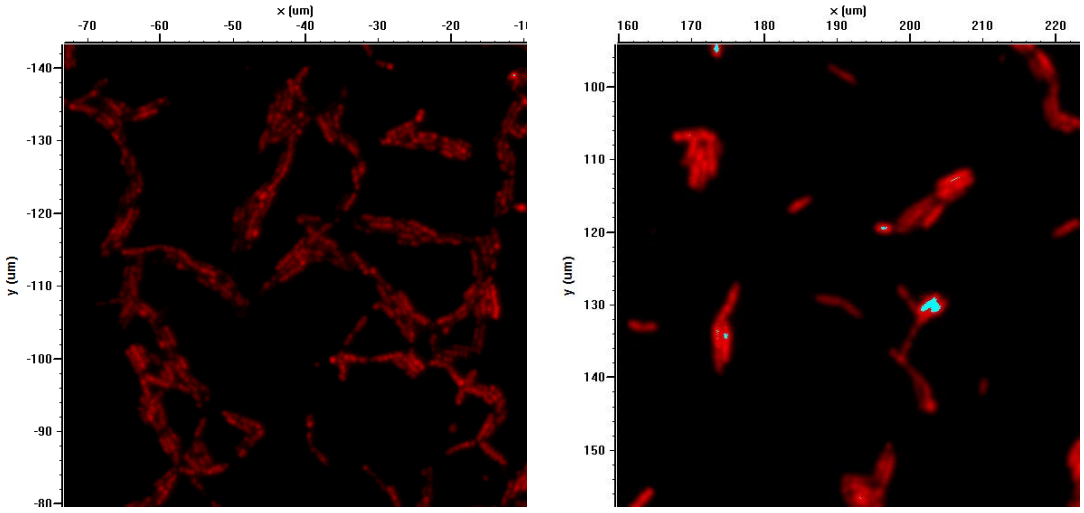

Figure 3. Confocal microscopy of cells of the *Bacillus* sp. AI 10 (left) and *Bacillus* sp. NRCXL 2.2 (right) at 460 nm

Analysis of functional groups of PHA produced by water hyacinth fermentation

The chemical structures of PHA polymers synthesized by the isolates AI 10 and NRCXL 2.2 using water hyacinth hydrolysate as a carbon source were evaluated using FTIR spectroscopy. FTIR spectra showed a sharp absorption band at 1719 cm^{-1} which corresponds to the ester carbonyl group. Other absorption bands at 2922, and 2851 cm^{-1} revealed the presence of an alkyl-CH_3 group. Moreover, four intense absorption bands at 1181, 1379, 1455, and 1272 cm^{-1} correspond to R-CO-O-C_2H_5 stretch, -CH_3, -CH_2, and -CH groups, respectively. As compared to the PHB standard, the most specific absorption bands of PHAs obtained from the isolates AI 10 and NRCXL 2.2 were similar to those of the PHB standard (Fig. 4), thus both *Bacillus* sp. strains produce poly(3-hydroxybutyrate) PHB from water hyacinth hydrolysate.

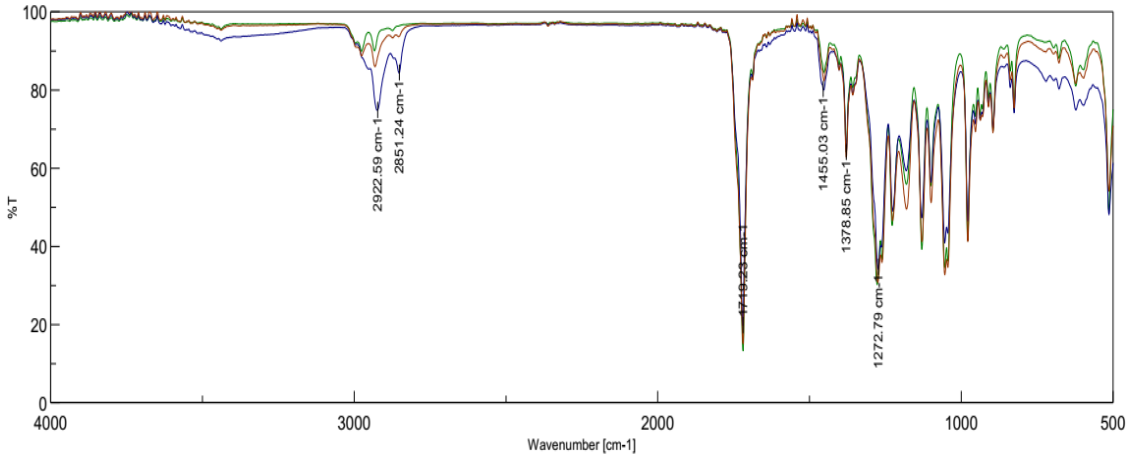

Figure 4. FTIR spectra of PHA produced by *Bacillus* sp. AI 10 (green line), *Bacillus* sp. NRCXL 2.2 (orange line) using water hyacinth hydrolysate as substrate, and standard PHB (Sigma Aldrich) (blue line), respectively. The x-axis represents the infrared spectrum, which plots the intensity of mid-range infrared spectra between 4,000 to 400 cm^{-1}

DISCUSSION

Water hyacinth (*Eichhornia crassipes*), the aquatic plant used to cause a menace to tropical and subtropical freshwater habitats due to its faster growth rate than any other plant hinders river transport. However, with 55–57% carbohydrate in dry biomass, water hyacinth is a high-potential biomass resource for processes of biofuels, animal feeds, and fertilizer from their manure (Nigam, 2002). To date, it has mainly been reported as a material source for bioethanol production. The pre-treatment of water hyacinth can be performed in many ways, each has its advantage and disadvantage. The dilute sulfuric acid (H_2SO_4) pretreatment often brings high efficiency which is premises for the enzymatic hydrolysis process to produce the highest final yields of sugar. Nevertheless, pre-treatment using diluted acid solutions at high concentration, temperature, and pressure can create toxic byproducts such as furfural, hydroxymethylfurfural, acetic acid, formic acid, and levulinic acid (Ganguly et al., 2012). These toxicants affect microbial metabolism in fermentation. Moreover, they cause DNA degradation that inhibits RNA and protein synthesis (Modig et al., 2002). Alkali pretreatments were noticed because of cheaper cost and energy savings. The sodium hydroxide (NaOH) pretreatment makes the internal surface of lignocellulose swell, leading to a decrease in the degree of polymerization, cellulose crystallinity as well as breaking down the lignin structure. Though, the amount of reducing sugars obtained after hydrolysis by NaOH was less than one by H_2SO_4. Among chemical pretreatment methods, $Ca(OH)_2$ pretreatment has some competitive advantages, namely, low-cost, reducing cellulose/hemicellulose degradation, not forming inhibitors in downstream processes, and can perform at the low-temperature condition (~ 100 ^oC). Furthermore, $Ca(OH)_2$ is a cheap chemical, easy to store and handle, less dangerous than others, yet its solubility decreases in hot water (Perry, 2011; Bajpai, 2016; Doan et al., 2021). The biggest of this method is the required amount of enzyme for later hydrolysis in comparison with others, therefore it can affect more or less to total cost of the biomass conversion process. In this study, we pretreated water hyacinth with $Ca(OH)_2$ (12 g/L) at 110 ^oC for 60 minutes before hydrolyzing with the mixture of Cellic®CTec2 cellulase and Cellic®HTec2 xylanase. The highest amount of reducing sugars was recorded at 409,5 mg/g which is equivalent to the result obtained from switchgrass pretreatment by $Ca(OH)_2$ (10 g/L) and hydrolysis by 2 enzyme cocktails NS 50013 + NS 50010 and Cellic CTec + Cellic HTec (433.0 mg/g and 412.3 mg/g, respectively) (Xu et al., 2011).

Thus far, there are quite a few publications on using water hyacinth hydrolysate to synthesize PHA, but bioethanol fermentation mainly. Manivannan & Narendhirakannan (2015) hydrolyzed water hyacinth biomass using *Trichoderma reesei* NRRL-3652 strain which can synthesis both cellulase and xylanase. After that, the hydrolysate was used as a substrate source for ethanol fermentation by *Saccharomyces cerevisiae* MTCC 173 and *Zymomonas mobilis* MTCC 2428 with the rates of 0.021–0.043 g/g. (Das et al., 2016). The *Pichia stipitis* NRRL Y-7124 utilized water hyacinth hydrolysate into ethanol at the rate of 0.35 g/g (Nigam, 2002). The reason for using yeast strains in bioethanol production is their ability to convert C5 and C6 sugar mixture while not many microorganisms can utilize C5 (Zhang et al., 2015). However, some of the *Bacillus* strains such as *Bacillus anthracis*, *Bacillus cereus*, *Bacillus megaterium*, *Bacillus firmus* were reported to be able to produce PHA from rice straw hydrolysate. The biomass yielded after hydrolysate fermenting *Bacillus megaterium* B-10 and *B. firmus* NII0830 was 4.67 g/L and 1.90 g/L with a PHA accumulated rate of 32.0% and 89% (Li et al., 2021; Sindhu et al., 2013). Recently, *B. cereus* VK92 and VK98 strains isolated from rice straw showed their capacity to synthesize PHA using rice straw hydrolysate as substrate sources. Rice straw was pretreated by 20% NH_3 at 80 ^oC for

10 hours before hydrolyzing by enzyme cocktail Celluclast 1.5 L, Novozyme 188, and Pentopan Mono BG. The received biomass yields were quite high, 5 and 5.42 (g/L), with PHA accumulation of 59.3% and 46,4% (Doan et al., 2021). In our study, two strains *Bacillus* sp AI 10 and *Bacillus* sp NRCXL 2.2 were fermented with water hyacinth hydrolysate and yielded 3.79 g/L and 2.84 g/L biomass. Their PHA accumulation was quite good, reached to 51.2% and 34.7%. Although these results are just the first step in using water hyacinth as a material source for bioplastic production, they can be the premise to conduct further studies to optimize the pretreatment, hydrolysis process as well as fermentation strategies to achieve the highest efficient PHA accumulation by bacterial strains isolated in Vietnam using the mixture of C5 and C6 sugars.

CONCLUSION

From the collection of 28 *Bacillus* strains, AI 10 and NRCXL 2.2 were screened that can synthesize PHAs from C5 sugars. Meanwhile, the total sugar of water hyacinth was pretreated by $Ca(OH)_2$ as well as hydrolyzed by the mixture of Cellic®CTec2 cellulase and Cellic®HTec2 xylanase 409.5 mg/g. This hydrolysate was used as a carbon source for the production of PHA by AI 10 and NRCXL 2.2 strains. Dry biomasses obtained from 2 strains were 4.79 g/L and 3.84 g/L with the PHA accumulation rates at 51.2% and 34.7%, respectively. The PHA accumulated from both two trains is PHB which is the most common biopolymer used in industry.

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