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Phosphorus treatment in wastewater by microorganisms isolated from cassava starch production waste

Nghiên cứu khả năng xử lý photpho trong nước thải của vi sinh vật phân lập từ nguồn thải của quá trình sản xuất tinh bột sắn

Research article

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In waste water, phosphorous (P) can exist in inorganic or organic forms. Depending on the concentration, P can cause eutrophication and severe environmental pollution. Microorganisms have the ability to use and accumulate P, so microorganisms are studied to treat P in waste water in general and wastewater from cassava starch processing plants in particular. Research results show that in the 20 samples of waste water and sludge of the plant has selected three strains of bacteria that can accumulate P in the form of granules in the cell. Among them, SHV22 has the highest P accumulation capacity, reaching $3.05x10^{-11}$ mg/cell, P removal efficiency in wastewater from cassava starch processing factory is 82.1%. The strain was identified as *Bacillus amyloliquefaciens*.

Trong nước thải P có thể tồn tại dưới dạng vô cơ hoặc hữu cơ. Tùy thuộc vào nồng độ, P có thể gây phú dưỡng và ô nhiễm môi trường nghiêm trọng. Vi sinh vật có khả năng sử dụng và tích lũy P, do đó vi sinh vật là đối tượng được nghiên cứu để xử lý P trong nước thải nói chung và nước thải của nhà máy chế biến tinh bột sắn nói riêng. Kết quả nghiên cứu cho thấy, trong 20 mẫu nước và bùn thải của nhà máy đã chọn lựa được 3 chủng vi khuẩn có khả năng tích lũy P dưới dạng hạt trong tế bào. Trong số đó, chủng SHV22 có khả năng tích lũy P cao nhất, đạt tới $3,05x10^{-11}$ mg/tế bào, hiệu quả loại bỏ P trong nước thải của nhà máy chế biến tinh bột sắn đạt 82,1%. Chủng đã được định danh là Bacillus amyloliquefaciens.

Keywords: cassava starch processing plant, microorganism, phosphorous treatment, waste water

1. Introduction

Phosphorus is a vital nutrient for life, present in all life-related activities and in many industries including industry of cassava starch processing. Depending on the concentration of phosphorus in the waste water of cassava starch processing factory, eutrophication may occur.

In the wastewater, phosphorus is present in the form of orthophosphate (PO_4^{-3}), inorganic poly-P or organic phosphorus. Sometimes it is also present in phosphorus granules. Small amounts of phosphorus exist in organic form; most are mineralized into inorganic (PO_4^{3-}) form (Roussos S. et al., 2003). Phosphorus compounds are considered as nutrients in wastewater and may be a serious pollutant for the environment.

Microorganisms can use phosphorus and store it as Poly-P granules in some bacteria (Fuhs and Chen, 1975). Poly-P particles in microbial cells are known as "colored seeds" or "volutinated particles," and they have a high affinity for dyes, which makes it possible to distinguish the presence of "volutinous" particles when dyeing Neisser (Gurr E., 1965).

Research on microorganisms capable of accumulating orthophosphate (PO_4^{3-}) in the treatment of phosphorus sources in waste water contributes to reduce environmen-

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tal pollution and has received the attention of scientists in the world.

2. Materials and methods

2.1. Materials

Waste water and sludge samples of cassava starch production were collected in Ha Noi (8 samples), Ninh Binh (6 samples) and Dak Lak (6 samples).

2.2. Research methods

2.2.1. Isolation of microorganism with ability of PO_4^{3-} accumulation

10g of sludge or 90ml of wastewater + 10ml of KH_2PO_4 0.1%, were mixed in a 250ml conical flask. Then the mixture was shaken 150 r/m at 25°C for 2 days.

Took 30ml of this mixture into 250ml bottle containing 75ml sterile broth and continued to shake for 5 more days. Diluted the sample successively from 10^{-1} to 10^{-9}.

Applied 0.1ml of sample at each concentration on Casitone Glycerol Yeast extract Agar (CGYA) (Yeas-extract 3g/l, 5 mg/l casein, 1 mg/l $MgSO_4.7H_2O$, agar 15g/l) and cultured at 25°C for 5 days.

Single colonies were selected and cultured on CGYA medium until pure colonies were obtained (Bosch and Cloete, 1993).

2.2.2. Determining microbial activity of PO_4^{3-} accumulation

+ Determination of volutin (poly-P) in microbial cells (Szabó et al., 2011).

- Principle: Based on the coloration of volutin (poly-P particles) with Neisser reagent forming the black-green particles in the microbial cells.

The colonies cultured in 100 ml of waste water from starch processing plant, centrifuged 3500r/m for 20 minutes to remove the precipitate. The filtrate was filtered through a 2µm filter, took 90 ml centrifuged filter and added 10ml KH_2PO_4 0.1%, then sterilized at 121°C for 20 minutes) on a 150r/m shaker for 5 days at 25°C. Reagents Neisser was used to observe the capable of capturing color of volutin under on optical microscope (county poly-P).

+ Determining the phosphorus concentration in the cells of microorganisms (Bosch and Cloete, 1993)

The colonies were cultured in 10ml pure environment at 25°C for 24 hours. Took 4 ml of culture fluid into a cone containing 96 ml of waste water (initial P concentration determined). The container was covered and anaerobically incubated at 25°C for 2 hours, then placed under aerobic condition for 5 hours by placing on a 150 r/m shaker at 25°C, pH 7.5. The culture media was filtered through a 0.22 µm filter to remove the cell, determining the P concentration by spectrophotometer at 660 nm.

P uptake in the cell (mg/l) = [P in original environment (mg/l) - P culture fluid VSV (mg/l)] / [density of cells in culture fluid (cells/ml) x1000].

2.2.3. Determination of cell density by counting the number of colonies growing on agar (Nguyen Lan Dung, 1983).

2.2.4. Evaluating the ability of isolated microorganisms to phosphorus compounds treatment in wastewater from cassava starch processing plants

The culture media was added to a triangular flask containing 100 ml of cassava starch wastewater, so that the cell density was about 10^5CFU/ml. Control formula was cassava starch waste water without added microorganism. The experiment was conducted at room temperature, on a shaker at a speed of 150 rpm. After 3-5 days, determined the total phosphorus (Pts) in waste water in experimental and control formulas.

Total P-treatment effect was compared with control = index of Total P reduced in experimental formula/index of total P in control formula x 100 (%).

2.2.5. Classification of microorganisms with ability to accumulate PO_4^{3-}

- Based on the morphological, physiological and biochemical classification of Bergey key (Stanley T. et al, 1989; Holt, J.G. et al, 2000; Holt, J.G. et al, 1994).

- Based on 16S rDNA gene sequencing. Determination of 16S rDNA sequences of microorganisms according to the method of Sakiyama et al., 2009.

3. Results

3.1. Isolation of microorganisms with ability of PO_4^{3-} accumulation

From 20 collected samples (10 samples of wastewater, 10 samples of sludge), 3 bacterial strains capable of phosphorus accumulation (PO_4^{3-}-) in the form of Poly-P (volutin) particles in the cell were isolated.

Table 1. Biological activity of microorganisms accumulating PO_4^{3-}

Symbol of microorganism strains	Neisser reagent reaction	Creation of volutinous particles	Absorbed in cells PO_4^{3-} (mg/cell)
SHV.18	Black green	+	1.31×10^{-11}
SHV.19	Black green	+	2.16×10^{-11}
SHV.22	Black green	+	3.05×10^{-11}

Note: (+) Positive reaction

Under the optical microscope at 1000x magnification, the isolates with volutinal particles (blackish-green grains on Neisser staining) were detected.

Analysis of PO_4^{3-} concentration of cultured isolates in wastewater environment after 5 days found that SHV.22 strain had the highest ability to absorb phosphorus. The PO_4^{3-} concentration in the cell reached $3.05x10^{-11}$mg/cell.

3.2. Microorganisms ability to treat phosphorus

Evaluation of phosphorus treatment ability in cassava starch wastewater of selective microorganisms were conducted by analysing total P in waste water and by adding selected microorganisms to wastewater (table 2), indicating that SHV.22 has the highest P total conversion potential (Pts efficiency of 82.1%).

Table 2. Total P-treatment capacity in cassava starch wastewater of microorganisms

No	Formula	Total P content (mg/l)	
		After treatment	Treatment efficiency compared to control (%)
1	Control (without microorganism)	16.1±0.5	-
2	SHV.18	6.2±0.08	61.7
3	SHV.19	7.6±0.3	52.6
4	SHV.22	2.9±0.2	82.1

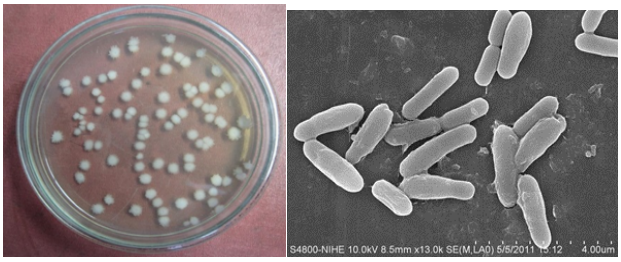
3.3. Microorganism identification

SHV.22 was selected and determined to species. The results showed that SHV.22 was Gram-positive bacteria, aerobic or anaerobic. On culture medium after 2 days of incubation, they formed rounded colonies with irregular edges, creamy, slightly wrinkled surface with a diameter of 0.4-1 mm. (Figure 1a).

On liquid culture medium, SHV.22 grew after 2 days and turned medium to yellow, creating a light brown scab on the wall. In static culture conditions, after 2-3 days of growth, strains SHV.22 made media opaque, made scum on the surface of the media. Observation under the Fe-SEM electrodeposition microscope (S-4800) at the National

Institute of Hygiene and Epidemiology revealed that SHV.22 is bacterial rod-shaped cells, oval spores, cell size 0.5x1.5-3.5µm (Figure 1b).

Along with assessing morphological characteristics of cells, the ability to assimilate the carbohydrates sources and some of the reactions necessary to determine the physiology, biochemistry of microorganisms also was conducted. Using the biochemical system of API 50 CHB (Bio Merieu, France), the results are summarized in Table 3.



(a) (b)

Figure 1. Colony and cell shape of bacteria SHV.22

Table 3. Ability to use hydrocarbon sources of bacteria SHV.22

Type of sugar	SHV.22	Type of sugar	SHV.22
Arabinose	+	Ramnose	+
D-glucose	+	Fructose	+
Lactose	+	Xylose	+
Mannose	+	Sucrose	+
Melibiose	-	Mannitol	+
Metyl α-D glycoside	+	Citrate	+
D-ribose	+	Starch hydrolysis	+
D-Turanose	-	Casein hydrolysis	+
Arginine dihydrolase	-	Gelatin hydrolysis	+
D-Raffinose	+	Denitrification	+

Note: (+) positive reaction; (-) negative reaction

SHV.22 bacteria used a variety of hydrocarbon sources and was capable of assimilating with most of the sugars characteristic for the classification of bacteria (glucose, lactose, mannitol, fructose, sucrose...) and it also had the ability to assimilate gelatine, starch hydrolysis, casein and to denitrate.

To identify the species names of the strains, the study used the molecular biological identification technique by identifying the 16S rDNA sequence of the SHV.22 strain The sequence of the SHV.22 strain had a nucleotide sequence of 1546 bp. NCBI-BLAST search results showed the highest sequence similarity with *Bacillus amyloliquefaciens MBE1283 (100%).*

Based on the results of identification by traditional methods (morphological, physiological and biochemical characteristics) and combined with identification results by molecular biology (based on the results of sequencing by nucleotide and genealogy) to confirm that SHV.22 strain is *Bacillus amyloliquefaciens.*

According to the World Health Organization (WHO) in 2004 and European Union Biosafety Directive 90/679/EEC of 26 November 1990, *Bacillus amyloliquefaciens* SHV.22 belongs to safety microorganisms in Highly bio-level (level 1), not causing adverse impacts on human health, animal and plant life and the environment, meeting the requirements of research into the production of microorganisms for agricultural production.

4. Conclusion

SHV.22 strain was isolated and identified as *Bacillus amyloliquefaciens*. SHV.22 with ability of orthophosphate (PO_4^{3-}) accumulation of in the cell reached $3.05x10^{-11}$ mg/cell. Total P removal efficiency after cassava starch processing was 82.1%.

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