

EXTRACTION AND CHARACTERIZATION OF STARCH FROM MICROALGAE AND COMPARISON WITH COMMERCIAL CORN STARCH

R N Ramli.¹,*C K Lee¹, M A Kassim¹

¹Bioprocess Technology Division, School of Industrial Technology,
Universiti Sains Malaysia, 11800 USM, Pulau Pinang, Malaysia.

*Email: rozinuraika@gmail.com ; cklee@usm.my

Abstract. Starch is the main component that consumed one third in the human diet. One-third of starch produced in the world is used in non-food application such as rubbers production and plastic. Plastic produced from starch which called as bio-plastic is biodegradable and helps in reducing the world pollution which non-degradable plastic now endanger the ecosystem either land or water. Microalgal the easy growing and environmental friendly have opened new potential of revolution for starch-based bio-plastic. The small granules of starch produced from microalgae possess an advantage to replace the starch from food-based, as the small granules able to yield a good quality of bio-plastic. In this study, marine microalgae had been studied for its starch content and could reach up to 19% of total starch content. However, in order to ensure the starch produced from marine microalgae is suitable for bio-plastic application, the characteristics of starch had been studied based on the size of starch granules, amylose/amylopectine content, swelling power and solubility and turbidity. These analyses were done with a comparison of corn starch that used widely in the production of bio-plastic. Hence, by determining these crucial characteristics, the application of starch from microalgae could be expended to various sustainable products.

1 Introduction

Starch is a natural carbohydrate storage polymer accumulated in plants. It is a completely biodegradable polysaccharide and one of the most abundant renewable resources. It has been considered as an excellent candidate to partially substitute synthetic polymer in packaging and other low-cost applications due to its abundance, biodegradability and low cost [1]. The molecular structure that consist of two D-glucose polymers: amylose and amylopectin. Amylose is a linear structure with α -1,4-linked glucan, and amylopectin is branched molecule characterized by α -1,4-linked and α -1,6 branching links [2].

The usage of starch in daily life is well-known either in food or non-food product. The sources of starch are from crops as potato, corn, and tapioca. Gifuni *et al.* [3] wrote in the study, two third of the starch used in food and one third in non-food.



The awareness on sustainable issue had hit the market as now starch crops had become the feedstock for the green product such as bioethanol and bioplastic. United State (U.S) on 2009 is the top producer for bioethanol production using corn as feedstock as constitute 50.7% from all world market while Brazil as the second producer, 40.2% production in market by using sugar cane as main sources [4]. These two sources are good in abundance and renewable source. However, these sources main is food sources which will lead to crisis in increasing of food price and also will create an issue between food sources and non-food production. In 2005, U.S was able to produce only 18.9 billion L of bioethanol from 20% of corn land in U.S. This production was counted only 1% from total fuel consumed in U.S per year [4]. Hence, in order to increase more the yield, demanding on these crops will increase as per the usage of land will be wider and will lead to deforestation. The process to produce these crops will never be sustainable even the product yield is green renewable product.

The chances of different starch properties will also affect due to the physical factors as the weather changes and uncontrollable environment conditions. The component inside the plant plays big role in product formation. Starch characteristics are determined by the granule size, amylase/amylopectin ratio, turbidity and solubility and swelling power [3].

These issues had lead to propose to use microalgae as the excellent candidates for starch accumulation and production. The advantages of being fast growing, easily grow and the capability absorbing more carbon dioxide and greenhouse emitting for photosynthesis [5]. The other prominent features of microalgae is it has simple growth requirement, can be easily grown in various aquatic environments such as fresh water, saline water or municipal waste water and also in controlled condition which will lead to consistency of cell produce without affecting from the outer environment condition [6,7]. Starch contents in microalgae species will be based on cultivation conditions and cultivation time. Generally, total carbohydrate content in microalgae is about 20% dry weight (DW) and starch content is about 10% DW [8]. The low-cost yet high yield from microalgae had reported by Brányiková *et al.* [9] by using freshwater alga *Chlorella vulgaris* which able to produce almost 60% of starch content since it accumulates large amounts of carbohydrates through photosynthesis and stores as starch in cells.

This study was to compare the characteristic of starch extracted from microalgae with corn starch in order to compare the ability of starch microalgae could be substituent to the starch from crop. The corn starch used in this study was commercial fully purified corn starch.

2 Methodology

2.1 Microorganism

Klebsormidium flaccidum was isolated from sea in Penang Island, Northern of Malaysia which further isolated in Bioprocess Division laboratory cell culture room. The cell was isolated using method of single-cell isolation by micropipette and dilution technique [10]. The medium used was Walne's medium (g/L) at 35 ppt: {Solution A: $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 1.5; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.4; H_3BO_3 , 33.6; $\text{Na}_2\text{-EDTA}$, 45; $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 20; NaNO_3 , 100, Solution B; 1 mL [in 100 mL: ZnCl_2 , 2.1g, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 2.0g, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 0.9g, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 2.0g, Concentrated HCl, 10mL], The strain was maintained in Walne's media at 35 ppt in room temperature (26°C-30°C). Solution C: [in 200 mL, Thiamine HCl (vitamin B_1), 0.2 g, Cobalamin (vitamin B_{12}), 10 mg.] Solution D: (in 1 liter) $\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$, 40.0g}. To complete the media, mix Solution A for 1.0 mL, Solution B for 0.001 mL, Solution C 0.1mL and Solution D 2.0 mL in per liter of seawater at pH 7.5. Solution D was added for isolation diatoms only [11]. The cultivation of cell was done in photobioreactor (Infors HT) at 12:12 hours of light and dark, at 125 rpm, temperature 30 °C, and carbon dioxide supply at 0.5 vvm. The light illumination was set at 2500 lux. The culture was harvested at end of exponential phase.

2.2 Extraction of Starch

Starch extraction was carried out using ethanol method [12]. Twenty five mg of microalgae biomass was extracted three times with 80% ethanol by vortex the samples for 1 minute then boil at 95°C for 10 minutes. After each extraction, the sample was centrifuged at 3500 rpm for 10 min. The sample

was repeated extracted using ethanol until colorless samples to removes interfering substances such as pigments since it will affect the starch values. Then, the sample was wash twice with deionized water and acetone to remove ethanol residue. Then the cell was dried under fume hood for overnight before proceed for further analysis

2.3 Starch Analysis

The starch extracted then analyzed the purity using Megazymes total starch analysis kit. 10 mg of starch was used from the starch extracted. The total starch and the purity were calculated as follows:

$$\text{Starch (\%)} = \Delta A \times \frac{F}{W} \times FV \times 0.9 \quad (1)$$

where, ΔA is absorbance reading against the reagent blank, F is 100 μg of D-glucose/absorbance for 100 μg of glucose (conversion from absorbance to μg), FV is final volume (10-100 ml) and W is weight (mg) of sample used.

$$\% \text{ Purity} = \frac{m_{\text{starch measured}}}{m_{\text{starch assayed}}} \times 100 \quad (2)$$

2.4 Characterization of Starch

2.4.1 Moisture Content

The moisture content was determined using method described by Lee *et al.* [13] with modification by drying the starch sample at 110°C for 24 hours or until the weight became constant.

2.4.2 Granule Morphology

The shape of starch granule was observed using scanning electron microscopy (SEM). The shape of granules was compared with corn starch granule [3].

2.4.3 Amylose Amylopectin Ratio

Amylose content was assayed using method used by Hassan *et al.* [14]. The starch sample of 0.1g was weighted into 100 ml of volumetric flask and added 1 ml 99% ethanol with 9 ml of 1M of sodium hydroxide (NaOH). The solution was mixed thoroughly then heated in boiling water for 10 minutes. After the solution was cooled, distilled water was added until the mark on volumetric flask and shaken to mix well. Then, 5 ml was pipette out from the sample and placed into new 100 ml of volumetric flask and 1ml of acetic acid and 2 ml of iodine solution were added to the new flask. Distilled water was added to the mark and shaken to mix well. The sample then was measured using spectrophotometer at absorbance 620 nm. The amylase and amylopectin content were calculated as below:

$$\text{Amylose content (\%)} = 3.06 \times \text{absorbance} \times 20 \quad (3)$$

$$\text{Amylopectin (\%)} = 100 - \% \text{ amylose content} \quad (4)$$

2.4.4 Turbidity

The turbidity was determined using method used by Hassan *et al.* [14]. Starch suspension of 1% (w/v) was heated in water bath at 90°C for 1 hour with constant stirring. Then the sample was cooled for another 1 hour until reach 30°C. Then the sample was stored for 4 days at 4°C and the turbidity absorbance reading was determined for each 24 hour for 4 days using spectrophotometer at 640nm using water as blank.

2.4.5 Solubility and Swelling Power

Solubility (g/g, dry basis) and swelling power (%) of the starch was determined using method described by Nadiha *et al.* [15]. Starch suspension of 1% (w/v) was preheated in water bath at 50°C, 60°C, 70°C, 80°C, 85°C and 90°C for 1 hour then centrifuged at 2000 rpm for 20 min. The supernatant then poured into an eppendorf tubes then dried at 100°C in oven until it reach constant weight. Where the sediment was weighted after the supernatant poured out. The solubility and swelling power were calculated as follow:

$$\text{Swelling Power } \left(\frac{g}{g} \right) = \frac{\text{Weight of the wet sediment (g)}}{\text{Weight of the dry starch (g)}} \quad (5)$$

$$\text{Solubility (\%)} = \frac{\text{Dry supernatant}}{\text{Dry starch mass}} \times 100 \quad (6)$$

3 Results

Klebsormidium flaccidum cell was harvested at the end of exponential as the highest starch accumulated at that phase. The biomass productivity of algae was 0.19 g/L.day. Total starch analyzed from the algae starch was 19.06% and commercial corn starch was 76.87%. The commercial corn starch was purchased as for market manufacturing for household. The starch powder produced based on optimized method for microalgae starch purification was characterized by 60% of purity. Microalgae species reported based on previous research, Rodjaroen *et al.* [16] was discovered for *Chlorella* sp. ranged of starch content 22% until 27%, *Chlorococcum* sp. was ranged 17% to 26%, *Scenedesmus* sp. reported the lowest from 7% until 23% starch content. The range was determined based on different species of the genus. Dragone *et al.* [17] had reported the starch content for *Chlorella Vulgaris* was 41%, the highest starch producer while Brányiková *et al.* [9] reported on *Chlorella vulgaris* able to produce almost 60% of starch content. Hence, in this study, *Klebsormidium flaccidum* was in the range of based on the previous reported microalgae starch and also could be extracted more the starch after undergo more purification process.

The moisture content of the samples shows the algae starch resulted in lower moisture content compare to corn starch as 24.8% and 50.8% relatively. Low moisture content makes easier storage as it could be stored in room temperature and less risk to bacterial contamination [18].

The shape of granule was observed under SEM in figure 1. The shape of starch from algae was observed to be relatively oval in figure 1(b) compare to corn starch shape was sphere showed in figure 1 (a). The aggregates of the algae starch was not made up from composite of other elements. The diameter of the granules were measured and resulted in the corn starch did had bigger granule size compare to algae starch. As shown in figure 1 (b) the diameter size of algae starch was measured nearly 1 µm which quite small as compare to granule from corn starch was nearly 7 µm. The application in industry for small granules now is growing interest in certain applications such as pharmaceutical, coating for fabric and film production [15]. The small granule is important in producing high quality of products due to its total specific area as the water uptake is increased and the hydration will lead to increase the viscosity, more sites for crosslinking reaction for starch modification and also high gelatinization temperature and resistance [3, 19]. The size of granules is influenced by the sources. The major sources of small granules could be found in sweet potato (5-10 µm), yam (5-15 µm), arrowroot (5-20 µm) that consists of 20% to 40% of small granules [20]. However, these sources has to undergo separation process which quite expensive and also since the sources are major source in food chain, hence limits the usage of these source [3]. The small granules found in algae would be the new source for the high quality of product.

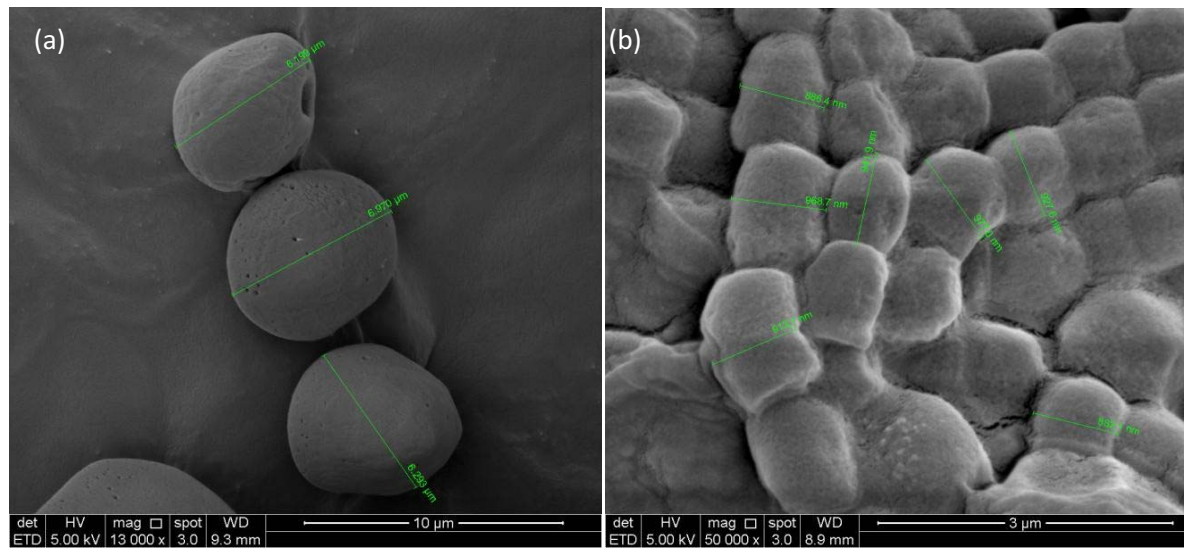


Figure 1. (a) Corn Starch granule was viewed under 13,000 magnifications with diameter of granules size and shape. 50,000 magnifications cannot be used on this sample since it will zoom too deep on granules. (b) *Klebsormidium flaccidum* starch granule was viewed under 50,000 magnifications with the diameter of granules and the shape. 13,000 magnifications cannot be used on this sample since it will show very small sizes of granules.

The amylose and amylopectin ratio was measured as the amylose content in algae starch was 25.52% and 74.48% of amylopectin content. Compare to corn starch which amylose content was 23.04% and the amylopectin was 76.96%. This shows almost similar content between corn and algae starch. In addition amylose/amylopectin ratio is mainly impacted by the biological origin and the environmental conditions which amylose content could be increased by controlling the microalgae culturing condition [3]. High amylose of starch content could lead to a strong and stiffer thermoplastic film with help by amylopectin that will form translucent paste that remains fluid when cooled [3, 14]. The turbidity of the corn starch was lower than the algae starch (figure 2). The turbidity measurement for each samples were increasing as the day of storage. This pattern was due to the swelling of granules and formation of network between amylose and amylopectin chains that were leached out of the granules during gelatinization [21]. Turbidity is used to characterize the retrogradation behavior of diluted starch paste [22]. During the first 24 hours of storage, the rapid increase of turbidity was due to the amylose chains were leached out from the granules during gelatinization and reduce the transmission of light. Then after 48 hours, the turbidity was unchanged due to the aggregation of amylose and amylopectin chain was completed [22].

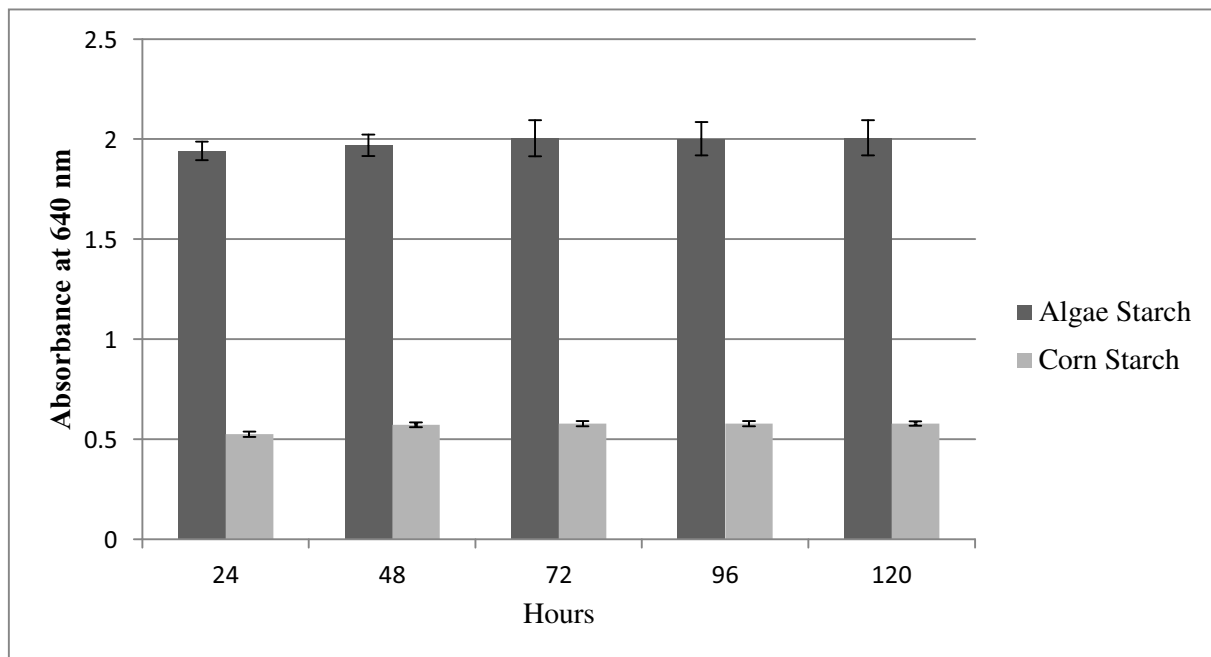


Figure 2. Turbidity of extracted starch from *Klebsormidium flaccidum* strain and commercial corn starch for 120 hours.

Swelling power is referred to the ability of water holding the starch granule and solubility is indicated as percent amount of starch leached out into the supernatant in the swelling volume determination [23]. The swelling capacity and water absorption happens when water adhered to the surface of starch granules and leads the granules to swell when exposed to higher temperature. The hydrogen bonds will be disrupted and replaced by water. The capacity of starch granules to swell will be depended on the capacity of starch molecules to hold water through the hydrogen bonding. This reaction is due to the amylose and amylopectin, given the water particles are trapped in amylopectin structure and also the difference in the distribution the chain length [24]. The granules size affects the gelatinization and the swelling capacity as the large granules will require more time to reach the gelatinization and small granules decreased it [25] due to the small granules have more efficient hydration than bigger one [26]. The hot water absorbs into the granules will caused them to swell. Then hydrogen bonds between water and hydroxyl groups of amylopectin and amylose are disrupted and trigger the swelling and solubilization of the starch granules. Subsequently, some of the amylose will be released from the granules and the granules will absorb more water and more swell [27]. The swelling power of starch from algae was lower compare to corn starch [Figure 3(a)] and the solubility of the corn starch was higher than algae starch. This as could due to the amylose content and the granule size as described by the Cythia *et al.* [27]. The difference in swelling power is due to the different in amylose content, which biomass with lower amylose content will possess high swelling power and the solubility will depend on temperature as higher temperature will result in higher solubility.

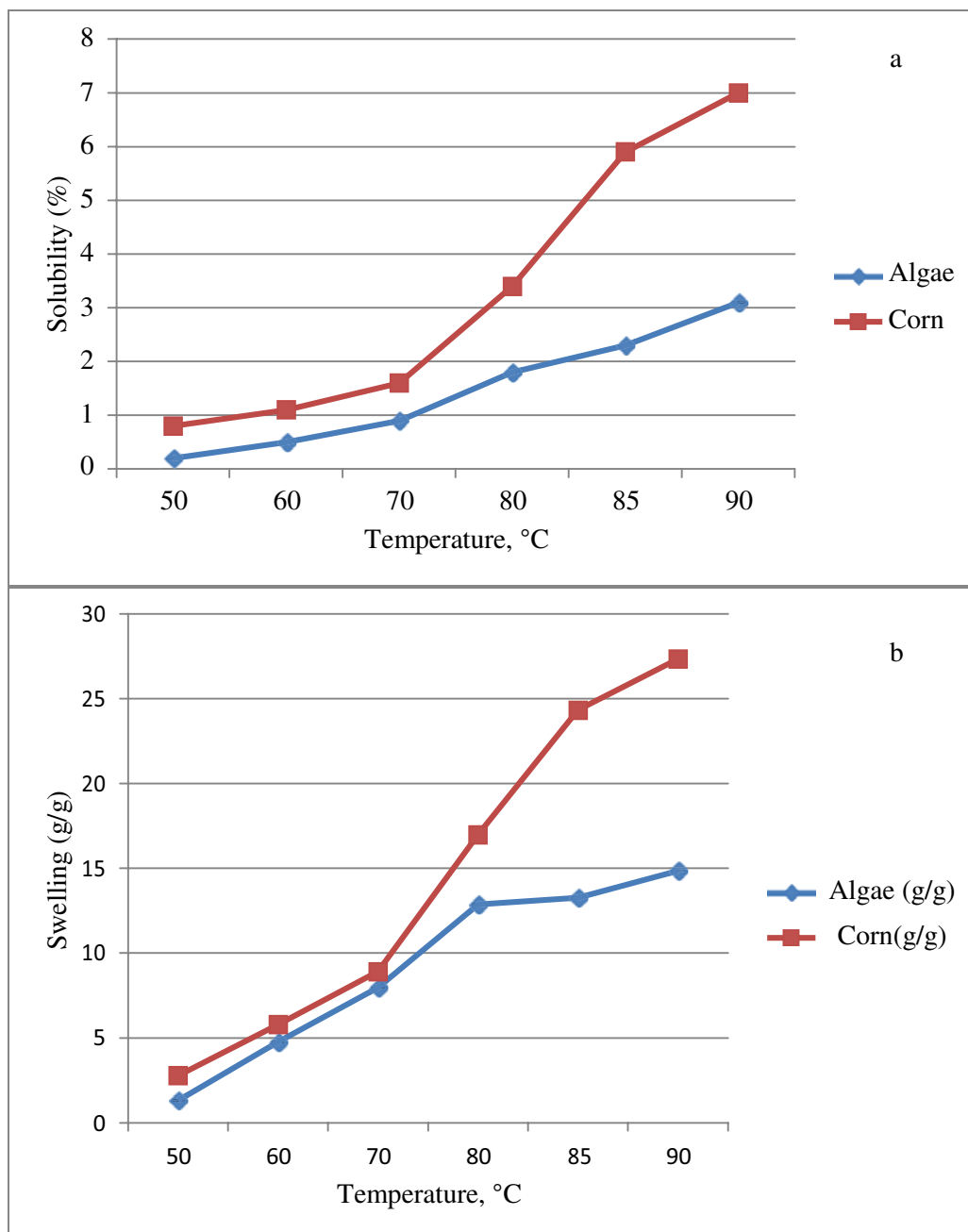


Figure 3. (a) Swelling power for the commercial corn starch and *Klebsormidium flaccidum*.
(b) Solubility of the commercial corn starch and *Klebsormidium flaccidum*.

4 Conclusion

Microalgae as the new renewable source is a replacement for the current starch source. The advantages show by the characteristics studied as it showed interesting features for the industrial application such as in pharmaceutical, cosmetics. *Klebsormidium flaccidum* is a strain of marine microalgae of inexpensive, green sources that can be returned to its natural state without any pollution after product making. The features show in this study, the starch from *Klebsormidium flaccidum* strain is shows quite close characteristics with commercial corn starch and the structural properties are surpass the

properties of corn starch. Therefore, more studies on the starch applications from *Klebsormidium flaccidum* strain would be a good evolution in industry.

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