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Research Article

Utilization of waste product from water chestnut stems to produce bioethanol

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Abstract

Bioethanol is one of the most promising replacements for fossil fuel since it is renewable and emits 85% less greenhouse gases compared to gasoline. Water chestnut (*Trapa natans* L., *sensu lato*) is an annual, floating-leaved aquatic plant of temperate and tropical freshwater wetlands, rivers, lakes, ponds and estuaries. Water chestnut corms are used for human consumption. The remaining part is waste product being discarded and abundant in the environment. The purpose of this research was to examine the feasibility of utilizing this waste to produce bioethanol. The dried water chestnut stem has moisture 10%; including cellulose 24.07%, hemicellulose 37.19%, lignin 7.82% and 30.92% others. The primary treatment of water chestnut stem powder is hydrolized by H_2SO_4 0.5% at 121^oC in 1 hour with ratio material: acid 1:10 (w/v) to get high reduced sugar 4g/l. Actinobacteria ACT 06 is used for hydrolysis and yeast SA.03 is used for fermentation. Actinobacteria ACT 06 is well performed at 35- 50^oC in neutral pH. At 3% supplementation, they can decompose CMC cirlce at diameter 40 mm after 3 cultivation days, cell density 8.53x10^8 CFU/ml and sugar concentration 5.10 g/l. Reduced sugar transformation efficiency 70-75% is noticed when fermenting at reduced sugar 3.0-5.0 g/l. Bioethanol is formed at 1.9-4.2% so water chestnut stems can be considered as potential material for bioethanol production.

Keywords: waste recovery, actinobacteria, yeast, hydrolysis, fermentation, *Eleocharis dulcis*, Vietnam**.**

Introduction

Bioenergy is renewable energy and is produced by using various biological organisms. Bioenergy is expected to solve the global warming problem by decreasing the carbon dioxide levels in the atmosphere [1]. A considerable amount of research is currently being conducted on the production of bioenergy due to the increasing demand for fossil fuel and its limited quantities in reserve. Recently, more research has focused on using non-edible biomass as raw materials including

lignocelluloses, celluloses and marine algae rather than the first generation biomass such as starch and sugar biomass [2, 3, 4]. Lignocellulosic feedstock is considered as an attractive raw material because of its availability in large quantities at low cost [5], not only for liquid transportation fuel but also for the production of chemicals and materials, i.e. the development of carbohydrate-based biorefineries [6, 7, 8, 9]. Besides terrestrial plants, aquatic plants are also a promising renewable resource [10]. Water chestnut (*Eleocharis dulcis*) is one of the world's worst aquatic weeds. It infests rivers, dams, lakes and irrigation channels, particularly in the Mekong river delta, Vietnam. Water chestnut is a grass-like sedge grown for its edible corms. The water chestnut is actually not a nut at all, but an aquatic vegetable that grows in marshes, underwater in the mud. It has tube-shaped, leafless green stems that grow to about 1.5 metres. The purpose of this research is to utilize the waste product from water chestnut production to obtain bioethanol through hydrolysis and fermentation.

Materials and Methods

Materials

Water chestnut stem was collected in Nga Nam District, Soc Trang Province, Vietnam.

Figure 1. Freshwater chestnut in Mekong river delta, Vietnam.

Research method

Physio-chemical method

Sun-drying and heat drying

- Natural drying under sun.
- Heat drying at 70^{o}C to basic weight.

Reduced sugar determination

- Reduced sugar is determined by Graxianop method

Microbial method

Microbial density

- Checking actinobacteria density on Gause medium
- Checking yeast density on Hansen medium

Microbial activity

Ability of cellulose decomposition is conducted by diffusion on agar Petri dishes.

Primary treatment

Water chestnut stem was chopped 2-3 cm and ground into powder. Weigh 50 gram of this powder into 1000 ml beaker. And then add H_2SO_4 0.5% at ratio 1: 10 (w/v) in 121^oC during 15, 30, 60 and 120 minutes. Neutrilize this solution by KOH and then filter it by filter paper/ absorbent cotton. The retentate obtained is the dried sample (CR1). Testing parameter includes the reduced sugar of CR1.

Hydrolysis

Hydrolysis by acid

50 grams was weighed of the dried sample CR1 put into beaker 1000ml, hydrolized by H_2SO_4 1%, 2% and 4% by ratio 1: 9; 1:10 and 1: 12 (w/v) at temperature 121^oC, in 60 minutes. Neutrilization was performed by KOH. After filtration of this compound, the filtrate and retentate was obtained. The retentate was then dried (CR2). Testing parameter includes the reduced sugar of CR2.

Hydrolysis by microorganism

After primary treatment and neutrilization, 50 grams of CR1 was weighed, put into beaker 1000ml, add 500ml of distilled water and supplement microorganism at ratio 1%, 3% and 5% (v/v). During hydrolysis microbial density and reduced sugar content was monitored after 1, 2, 3, 5, 7 days. The retentate after filtration is called CR3 (drying).

Fermentation method

Fermentation fluid containing *Saccharomyces cerevisiae* was selected to shake for 2 days. Each fermentation batch has volume 1000ml. Yeast supplementation 10% (v/v), temperature 30^oC; pH= 5.5, duration in 5 days are executed. Testing parameter includes pH, reduced sugar content and ethanol.

Statistical analysis

All data are processed by Excel 2003.

Results and Discussion

Microorganism to hydrolize hydrocarbon

During cultivation 0-72 hours, the bacterial cell density and decomposition circle diameter on CMC were observed.

Table 1. Bacterial cell density and activity of 4 antinobacteria strains on CMC.

Actinobacteria strain	Cell density (CFU/ml)			Decomposition circle diameter on CMC (mm)		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
ACT 01	$5.77x10^7$	$6.20x10^8$	$4.14x10^8$	25	31	33
ACT 06	$2.47x10^6$	$7.31x10^8$	$6.12x10^8$	28	33	40
ACT 17	$2.18x10^8$	$8.34x10^8$	$5.22x10^8$	26	30	35
ACT 18	$1.87x10^6$	$3.56x10^8$	$2.34x10^8$	26	32	37

From Table 1 it can be seen that all 4 actinobacteria strains are well proliferated after 48 hours. Among them, the strain ACT 06 has the highest activity compared to ACT 01, ACT 17 and ACT 18. ACT 06 strain was thus selected as biological agent for hydrocarbon biotransformation. The bioactivity of ACT 06 on CMC can be seen in Table 2.

Table 2. Bioactivity of actiobacteria ACT 06 on CMC.

Cultivation time (days)	Decomposition circle diameter (mm)	Cell density (CFU/ml)
2	32	$3.10x10^8$
3	40	$6.33x10^8$
5	42	$6.70x10^8$
7	45	$6.80x10^8$
10	29	$2.14x10^8$
15	30	$8.07x10^8$

After 3 days of cultivation, actinobacteria cell density is presented at $6.33.10^8$, decomposition circle diameter on CMC reachs 40mm. After 7 days of cultivation, actinobacteria cell density is presented at $6.80x10^8$, decomposition circle diameter on CMC reachs 45 mm. After 10-15 days of cultivation, actinobacteria cell density is nearly stable, decomposition circle diameter on CMC down to 29-30mm.

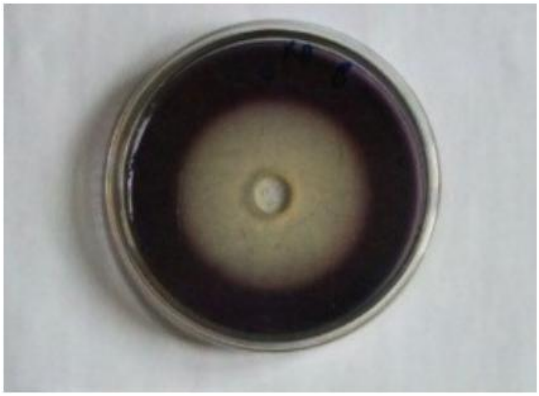

Figure 2. Decomposition circle diameter of actinobacteria ACT06 on CMC after shaking for 3 days.

Table 3. Effect of temperature on growth of ACT 06 after shaking for 3 days.

Cultivation temperature (^oC)	Actinobacteria cell density (CFU/ml)
25	$2.67x10^5$
30	$8.15x10^6$
35	$5.20x10^8$
40	$9.40x10^8$
45	$7.23x10^8$
50	$5.34x10^8$
55	$4.56x10^5$

As can be seen from Table 3, the actionobacteria strain ACT06 grows well in the temperature range 35-50^{o}C. pH also influences growth of ACT 06, this research uses buffer McIivaine to adjust pH value. The results show that actinobacteria can grow effectively in range pH 7.0-7.4 (see Table 4).

Table 4. Effect of pH to growth of ACT 06 after shaking for 3 days.

pH	Actinobacteria cell density (CFU/ml)
4.4	$8.35x10^{3}$
5.0	$2.57x10^{5}$
5.4	$7.22x10^{5}$
6.0	$3.31x10^{6}$
6.4	$4.20x10^{6}$
7.0	$8.71x10^{8}$
7.4	$6.45x10^{8}$
8.0	$8.89x10^{7}$

From the above results, it can be seen that the actinobacteria strain ACT 06 can transform hydrocarbon strongly at temperature range 35-50^{o}C in neutral pH.

Bioethanol production from water chestnut stem

Primary treatment

During primary treatment, H_2SO_4 0.5% was used and the reaction monitored during 0-2 hours at temperature 121^{o}C. Effect of H_2SO_4 0.5% and reaction time to the reduced sugar content is shown in Figure 3.

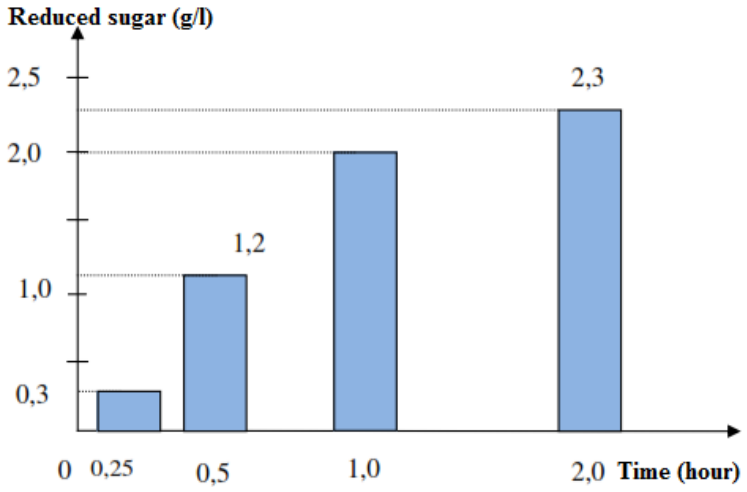

Figure 3. Effect of reaction time to the reduced sugar content at 121^{o}C with H_2SO_4 0.5%.

As can be seen from Figure 3, during 0-2 hours the reduced sugar content is inversable to reaction time. After 1 hour the reduced sugar content is optimal (2.0g/l). After 2 hours, the reduced sugar content is changed insignificantly (2.3 g/l), thus the best condition selected was H_2SO_4 0.5% at 121^{o}C in 1 hour.

Effect of acid concentration during hydrolysis

The effect of different acid H_2SO_4 concentrations (1 %, 2% and 4%) in 1 hour at 121°C to the reduced sugar content was investigated.

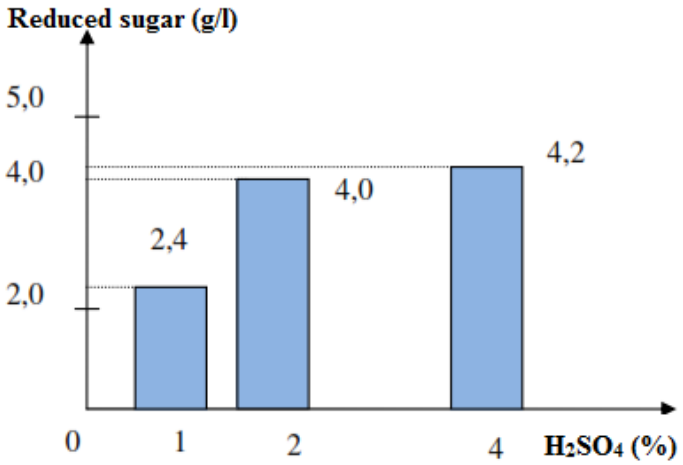

Figure 4. Effect of H_2SO_4 concentration to the reduced sugar during reaction at 121°C in 1 hour.

From Figure 4, after 1 hour of reaction with H_2SO_4 4%, the highest reduced sugar content was obtained (4.2 g/l) following H_2SO_4 2% (4.0g/l) and H_2SO_4 1% (2.4 g/l). To save time and acid solution, H_2SO_4 2% was chosen for hydrolysis.

Hydrolysis by microorganism

Actinobacteria cell density and the reduced sugar content were monitored after 1, 2, 3, 5 and 7 days with actinobacteria supplementation 1%, 3% and 5% of ACT 06 after shaking for 3 days. Results are shown in Table 5.

Table 5. Actinobacteria cell density and the reduced sugar content by time.

Time (day)	Actinobacteria cell density (CFU/ml)			Reduced sugar content (g/l)		
	CT1	CT1	CT3	CT1	CT1	CT3
1	$5.40x10^8$	$5.45x10^8$	$5.62x10^8$	1.68	1.76	1.93
2	$6.03x10^8$	$7.15x10^8$	$7.41x10^8$	2.34	3.43	4.01
3	$7.92x10^8$	$8.53x10^8$	$8.62x10^8$	4.27	5.10	5.27
5	$4.23x10^7$	$4.76x10^7$	$5.13x10^7$	3.89	4.56	4.48
7	$7.03x10^6$	$7.03x10^6$	$7.03x10^6$	3.17	3.08	2.77

Whereas:
CT1: 50g CR1 + 500ml distilled water + 1% ACT 06 shaking in 3 days (v/v)
CT2: 50g CR1 + 500ml distilled water + 3% ACT 06 shaking in 3 days (v/v)
CT3: 50g CR1 + 500ml distilled water + 5% ACT 06 shaking in 3 days (v/v)

As shown in Table 5, the optimal condition for hydrolysis is 3 days with 3% (v/v) ACT 06 supplementation.

Biotransformation of hydrocarbon into simple sugar in the water chestnut stem.

Table 6. Main components in raw material after primary treatment and hydrolysis.

No	Component	Percentage (%)			
		Raw material	Primary treatment (H_2SO_4 0.5%, 121°C, 1 hour)	Hydrolysis by acid (H_2SO_4 2% at 121 °C in 1 hour)	Hydrolysis by actinobacteria, 3% ACT 06 in 3 shaking days
1	Cellulose	24.07	37.67	39.83	18.80
2	Hemicellulose	37.19	22.90	9.52	24.01
3	Lignin	7.82	6.77	8.58	8.22
4	Others	30.92	32.66	42.07	49.33
	Total	100,00	100,00	100,00	100,00

From Table 6, it can be seen that percentages of cellulose, hemicellulose, lignin and others are changed compared to the initial values after primary treatment. When hydrolysis is performed by H_2SO_4 2%, percentage of hemicellulose is decreased dramatically. Meanwhile, hydrolysis by actinobacteria, percentage of cellulose is more reduced to hemicellulose. To clearly see the biotransformation of cellulose, hemicellulose and lignin during treatments, these components were compared in the raw material and material after primary treatment (Table 7); content of celluose, hemicellulose and others in material after primary treatment and hydrolysis (Tables 8 and 9).

Table 7. Biotransformation of components after primary treatment.

Component	Initial raw material (g)	After primary treatment (H_2SO_4 0.5%, 121°C, 1 hour) (g)	Transformation	
			g	%
Cellulose	12.04	11.50	0.54	4.5
Hemicellulose	18.60	6.99	11.61	62.4
Lignin	3.54	2.17	1.37	38.7
Others	16.36	9.96	6.40	39.1
Total	50.00	30.52	19.48	39.0

It can be seen from Table 7 that after the primary treatment a large amount of hemicellulose is hydrolysed so the hemicellulose reduction is to 62.4%. Following that is lignin 38.7%. However, this process doesn't significantly affect to cellulose in the water chestnut stem (transformed 4.5% cellulose).

The effect of H_2SO_4 2% to biotransformation of components is shown in Table 8.

Table 8. Transformation of hydrocarbon during hydrolysis by acid solution.

Component	Initial raw material (g)	After primary treatment (H_2SO_4 2%, 121°C, 1 hour) (g)	Transformation	
			g	%
Cellulose	18.84	14.26	4.58	24.3
Hemicellulose	11.45	3.41	8.04	70.2
Lignin	3.39	3.07	0.32	9.4
Others	16.32	15.06	1.26	7.7
Total	50.00	35.80	14.20	28.4

From Table 8 it can be seen that at 121^{o}C in 1 hour, sulphuric acid 2% can transform 70.2% hemicellulose, 24.3% cellulose and 9.45% lignin in raw material. It can be predicted that the reduced sugar formation by acid hydrolysis is sugar 5-carbon.

Table 9. Transformation of hydrocarbon during hydrolysis by ACT 06.

Component	Initial raw material (g)	Hydrolysis by actinobacteria (g)	Transformation	
			g	%
Cellulose	18.84	6.98	11.86	63.0
Hemicellulose	11.45	8.91	2.54	22.2
Lignin	3.39	3.05	0.34	10.0
Others	16.32	14.60	1.72	10.5
Total	50.00	37.12	12.88	25.76

From Table 9, the high transformation of cellulose is observed, owing to ACT 06. This actinobacteria uses cellulose as feed to transform into sugar.

From the above results, the treatment with acid H_2SO_4 hydrolizes 88.8% hemicellulose, 27.7% cellulose. Combination of acid and actionbacteria ACT 06 can hydrolize 70.7% celluose and 64.7% hemicellulose in the water chestnut stem. This combination method was chosen for hydrolysis to get the highest hydrocarbon biotransformation.

Fermentation efficiency

The fermentation capability of yeast *Saccharomyces cereviasiae* SA.03 was examined in 5 formulas at temperature 30^{o}C, pH= 5.5 in 5 days, fermentation batch 1 litre with 10% yeast.

- LM1: Fermentation fluid is the hydrolysis solution from the primary treatment by H_2SO_4 0.5% at 121^{o}C in 1 hour.
- LM2: Fermentation fluid is the hydrolysis solution from H_2SO_4 2% at 121^{o}C in 1 hour.
- LM3: Fermentation fluid is the hydrolysis solution from the primary treatment by H_2SO_4 0.5% at 121^{o}C in 1 hour + the hydrolysis solution from H_2SO_4 2% at 121^{o}C in 1 hour.
- LM4: Fermentation fluid is the hydrolysis solution from the primary treatment by H_2SO_4 0.5% at 121^{o}C in 1 hour + the hydrolysis solution from actinobacteria supplemented 3% ACT 06 after shaking for 3 days.
- LM5: Fermentation fluid is the hydrolysis solution from actinobacteria supplemented 3% ACT 06 after shaking for 3 days.

During fermentation the change of pH and reduced sugar content were monitored (Tables 10 & 11).

Table 10. Change of pH during fermentation.

Formula	LM1	LM2	LM3	LM4	LM5
Fermentation time (day)					
1	5.4	5.1	5.2	5.3	5.0
2	5.0	4.9	5.1	5.0	4.8
3	4.7	4.4	4.8	4.6	4.1
4	4.4	4.0	4.3	4.2	3.9
5	3.8	3.9	3.7	3.8	3.7

As can be seen from Table 10, after 4 days of fermentation, pH gradually decreased. Until the 5th day, pH of the fermented batch is lower than 4 so it limits yeast growth. Fermentation for 4 days was thus selected as the most suitable parameter. Transformation efficiency of the reduced sugar under fermentation is illustrated in Table 11.

Table 11. Transformation efficiency of the reduced sugar in 4 days of fermentation.

Formula of fermentation	Reduced sugar (g/l)			Biotransformation efficiency (%)
	Before fermentation	After fermentation	Biotransformation	
LM1	2.0	1.03	0.97	48.5
LM2	4.2	1.16	3.04	72.4
LM3	3.1	0.86	2.24	72.3
LM4	3.6	0.87	2.68	72.7
LM5	5.1	1.23	3.75	75.9

Ethanol formation after fermentation

Ethanol produced from fermentation was analysed by the boiling point and hydrometer method (see Table 12).

Table 12. Ethanol formation after fermentation.

No	Formula of fermentation	Ethanol formation (%V)		
		Boiling point	Hydrometer	Average
1	LM1	2.1	1.7	1.9
2	LM2	2.8	2.4	2.6
3	LM3	2.7	2.2	2.5
4	LM4	3.3	2.9	3.1
5	LM5	4.3	3.9	4.2

It can be seen from Table 12 that ethanol formation is in the range 1.9-4.2%V. Although this ethanol content is lower than ethanol produced from starch, it is shown that the water chestnut stem can be utilized to produce ethanol. This approach has potential in reduction of environmental pollution.

Conclusion

Due to rapid growth in population and industrialization, worldwide ethanol demand is increasing continuously. Conventional crops such as corn, cassava and sugarcane are unable to meet the global demand for bioethanol production due to their primary value as food and feed. Therefore, lignocellulosic substances such as agricultural wastes are attractive feedstocks for bioethanol production. Agricultural wastes are cost effective, renewable and abundant. Bioethanol from water chestnut waste could be a promising technology though the process has several challenges and limitations such as biomass transport and handling and efficient pretreatment methods for total delignification of lignocellulosics. Water chestnut popularly known as *Nang* in Vietnam, is an aquatic angiosperm. It belongs to the family *Trapaceae*, one of the free-floating plants, grown in shallow water fields, ponds or swampy lands in tropical and sub-tropical countries. This waste product has been successfully utilized to produce bioethanol.

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