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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 green-house 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 corm is used for human consumption. The remaining part is waste product being discarded and abundant in environment. Purpose of our research is to utilize this waste to product 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 ^0C 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 we can consider water chestnut stems as potential material for bioethanol production.

Keywords: Water chestnut stem, actinobacteria, yeast, hydrolysis, fermentation, bioethanol.

1. 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 (McKendry *et al.*, 2002) [6]. 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 (Lewandowski *et al.*, 2003; Mustafa Balat, 2011; Marina O.S. Dias *et al.*, 2014;) [3, 7, 5]. Lignocellulosic feedstock is considered as an attractive raw material because of its availability in large quantities at low cost (Parisi *et al.*, 1989) [10] not only for the liquid transportation fuel but also for the production of chemicals and materials, i.e. the development of carbohydrate-based biorefineries (Farrell *et al.*, 2006; Parameswaran Binod *et al.*, 2010; Farid Talebnia *et al.*, 2010; Nibedita Sarkar *et al.*, 2012) [2, 9, 1, 8]. Besides terrestrial plants, aquatic plants are also promising renewable resource (Mamta Awasthi1 *et al.*, 2013) [4]. Water chestnut (*Eleocharis dulcis*) is one of the world's worst aquatic weeds. It infests rivers, dams, lakes and irrigation channels on Mekong river delta, Vietnam. Water chestnut more often called simply the 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. Purpose of our research is utilize waste product from water chestnut stem to get bioethanol through hydrolysis and fermentation.

2. MATERIAL & METHOD
2.1 Material
Water chestnut stem is collected in Nga Nam District, Soc Trang Province, Vietnam.


Fig 1: Fresh water chestnut in Mekong river delta, Vietnam

2.2 Research method

2.2.1 Physio-chemical method

2.2.1.1 Sun-drying and heat drying
- Natural drying under sun.
- Heat drying at 70 ^{0}C to basic weight.

2.2.1.2 Reduced sugar determination
- Reduced sugar is determined by Graxianop method

2.2.2 Microbial method

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

2.2.2.2 Microbial activity

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

2.2.3 Primary treatment

Water chestnut stem is chopped 2-3 cm and grinded 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 ^{0}C during 15, 30, 60 and 120 minutes. We neutrilize this solution by KOH and then filter it by filter paper/ absorbent cotton. The retentate we get is the dried sample (CR1). Testing parameter includes the reduced sugar of CR1.

2.2.4 Hydrolysis

2.2.4.1 Hydrolysis by acid

We weigh 50 gram of the dried sample CR1 put into beaker 1000ml, hydrolize by H_2SO_4 1%, 2% and 4% by ratio 1: 9; 1:10 and 1: 12 (w/v) at temperature 121^{o}C, in 60 minutes. Neutrilization is performed by KOH. After filtration this compound, we get the filtrate and retentate. The retentate is then dried (CR2). Testing parameter includes the reduced sugar of CR2.

2.2.4.2 Hydrolysis by microorganism

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

2.2.5 Fermentation method

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

2.3 Statistical analysis

All data are processed by Excel 2003.

3. Result & Discussion

3.1 Microorganism to hydrolize hydrocarbon

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

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

In table 1, we see 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. So we choose this ACT 06 strain as biological agent for hydrocarbon biotransformation.

About the bioactivity of ACT 06 on CMC, we see the table 2 below.

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.

Fig 2: Decomposition circle diameter of actinobacteria ACT06 on CMC after 3 shaking days

Table 3: Effect of temperature to growth of ACT 06 after 3 shaking days

Cultivation temperature (^{0}C)	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	$4.56x10^5$

From table 3, the actionobacteria strain ACT06 grows well in the temperature range 35-50 ^{0}C pH also influences to growth of ACT 06, this research uses buffer Mc Iivaine to adjust pH value. Our result shows 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 3 shaking 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 above results, we see that the actinobacteria strain ACT 06 can transform hydrocarbon strongly at temperature range 35-50 ^{o}C in neutral pH.

3.2 Bioethanol production from water chestnut stem

3.2.1 Primary treatment

During primary treatment, we use H_2SO_4 0.5% and monitor the reaction during 0-2 hours at temperature 121 ^{0}C. Effect of H_2SO_4 0.5% and reaction time to the reduced sugar content is expressed in figure 3.

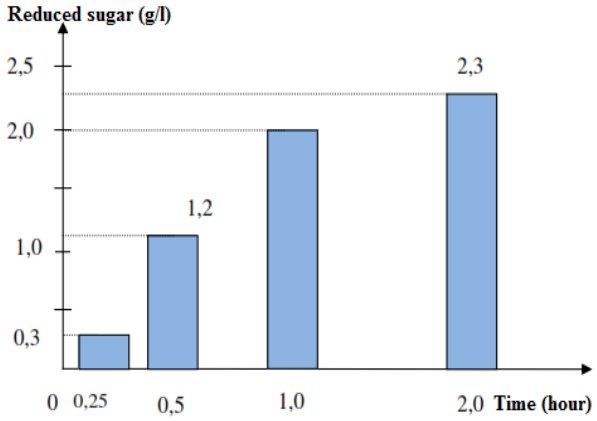

Fig 3: Effect of reaction time to the reduced sugar content at 121 ^{0}C with H_2SO_4 0.5%

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) so we choose condition H_2SO_4 0.5% at 121^{0}C in 1 hour.

3.2.2 Effect of acid concentration during hydrolysis

We investigate the effect of different acid H_2SO_4 concentrations (1 %, 2% and 4%) in 1 hour at 121^{o}C to the reduced sugar content.

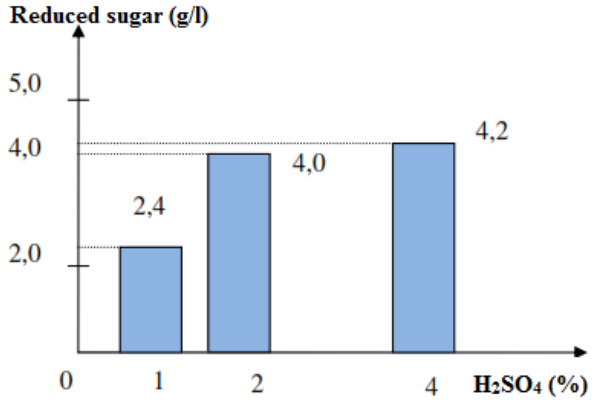

Fig 4: Effect of H_2SO_4 concentration to the reduced sugar during reaction at 121^{o}C in 1 hour

From figure 4, after 1 hour of reaction with H_2SO_4 4% we get the highest reduced sugar content
(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, we choose the H_2SO_4 2% for hydrolysis.

3.2.3 Hydrolysis by microorganism

We monitor actinobacteria cell density and the reduced sugar content after 1, 2, 3, 5 and 7 days with actinobacteria supplementation 1%, 3% and 5% of ACT 06 after 3 shaking days. Results are expressed 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)
From table 5, the optimal condition for hydrolysis is 3 days with 3% (v/v) ACT 06 supplementation.

3.2.4 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, we see that percentages of cellulose, hemicellulose, lignin and others are changed comparing to the initial after primary treatment. When hydrolysis 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, we compare these components in the raw material and material after primary treatment (table 7); content of celluose, hemicellulose and others in material after primary treatment and hydrolysis (table 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

From table 7, after the primary treatment a huge amount of hemicellulose is hydrolysed so the hemicellulose reduction 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).
Effect of H_2SO_4 2% to biotransformation of components is expressed 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, we see that at 121°C in 1 hour, sunfuric acid 2% can transform 70.2% hemicellulose, 24.3% cellulose and 9.45% lignin in raw material. We can predict 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, we see the high transformation of cellulose is owing to ACT 06. This actinobacteria uses cellulose as feed to transform into sugar.

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

3.2.5 Fermentation efficiency

We examine the fermentation capability of yeast *Saccharomyces cereviasiae* SA.03 in 5 formulas at temperature 30ºC, 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 ^{0}C in 1 hour.

LM2: Fermentation fluid is the hydrolysis solution from H_2SO_4 2% at 121ºC in 1 hour.

LM3: Fermentation fluid is the hydrolysis solution from the primary treatment by H_2SO_4 0.5% at 121 ^{0}C in 1 hour + the hydrolysis solution from H_2SO_4 2% at 121^{0}C in 1 hour.

LM4: Fermentation fluid is the hydrolysis solution from the primary treatment by H_2SO_4 0.5% at 121 ^{0}C in 1 hour + the hydrolysis solution from actinobacteria supplemented 3% ACT 06 in 3 shaking days.

LM5: Fermentation fluid is the hydrolysis solution from actinobacteria supplemented 3% ACT 06 in 3 shaking days.

During fermentation, we monitor the change of pH and reduced sugar content (table 10 & 11).

Table 10: Change of pH during fermentation

Formula / Fermentation time (day)	LM1	LM2	LM3	LM4	LM5
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

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. So we choose the fermentation is 4 days. Transformation efficiency of the reduced sugar in fermentation is expressed 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

3.2.6 Ethanol formation after fermentation

Ethanol produced from fermentation is analized 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

From table 12, we see the ethanol formation in range 1.9-4.2%V. Although this ethanol content is lower than ethanol produced from starch, we can utilize the water chestnut stem to produce ethanol. This approach is very potential in reduction of environmental pollution.

4. Conclusion

Due to rapid growth in population and industrialization, worldwide ethanol demand is increasing continuously. Conventional crops such as corn and sugarcane are unable to meet the global demand of bioethanol production due to their primary value of 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. We have successfully utilized this waste product to produce bioethanol.

5. Reference

1. Farid Talebnia, Karakashev D, Angelidaki I. Production of bioethanol from wheat straw: An overview on pretreatment, hydrolysis and fermentation. Bioresource Technology 2010; 101(13):4744-4753.
2. Farrell AE, Plevin RJ, Turner BT, Jones AD, O'Hare, M, Kammen DM. Ethanol can contribute to energy and environmental goals. *Science* 2006; 311(5760):506-508.
3. Lewandowski I, Scurlock JMO, Lindvall E, Christou M. The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. Biomass and Bioenergy 2003; 25(4):335-361.
4. Mamta Awasthi1, Kaur J, Rana S. Bioethanol production through water hyacinth, eichhornia crassipes via optimization of the pretreatment conditions. International Journal of Emerging Technology and Advanced Engineering 2013; 3(3):46-49.
5. Marina O.S. Dias, Marcelo Modesto, Adriano V. Ensinas, Silvia A. Nebra, Rubens Maciel Filho, Carlos E.V. Rossell. Improving bioethanol production from sugarcane: evaluation of distillation, thermal integration and cogeneration *systems*. *Energy* 2013; 1(1):1-13.
6. McKendry P. Energy production from biomass (Part 1): overview of biomass. Bioresource Technology 2002; 83(1):37-46.
7. Mustafa Balat. Production of bioethanol from lignocellulosic materials via the biochemical pathway: A review. Energy Conversion and Management 2011; 52(2):858-875.
8. Nibedita Sarkar, Ghosh SK, Bannerjee S, Aikat K. Bioethanol production from agricultural wastes: An overview. Renewable Energy 2012; 37(1):19-27.
9. Parameswaran Binod, Sindhu R, Singhania RR, Vikram S, Devi L, Nagalakshmi S *et al.* Bioethanol production from rice straw: An overview. *Bioresource Technology* 2010; 101(13):4767-4774.
10. Parisi F. Advances in lignocellulosics hydrolysis and in the utilization of the hydrolysates. Advances in Biochemical Engineering/ Biotechnology 1989; 38:53-87.