

# Producing fuel ethanol from energy hygrophyte duckweed

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**Abstract:** through Component analysis to kind of duckweeds, 3 High starch varieties were screened out: *Spirodelia oligorrhiza* S3, *Spirodelia polyrrhiza* V7, the corresponding starch contents were 47.86%, 41.45% and 39.69%. According to the structural characteristics of duckweed, hydrolysis efficiency of enzymatic hydrolysis and different concentrations of acid hydrolysis, was compared, then, hydrolysis technology was confirmed when the boiling water bath for 2 h and acid concentration was 4 mol/L, the hydrolysis efficiency reached 93.52%. Through studying on ethanol fermentation found that the reason that hydrolyte of duckweed couldn't be fermented normally and ethanol fermentation efficiency of ethanol reached 91.83% when using water extract to hydrolyze.

**Keywords:** Energy hygrophyte duckweed; Starch; Fuel ethanol sewage treatment

## Introduction

With the growing scarcity of fossil fuels and the days of eco-environmental load benefit-add, world countries have implemented alternative energy development as an important energy policy for sustainable economic development. In fuel ethanol production, raw material cost to total production cost 70%~80%, and mainly from corn and other food crops, have threatened global food security. All cassava, sweet potato, non-grain ethanol, such as sugarcane, can avoid food consumption, but not highly industrialized, variety area limit obvious. Combine local resources, select reliable production material, take the road to pluralism, to

The development of biomass energy is of great practical significance.

Aquatic plant growth does not occupy land, solar energy autotrophic, to absorb nitrogen, phosphorus, and other nutrients in wastewater, growth fast, growth period length, containing starch that can be converted to fermented sugar, components such as cellulose, is potential new materials applied to fuel ethanol development. Current, only fewer report on the production of fuel ethanol using aquatic energy plant fermentation. Nigam<sup>[1]</sup>, D. Mishima<sup>[2]</sup> and Ashish Kumar<sup>[3]</sup> will aquatic plant water hyacinth, fiber ingredients in cabbage raw materials fermentation production fuel ethanol; Chen Y try to ferment the starch from duckweed out of ethanol, yields 25.8% (dry weight)<sup>[4]</sup>.

Duckweed (Lemnaceae) plant", acronym duckweed, a total of 4 a species, worldwide distribution. Most of them are floating. Long in water flow relatively flat Lake River bend, through root or leaf the body absorbs the necessary nutrients such as nitrogen and phosphorus from water. Duckweed birth long, can grow year-round in many warm tropics, propagation speed fast, 2~7 d breeding generation, growth process consuming air CO<sub>2</sub> and water N, P, to reduce greenhouse gases, purify the body of water, adsorption heavy metal in water, can also be used to manage and beautify the environment, build manual wetlands etc. reported, 1 g duckweed raw dry matter 7 d build \$ g dry matter (cumulative corn biomass 2.3 g/g)<sup>[5]</sup>. H. Oron try show, \*Sewage can produce duckweed per year/hm<sup>2</sup>. Bielecki reports that, the purple-backed duckweed starch produced by sewage treatment contains 83%~75%<sup>[1]</sup>, is a new type of starch for fuel ethanol. Quality raw

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materials, can be used directly for energy production<sup>[9]</sup>, to form a governance environment and The sustainable development model of energy reuse.

is currently, There is no domestic use of aquatic plant fuel to produce BA alcohol related reports. This paper studies Aquatic Energy Plant duckweed production Fuel ethanol technology, filter for high starch varieties, test appropriate front Rationale and hydrolysis process, developing fuel ethanol production technology, Solid Efficient ethanol conversion of current aquatic energy plants, for fuel ethanol-Raw produce alternative raw materials.

## 1. materials and methods

1.1 Duckweed from multiple regions at home and abroad (Table 1), bacteria spawn wine yeast Y-16, This is the high ethanol obtained from mutation breeding in this laboratory producing strains. liquefying enzyme 1 to liquezymesupra, purchased from Norway Letter Company, enzyme Alive \$knu/g (1 KNU pointing tot., PH 5.6 Launch the water 5.26g/hamylase content); liquefaction Enzymes 2 purchased from Chengdu Kelon Reagent company. glucoamylase from Novozymes company, enzyme activity Force \$aug/ml (AUG to T., PH 4.3 Launch Solution 1 mmol/min enzyme content for maltose). Other chemicals purchased from all Long March chemical reagents company, is parse pure.

### 1.2 Instrumentation and devices

752 spectrophotometer (Shanghai Kepler Instrument Limited public Division) 2200 Kjeldahl nitrogen Meter (Swedish fox company), multin/C2100 Total organic carbon/Total nitrogen Analyzer (German Jena Analyzer Joint AG), full 9790 gas chromatograph (Zhejiang Fauli-Analysis Instruments Co., Ltd.).

## 1.3 test Methods

### 1.3.1 duckweed Plant and its hydrolysate composition analysis

through the duckweed starch, protein, Cellulose, Fat, Gray All-component analysis, System classification and physiological ecology identification etc Research, Analyzing collection of species, variety features and main component Components, using HPLC Analysis Duckweed and its hydrolysates sugar Components, qualitative fermentation sugar species.

### 1.3.2 screening of high starch duckweed varieties

to determine the starch content of the collected varieties, Filter High The duckweed of starch content, as raw material for ethanol fermentation.

### 1.3.3 duckweed pretreatment process

enzymatic hydrolysis: Grind The dried duckweed sample into a grinding, To apply amount to triangle bottle, Press 1:2 Add water blending, on 121 Zhang cook/min, take out. then heat in a water bath to the 80~90 Zhang, Add Connaught D-letter liquefaction enzyme, stirring, to iodine liquid detection is not blue. Cold but to room temperature, Add 1 Seeglucoamylase Action 4h. using salicylic acid colorimetric Determination of reducing sugar content in samples, Calculate enzymatic hydrolysis efficiency.

acid hydrolysis: hydrolysis of samples using hydrochloric water solution, acid Strong degrees 1.2, 2, 4, 6 mol/l, per feed ratio 1:2 Add acid "", Boiling water bath 2H. Use Salicylic Acid Colorimetric method to determine The reducing sugar content, Calculates acid hydrolysis efficiency.

### 1.3.4 duckweed ethanol fermentation

(1) Take the right amount of dried dry powder, Press 1.3.3 pretreatment Process water Solution Samples, after cooling, adjustment pH to 5, + Zhang sterilization min, prepare for fermentation with.

(2) After the sample is hydrolyzed, Centrifuge collect supernatant, Adjust pH to 5, sterilization min, prepare for fermentation. yeast seed liquid

Culture 12~16H, 10% inoculation to fermentation medium, in Zhang, R/min Culture under shaker conditions H. sampling test ethanol content, and calculate fermentation efficiency.

## 1.4 Profiling methods

acid hydrolysis-DNS method to determine the amount of starch in duckweed; Mining with Sweden FOSS 2200 Determination of protein by automatic Kjeldahl nitrogen meter; by GB/T 5009.10-2003 To determine cellulose; Follow the referenced document [ten] determining fat, Ash; using the German multi/c2100 type Total carbon Analyzer to determine carbon elements (C%); Follow the GB 7887- To determine phosphorus elements (P%); Determination of using high pressure liquid chromatography monosaccharide components in hydrolysate, mobile phase to water, flow rate 0.3 ml/min, column Temperature Zhang; Use Salicylic Acid colorimetric method to determine reducing sugar; take the Gas chromatograph Determination of ethanol, load is Team, Mobile phase is: Empty gas = 1:1 (1 ml/min), column Temperature Zhang, Sample Feed Zhang, Check probe Zhang, restore sugar content starch Content x 1.1 fermentation efficiency = Results and discussions 2.1 Analysis of main components of duckweed plants

Aquatic plants and dryland energy sources have different properties and features, need to develop compatible fuel ethanol pretreatment and fermentation process. Analyze the main components of duckweed, parse its group Texture features, targeted pretreatment process, hydrolysis worker Arts, Research on fermentation technology, To provide the duckweed for energy production revelation. from table 2 to see, The main ingredient in duckweed is starch, protein, cellulose, Ash, Low fat content, 5 Ingredient Total content to 40% c~90% C, different varieties, Collection and culture conditions There are differences in the sample composition of the, like little root violet V5 and multiple violet V5-1 total content less than 60%, is lower than another 4 breed. duckweed element analysis shows, C, H, N, P, K elements have a certain amount of (table 3), can be used directly for yeast growth and fermentation of ethanol.

accumulates, selection of high starch varieties for aquatic energy plant production Fuel Ethanol laying the foundation, for its development in energy and environmental protection The and leverages a large number of fine varieties. This experiment has taken a large number of duckweed samples at home and abroad, areas. (Table 1), includes green-ping, Violet back duckweed, wuping etc 4 variety of species. Little root purple-ping S3 starch content to 47.86%, is an excellent variety for ethanol production, less root purple-S1 and multi-root purple Ping V7 also high starch content type (Chart 1). S3 and S1 all same less root purple ping, others samples, especially green Ping did not select high starch products. Different varieties also have different effects in sewage treatment, Green All grow fast, Clear Treatment effect, But active biomass content Lower, Violet Ping due to volume and leaf surface area generally greater than green ping, more tolerant in sewage than green-ping. Sewage treatment effect and health substance starch content is the key factor for selecting good and practical varieties.

diagram 1 Comparison of starch content of different duckweed varieties Fig. 1 Comparison to starch content from different duckweed varieties

### 2.3 duckweed pretreatment process

using duckweed varieties S3 hydrolysis Test. uses two kinds of non-the same liquefaction enzyme treatment substrate, Hydrolysis efficiency difference is not obvious (Chart 2), Highest value is 87.76%. hydrolysis rate of acid hydrolysis process is significantly not

and, acid concentration is 1. 4~4 mol/L, increases with acid concentration,

diagram 2 Two kinds of enzyme water Compare efficiency comparisons

Fig. 2 Comparison to hydrolysis efficiency to two enzymes DE (dextrose equivalent): glucose value, Table non-starch hydrolysis degree, next Same. 1, 2: NOAA liquefaction treatment 3, 4: kelon liquefying enzyme treatment. 80~, action until iodine is not changed blue. 4 Group with glucoamylase room temperature treatment 4h.

hydrolysis efficiency gradually increases, 4 mol/L and 6 mol/L hydrolysis Effect rate is essentially the same (Chart 3), where 4 mol/L with the highest hydrolysis efficiency to 93.52%. results show, Current enzymatic hydrolysis is not completely hydrolysis of duckweed, to meet the need for fuel ethanol fermentation, and expensive; and acid water solution simple, Easy to implement, hydrolysis High Efficiency. test to confirm 4 mol/L Hydrochloric acid boiling water bath hydrolysis 2h for best hydrolytic process, to prepare samples for fermentation using this method.

diagram 3 does not Comparison of acid hydrolysis methods

Fig. 3 Comparison to different methods to acid Hydrolysis

## Concentration

duckweed Water extracts are mainly fiber sugar and other small amounts of polysaccharides, Basic glucose-free monosaccharide. hydrolysate HPLC points The analysis results indicate that, Duckweed In the extract of acid hydrolysis and enzymatic hydrolysis in glucose and small Arabic sugar, xylose, glucose is predominantly(), % total reducing sugar 75% around, Enzyme hydrolysis Product more complex than acid hydrolysate.

## 2.4 duckweed ethanol fermentation

### 2.4.1 duckweed full residue ethanol fermentation

fermentation results of different species of duckweed and glucose control as table 4:, Duckweed has the highest fermentation efficiency 48.1%, minimum 13.3%, below glucose control 41%~76%. full-time fermentation is the most commonly used raw material fermentation method, To avoid extracting Add to the cost of fermenting sugar and complex operations, also the provides many other nutrients. experiment\_Direct try all Study on the method of residue fermentation for producing fuel ethanol from duckweed. liquid ratio to 1:10 time, fermentation efficiency 48.08%; The liquid ratio is 1:2, fermentation efficiency is 37.56%. fermentation results show, duckweed full slag straight Connect to ferment, efficiency is inversely proportional to the material ratio, The larger the concentration, fermentation effect The rate is significantly lower.

when fermenting with duckweed residue, fermentation basic cannot be often, the presence of a large amount of chlorophyll makes the ferment dark green, Leaf Green element, anthocyanin, fermentation of non-starch such as flavonoids Possible suppression of procedures<sup>[one]</sup>,<sup>[A]</sup>. also, on hydrolyzed substrate over in, Dilute acid not only hydrolysis of starch, will also be used for cellulose and so on to produce Toxic substances such as furfural<sup>[A]</sup>, suppresses fermentation, or is pretreatment acid or enzymatic hydrolysis not completely, cause low fermentation efficiency. tests solid-Liquid separation of duckweed hydrolysates, looking for increased fermentation effect rate, and analyze the reason why the fermentation efficiency of duckweed residue is low. 2.4.2 duckweed hydrolysis solid residue ethanol fermentation

to add the residue of the duckweed hydrolysate after solid-liquid separation 18% Portuguese grape sugar, ethanol fermentation, ferment after, Test group fermentation maximum efficiency 34.7%, Lowest 29.6%, is lower fermentation efficiency (table 5), The fermentation efficiency of the control group is 89.8%, indicates duckweed hydrolysis residue cannot direct fuel ethanol fermentation, which may be saved on substances inhibiting yeast fermentation.

because Duckweed is traditional Chinese medicine, where the medicinal substances of the Osmanthus Lavender, celery to multi-hydroxy flavonoids<sup>[A]</sup>, has bacteriostasis<sup>[a]</sup>, Total residue fermentation cannot avoid these ingredients to yeast growth and produce B Effect of the alcohol process. in the hydrolysis and fermentation process, These ingredients are not The break out increases the likelihood of suppressing fermentation.

Experiment to further study the morphology of yeast in fermentation residue, View to observe the presence of yeast inhibition. with Xmoil Mirror The fungus in the culture medium, in culture medium containing duckweed slag yeast germ body diameter It's the same. 1mm, is significantly smaller than the control group of bacteria large Small, and there is a phenomenon of yeast fragmentation (4(B)), Portuguese grape sugar Control body intact. The result shows that,, duckweed residue, in the bacteriostatic component, causes yeast to take shape and physiology changes, To inhibit ethanol fermentation.

### 2.4.3 duckweed Hydrolysis solution ethanol fermentation

take a little root violet S3 Ethanol fermentation, starch content is 47.86%, extracts hydrolysis supernatant fermentation, maximum fermentation efficiency is 91.83% (Table 6). Solid-liquid separation of duckweed hydrolysates, with soluble sugar liquid fermentation, promoting duckweed ethanol fermentation.

Table 6 Comparison of fermentation efficiency of two hydrolysis modes

Table 6 Comparison of hydrolysis efficiency of process of extracting sugar after hydrolysis, The adds the fermented sugar to the Other ingredients separate use, help increase ethanol fermentation efficiency, and For Other useful ingredients (such as flavonoids) further application of.

### 3. Epilogue

with the continuous development of our society, large amounts of contaminated water and the production of domestic sewage becomes environmental and ecological balance catastrophe. Developing aquatic energy plants can combine these two aspects. This trial shows the use of aquatic energy plant duckweed for renewable energy availability of fuel ethanol production, after the duckweed is hydrolyzed to the whole plant, mentioning take sugar liquid for fuel ethanol fermentation, max fermentation efficiency up to 91.83%. Clean water quality, also provides raw materials for energy production, real Technology development coupled with current energy production and environmental governance.

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