


A detailed microscopic image of plant tissue, showing a cross-section of cells with prominent cell walls and internal structures. The image is dominated by shades of green, with some areas appearing more translucent or yellowish. The cells are arranged in a somewhat regular pattern, with some larger, more rounded cells in the foreground and smaller, more elongated cells in the background.

— ENERGY

# Alternative vinasse

Production waste from  
ethanol may be used to  
produce biodiesel

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Combining microalgae and vinasse to produce biodiesel is the challenge that has been undertaken by the São Paulo enterprise Algae Biotecnologia. Although companies in the United States have been successful in producing biodiesel from algae, the use of vinasse by this company is a new feature. The waste produced in the manufacture of ethanol is characterized not only by the highly noxious smell it emits but also its richness in mineral salts, mainly potassium, and its high levels of acidic organic material. Also called dunder, in the mid-1970s, vinasse became the villain of Proálcool, the government program that introduced ethanol as a fuel. Thrown into rivers and lakes as an effluent, this byproduct killed fish and polluted water and even reached the water table in some locations. After 1978, specific rules and legislation at the federal and state levels, prepared mainly by the Companhia de Tecnologia de Saneamento Ambiental (Cetesb) [Environmental Sanitation Technology Company] of the State of São Paulo, forced producers to develop an environmentally sound and commercially appealing use of the waste. The solution that was developed was to use vinasse to fertilize sugar cane plantations. Since then, vinasse has been sprayed onto plantations using irrigation piping, in a process called 'fertirrigation', or taken by truck to be put directly onto the crops. Although this process is fairly common in the sugar and alcohol industry, the volume of vinasse has been growing exceptionally quickly. For each liter of ethanol that is made, at least 10 liters of vinasse are produced. In 2010, 25 billion liters of ethanol were produced and consequently yielded more than 250 billion liters of vinasse from the distillation of the 'wine' obtained from the cane juice fermentation process. The large volume indicates that alternative uses besides fertilizer should be identified. However, to produce more profitable ethanol on some large properties that incur



Experimental cultivation of algae at the biotechnology company

large expenses transporting the vinasse, a new process, developed by Fermentec from Piracicaba, in upstate São Paulo, has emerged to reduce the amount of waste by increasing the level of alcohol in the fermentation phase. “With this increase, it’s possible to cut vinasse production by half”, says agronomist Henrique Amorim, a Fermentec partner and retired professor from the Higher School of Agriculture Luiz de Queiroz (*Esalq*) at the University of São Paulo (USP).

However, although the volume of vinasse is reduced in this new process, at least 160 billion liters will be left over every year. This large excess represents a raw material that can be used to produce biodiesel from microalgae, a process that has been proven to be effective in Algae’s laboratories. “We’ve already had great results, and the challenge now is to scale up oil production in pilot plants until 2012 and then start testing in a distillery in 2013 and 2014,” says Sergio Goldemberg, the company’s technical manager. The oil is extracted from the biomass that is formed by the multiplying microalgae cultivated in the vinasse. The microalgae consume the nutrients from the liquid and grow. Some species double their population in just one day.

To extract the oil, a centrifugation system is needed to separate the lipids (fats) from the biomass. The material then goes to a drier, and the oil is ex-

tracted using mechanical or chemical techniques. The level of lipids from the microalgae biomass reaches 30%, compared with 18% from soybeans or 40% from jatropha. Microalgae also have another advantage. Their productivity can reach 40,000 kg of oil per hectare (kg/ha), while the productivity of soybeans reaches 3,000 kg/ha and that of jatropha reaches 3,500 mil kg/ha. Also

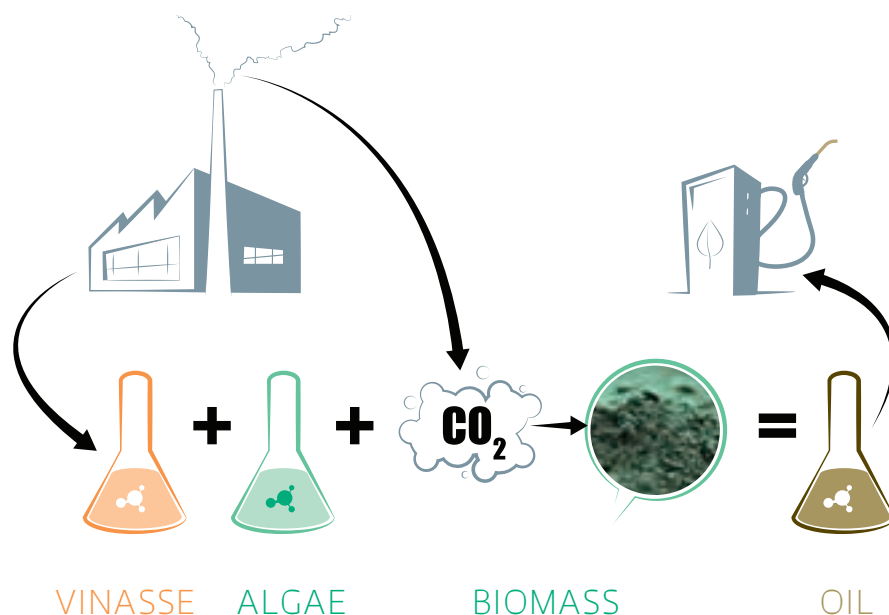
in favor of the microalgae is the fact that the CO<sub>2</sub> produced by the distilleries during fermentation, which is absorbed by the sugar cane plantation itself, can be used during the production of biomass because the microalgae require CO<sub>2</sub> to multiply. Any surplus protein produced in the process can be used as feed for fish farming, providing an additional benefit for the producers. To produce biodiesel, any type of oil, including oil from the microalgae, goes through transesterification, a chemical reaction between one type of alcohol – methanol or ethanol – and a lipid, which results in biodiesel.

#### RIGHT CHOICE

Goldemberg explains that the researchers involved in the project are now trying to develop studies and solutions to improve the entire system’s effectiveness. The search begins with the choice of microalgae or cyanobacteria, organisms that are similar to algae. “We’re researching a lot of species, mainly those that live in fresh water,” says Goldemberg. “Afterwards, we select some to find out which ones adapt best to the vinasse and produce microbial biomass with a high lipid content,” says Professor Reinaldo Bastos, from the Center of Agrarian Sciences in Araras, at the Federal University of São Carlos (UFSCar), an Algae partner in the research, along

## Path to biodiesel

Vinasse produced in the distillery is transformed into biomass by algae



with a group led by Professor Eduardo Jacob-Lopes, from the Federal University of Santa Maria, in Rio Grande do Sul. “We already have some 20 species, many collected from the environment, that are being tested in cultures with vinasse,” says Bastos.

Vinasse works as a culture for growing and multiplying microalgae. In experiments in other countries, particularly in the United States, companies that grow algae must add mineral salts and nutrients to the water during the production process. “We have advantages relative to them because we have really economic waste for production,” says Goldemberg. In the United States, various companies use algae to make biofuels, including aviation biokerosene, although still not on a commercial scale. Examples include: Solazyme, which has investments from the large oil and energy corporation, Chevron; Algenol, which has partnerships with Dow; and Sapphire, which has an investment from Cascade, a company belonging to Bill Gates, Microsoft, and the Rockefeller Foundation. All three companies also receive funding from the United States Department of Energy. Initial studies in the use of algae to produce biofuels took place during the 1980s at the National Renewable Energy Laboratory (NREL) in the United States. “But at the time, the energy problem and excess CO<sub>2</sub> were not important,” says Goldemberg, who is an agronomist and has already worked with vinasse in ethanol distilleries, before establishing Algae. The wave of projects with government support, mainly in companies in the United States, began in the 2000s.

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## THE PROJECT

Selection of tolerant yeasts in fermentation processes with a high alcoholic strength to reduce vinasse and save energy – nº 09/52427-2

### TYPE

Technological Innovation in Small Businesses (Pipe)

### COORDINATOR

Henrique Amorim – Fermentec

### INVESTMENT

R\$ 202,923.42 and US\$ 135,310.28 (FAPESP)

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## “We verify which genes are related to the capacity of the organism to remain viable with a high alcohol content,” says Márcio Silva Filho, from USP

“We could have repeated what is done abroad, although there are still no products for sale, but we decided to have our own ideas and follow a new path with vinasse,” says Goldemberg, who is the son of José Goldemberg, a professor from the University of São Paulo (USP), former Education Minister and former Secretary of the Environment of the State of São Paulo. Algae receives R\$ 2.5 million in funding for its research from the Studies and Projects Funding Agency (*Finep*), in an Economic Grant Program project, and has received a second grant for R\$ 3.2 million over three years from the National Economic and Social Development Bank (BNDES), from its Technology Fund (Funtec), to be used with UFSCar, which also received R\$ 400,000 from the company. Algae was set up in 2007, and since 2009, the company has been a joint venture with the Ecogeo Group, a conglomerate that operates in the areas of consultancy and environmental engineering and had revenue of R\$ 50 million in 2010.

### ALCOHOLIC YEAST

Biodiesel production from vinasse, in addition to leading to new benefits in the end product, may also eliminate additional expenses for the ethanol producers from pumping or carrying this waste transformed into fertilizer over long distances. Fermentec’s proposal to cut vinasse production by half may

lead to savings for distillery owners. “Taking vinasse up to 35 km away from where it is produced pays for the fertilizer, mainly potassium chloride, which is mostly imported. More than this distance, it produces a loss,” says Amorim from Fermentec. The company’s goal is to increase the level of alcohol to 16% from the average 8% by the end of the fermentation process, the phase at which *Saccharomyces cerevisiae* yeast transforms sugar into alcohol. Afterwards, in the distillation phase, the alcohol is separated from the vinasse.

The company, which has revenues of R\$ 10 million per year, has been selecting strains of *Saccharomyces* since 1990 and uses almost 80% of the yeasts used in Brazil’s distilleries. For the last six years, the company has been studying the temperature of the fermentation process, particularly as it relates to the selection of these microorganisms. To do so, it has brought together researchers, such as professors Luiz Carlos Basso and Márcio de Castro Silva Filho, from Esalq, Pio Colepicolo, from the Institute of Chemistry at USP, and Boris Stambuck, from the Federal University of Santa Catarina. Coordinated by Silva Filho and with funding from the National Council for Scientific and Technological Development (CNPq), a study was performed to understand how yeasts can adapt to the high alcohol levels during fermentation. By analyzing 6,000 genes of these yeasts, it was possible to identify the genes that are related to the organism’s capacity to remain viable at high alcoholic strengths. “We’ve already identified a series of genes, and in the long-run we’ll be able to introduce or modulate the expression of these genes in yeast strains,” says Silva Filho. To select new yeasts that operate at high alcoholic strength, in 2009, Fermentec requested a project from FAPESP’s Technological Innovation in Small Businesses (Pipe) Program. “We want to find better yeasts than the current ones that can function at an alcoholic strength of 18% for use in the new fermentation process,” says Amorim.

A study was successfully performed at the Pedra Distillery in Serrana, São Paulo. With fermentation at 16%, a saving on vinasse of R\$ 7 million per crop in this distillery was estimated. “We’re now ready to market the process,” says Amorim. ■