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Biofuels: A Molecular Perspective

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Abstract. What is the best biomass source and conversion process to create the biofuels to power our country in the future? Specifically, is cellulosic ethanol capable of becoming a viable biofuel? To answer these questions, the processes used to convert biomass into fuel, as well as the biomass itself, were examined on a molecular level, focusing on the chemistry behind the reactions. After that, developing technologies were examined to determine their potential. Lastly, alternative biomass sources were examined. Given recent mandates and developing technologies, the United States is capable of developing a thriving cellulosic ethanol industry. In addition, algae has shown potential as an environmentally friendly biomass source and the hybrid hydrogen-carbon conversion process is promising. However, each facet of biofuels production warrants more research in order to discover the ultimate solution to our energy woes.

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In a time fraught with concerns about global warming and energy independence, a number of questions are raised about the future of our fuel supplies. Many are looking to biofuels to alleviate these problems. However, there are currently a number of hurdles to be overcome in making biofuels a viable energy source, free of subsidies and tax breaks. But what advances are being made in order to increase the yields of biofuels? What other possible crops and conversion processes could be used to make biofuels a more viable energy source? Will cellulosic ethanol ever be economically feasible? In order to answer these questions, one needs to cut through the political agendas, ignore corporate interests, and examine these procedures on a molecular scale, considering both the biochemical and thermochemical components of the process. This paper will focus on the various processes currently being used to extract energy from biomass, as well as those being developed.

First, I would like to examine the processes currently being used for biomass energy extraction, including the underlying chemistry. After assessing our current situation, I will investigate new methods. After perusing this information, I will attempt to determine the best source of biomass, as well as the best method for processing it. Ultimately, I plan to address the question: Are biofuels realistic candidates to assuage our energy problems and how efficient can they become?

Current Technologies

Generally, ethanol production involves three main steps. The first step is the breakdown of a plant structure into fermentable sugars. The second step involves the fermentation of those sugars to produce ethanol. Lastly, the ethanol must be purified.

In ethanol production from sugar feedstocks, fermentation of a crop is induced to convert sugars from the plant into ethanol. In fermentation, microorganisms use the fermentation sugars as food and create ethanol as a byproduct. There are several intermediates involved in the fermentation, but the net result is ethanol and energy for the yeast. It is crucial that this reaction be carried out in the absence of oxygen or else the yeast will undergo aerobic respiration and produce carbon dioxide and water. The most common organism used for fermentation is *Saccharomyces cerevisiae*, more commonly known as baker's yeast. Common sugar feedstocks are sugar beets, sweet sorghum, and sugarcane. The most suitable sugars for ethanol production are those of the six carbon variety, with glucose being one of the most useful. For this reason, crops that are concentrated in glucose are preferred for ethanol production. However, sugar crops account for a large portion of our food supply, and are thus relatively expensive compared to other fuel sources.

Ethanol production from starch sources differs in a few key areas. Starch consists of long chains of glucose molecules. These chains must be broken in order to use the fermentable glucose. Currently, starches are broken down through hydrolysis. This involves the splitting of a water molecule in order to decompose a starch molecule into its glucose monomers. In the starch chain, the glucose molecules are bonded to one another by oxygen molecules. The two molecules are split with the aid of an enzyme, and then the alcohol group (OH) from part of the water molecule attaches to one of the glucose molecules and the hydrogen attaches to the other glucose molecule in the pair. This reaction will only occur in the presence of an amylase enzyme and heat. An enzyme functions by simply binding to one or more of the reactants in a reaction. The enzyme brings the reactants together when under normal conditions the reaction would have proceeded much more slowly, or not at all. The exact amylase enzyme will vary and improvements are being made in efficiency and costs of enzyme production.

The sugars required for fermentation can also be obtained from other cellulosic parts of the plants, such as corn stalks, wood, or switchgrass. Cellulosic ethanol is identical to corn ethanol and is attractive because the cellulose is available from a variety of sources and is the most abundant source of biomass on the planet. Even with current methods, the U.S. Department of Energy believes that modest changes in crop practices could yield enough biomass to replace thirty percent of the gasoline used in the United States by 2030. Cellulosic ethanol is particularly attractive because it uses less fossil fuel than ethanol produced from other sources. Although the process is currently less efficient than corn ethanol production, biomass contains more potential energy and residues from the biomass process can be used to produce heat and power for the processing facility, creating an energy cycle.

Cellulose, like starch, is composed of chains of at least 500 glucose molecules. However, cellulose is much stronger because it is composed of beta linkages between glucose molecules rather than alpha linkages like starch. This simply means that the glucose units are oriented in different directions along the chain, rather than all in one direction. This allows the glucose chains to form sheets and a crystalline structure with considerable tensile strength and resistance to hydrolysis. This strength is also due in part to hydrogen bonding, which is the strongest intermolecular force. This force arises from the attraction between hydrogen atoms on different molecules. Many organisms possess the ability to digest alpha bonds between glucose molecules, but few possess the enzymes required to break down the beta linkages and hydrogen bonds found in cellulose.

A number of other polymers are responsible for cellulose's rigidity and durability. These properties are due in part to the polymer known as lignin. Often referred to as "nature's cement," lignin allows plants to stand upright and absorb the sun's rays. Lignin has a complex chemical structure that can take on a variety of forms with huge molecular weights. It's strength and stability is due in large part to its resonance stabilized aromatic rings. A resonance stabilized molecule is one in which the electrons bonding the constituent atoms are in constant motion, moving from one bond to the next, creating double bonds along the way. This delocalization reduces the energy within the molecule, thereby reducing the chances of the bonds breaking and allowing for a more stable molecule.

In addition to cellulose, cellulose-rich plants are often a source of hemicellulose. Unlike a glucose polymer which contains only glucose monomers, hemicellulose is composed of several different monomers and is covalently bonded to lignin. Also, hemiceullose is easily broken down through hydrolysis into basic sugars. However, current yeast species are unable to ferment the sugars that are yielded, but I will return to this point later in the paper.

Plants evolved these tough coatings over millions of years to protect themselves from pathogens so it is no surprise that it is difficult to extract energy from the polymer. Lignin especially provides resistance to attack by microorganisms. Unfortunately, this also makes lignin resistant to breakdown by anaerobic processes. Currently, the prospect of cellulosic ethanol is bleak, due to the fact that enzymes capable of cellulose digestion are expensive and inefficient.

The first step in producing cellulosic ethanol is the pretreatment. This step involves making the cellulose chemically accessible by removing the lignin seal and compromising the layered crystalline structure. Not only is it important to expose the cellulose, but an effective pretreatment also minimizes the number of byproducts that can impede the rest of the biomass processing. One technique is known as the ozone pretreatment. This treatment degrades the lignin overcoat by attacking the aromatic rings through oxidation, which is simply the addition of an oxygen atom to a molecule, resulting in the cleavage of the double-bonded carbon atoms. Another pretreatment method is known as wet oxidation. Once again, the lignin is oxidized with air and oxygen at temperatures exceeding 120 degrees Celsius in an alkaline environment.

A more violent, forceful pretreatment technique is known as steam explosion. In this method, biomass is exposed to high-temperature water vapor and discharged into the air. The steam explosion is capable of breaking the strong hydrogen bonds between the molecules in the biomass.

Another pretreatment process is known as ammonia fiber expansion (AFEX). AFEX is an alkali based treatment, like wet oxidation. Heat, ammonia, and biomass are combined in a reactor to produce the treated biomass. However, the exact mechanism through which AFEX functions is not understood. It is known that lignin is broken down on the surface and the ammonia cleaves the hemicellulose into oligosaccharides, which are linked sugar monomers with chains of three to ten units. As with all of the pretreatment processes mentioned thus far, there are byproducts that inhibit the enzyme functions including acids and aromatic rings. Acids can decompose the enzymes and aromatic rings can interfere with binding sites. All of the pretreatment processes mentioned involve chemical or physical means of degrading the robust lignin cover, but some processing facilities apply both techniques in conjunction with another to achieve greater yields.

The next step in the creating of cellulosic ethanol is known as the cellulolytic process and the purpose is to break down the cellulose molecules after they have been exposed for interaction by the pretreatment process. There are two different cellulolytic methods that are currently used. One involves acid hydrolysis, and the other uses enzymatic reactions.

The hydrolysis breakdown is an acidic process dating back to the late 19th century. The reaction proceeds in three steps that are very similar to the hydrolysis reaction mentioned previously, except for the addition of an acid. Because of the acid, the biomass must be neutralized before proceeding to the fermentation process. Moving bed chromatography is often used to purify the products before fermentation. This involves using a solvent to move the unpurified product down through a silica gel column. The different components of the product will move through the column at different speeds based on their attraction for the solid phase (silica gel) of the column. The desired product can be recovered when it exits the bottom of the column. This purification step is crucial because the yeast are sensitive to the acid as well as other potential toxic byproducts. These problems reduce the efficiency of cellulose decomposition through hydrolysis.

The enzymatic breakdown is a relatively new process for releasing glucose molecules from cellulose. Normal starch fermentation requires the use of amylase enzymes, but cellulosic ethanol production involves the use of cellulase enzymes. While corn ethanol production is accomplished through the use of just a single enzyme, cellulosic enzyme breakdown requires a complex array of enzymes working in conjunction with one another. These include cellulases, hemicellulases, and other glycosyl hydrolases. Glycosyl hydrolases are involved in the breakdown of cell walls and several different categories can exist in an enzyme cocktail designed for cellulosic ethanol production including: exocellulases, endocellulases, exoxylanases, endoxylanases, cellobiases. Obviously, the enzymatic breakdown of cellulose is a very complicated process. This reaction occurs in the stomachs of ruminants and ultimately results in the hydrolysis of the cellulose as described in the acid process in the previous paragraph. However, the enzymatic breakdown can be carried out industrially without creating toxic, yeast inhibiting byproducts. To make the enzymatic breakdown of cellulose economically feasible, however, it has been postulated that a five to tenfold increase in enzyme efficiency is required.

Unfortunately, manufacturers are very secretive about the processes they use to produce these enzymes on large scales. However, it is common knowledge that enzymes are most often produced through the use of microbes. These are popular because of their short generation time and high yields. In addition, microbes produce extracellular enzymes which are very easy to harvest. The genomes of these microbes have been sequenced, allowing for accurate genetic manipulation.

There are two types of fermentation used in the production of enzymes: submerged liquid fermentation and solid substrate cultivation. In submerged liquid fermentation, the microbes are simply submerged in an aqueous medium that contains all the nutrients needed for growth. Solid substrate cultivation is a much older process that involves using a water insoluble medium with variable amounts of water on the surface. Studies have shown that there are differences between the genetically identical enzymes being grown on different substrates. The solid substrate enzymes have shown greater heat stability, but the process of using a solid substrate is more difficult. Cultivation times are longer and the organisms must be capable of surviving in environments with reduced moisture levels.

After the breakdown is complete, whether through enzymatic or hydrolytic means, fermentation is induced. This fermentation process is essentially the same as described previously. However, the breakdown of cellulose material yields an array of sugars due to the presence of hemicellulose, including xylose and arabinose. These are both five carbon sugars whose energy currently goes unused due the inability of yeast to ferment these sugars. Technological advancements are needed to take advantage of these energy sources.

Another process, known as gasification, can be used to extract energy from cellulose. The cellulose chain is not broken down by chemical means, but rather is converted into what is known as a synthesis gas. A gasifier is capable of converting the biomass into carbon monoxide and hydrogen through the addition of high pressure, oxygen, and heat. The synthesis gas can then be converted to a number of different products, including ethanol. The National Renewable Energy Laboratory (NREL) uses a method that involves several steps. First, the biomass is dried before being placed into an indirect gasifier. The product is then subjected to a tar reformer, followed by cleanup and compression of the syngas. The gas must be cooled before being fed to microorganisms because it exits the gasifier at roughly 2,350 degrees Fahrenheit. The waste heat generated from cooling the gas can be used to power steam-driven turbines. A small amount of ash is generated, but it is negligible in comparison to the waste products generated by other common means of creating liquid fuels. The gas created is fed to a microorganism capable of synthesizing the carbon dioxide, carbon monoxide, and hydrogen into ethanol. This process is promising because up to one-third of the mass contained in biomass cannot be converted through simple biochemical means and must be converted by thermochemical reactions. The gasification process is not harmful to the environment, creating no air emissions.

There are two main types of gasifiers. They consist of draft gasifiers and fluid bed gasifiers. Draft gasifiers can handle larger volumes of biomass at once, but are less suitable for creating alcohols, such as ethanol, because they tend to produce less refined products. Fluid bed gasifiers work by creating a highly turbulent mixing zone for the reaction. The products from fluid bed gasifiers are more refined and therefore better suited for the synthesis of ethanol and hydrogen. Unfortunately, fluid bed gasifiers cannot handle large volumes of biomass. Currently, neither gasification process is feasible on an industrial scale. The syngas would have to be cleaned of contaminants, which is currently an expensive task.

While cellulosic ethanol holds some promise, technological leaps must be taken before it can compete with the price of corn ethanol. The enzymes required to produce a gallon of corn ethanol cost three cents. The enzymes required to produce cellulosic ethanol cost between thirty and fifty cents to produce a gallon. The department of energy estimates that it costs nearly twice as much to produce a gallon of cellulosic ethanol as it does to produce a gallon of corn ethanol. However, there are numerous advances on the horizon that will be elaborated upon later in the paper.

Purification

No matter which method of ethanol production is used, it must be refined before reaching our vehicles. The main component of the unpurified ethanol that needs to be removed is water, creating what is known as anhydrous ethanol. Currently, fractional distillation is used to purify ethanol for fuel use. Fractional distillation takes advantage of the different boiling points of compounds. Because compounds have different vapor pressures due to intermolecular forces, they will vaporize and condense in the distillation column at different temperatures. As the temperature of the mixture increases, the undesired compounds will distill at different temperatures and can be collected in fractions.

Ethanol boils at 78.3 degrees Celsius and is generally the third fraction collected during fractional distillation. However, this will only purify the ethanol to roughly 95 percent and form an azeotrophic mixture. This means that further distillation will not purify the product further. A material separating agent, such as benzene, must be added to change the properties of the mixture to allow further distillation to be effective. Water, ethanol, and benzene form an azeotrope that boils at a lower temperature than ethanol. This more volatile mixture can then be distilled out, removing the water.

Ethanol can also be purified through the addition of what is known as a hygroscopic agent, which is basically a water attracting molecule. Possible desiccants are glycerol, lime, and rock salt. The desiccant will attract the water and form a precipitate, calcium hydroxide in the case of lime that can be separated from the ethanol mixture. In this reaction, the hydroxyl (OH) group from water is removed and forms with lime, CaO, to form hydrated lime, $Ca(OH)_2$. A zeolite can also be used to filter the water from the ethanol mixture. Zeolites are minerals that have extremely small pores. These pores can be used to trap water while not absorbing ethanol. This method is useful only for removing water from ethanol, but fails to remove other contaminants.

Current Research

The United States continues to make huge investments in research into renewable fuels, but is this research in vain? I will first examine advances in cellulosic ethanol and biomass conversion, and then examine more exotic potential sources of renewable fuels and biomass conversion processes currently under development.

NREL is at the forefront of advances in biomass processing technology. The primary areas of current conversion research are to make enzymes cheaper and more efficient, requiring fewer enzymes per ton of biomass processed. Unfortunately, cellulase enzymes function very slowly, making it essential to select the best enzymes for the conversion process. Currently, the most efficient group of cellulase enzymes is known as the cellobiohydrolase I (CBHI) family. NREL has discovered enzymes from this family that are twice as effective as those currently used industrially. These new enzyme “cocktails” have reduced the costs of converting biomass to cellulosic ethanol twenty fold.

Recently, NREL has employed scanning electron microscopy to examine the reactants and products in biomass conversion on a molecular level. For example, it was discovered that even after lignin had been removed in the pretreatment process, it re-deposited back onto the cellulose, reducing enzymatic access to cellulose and the sugar yield. The Biomass Surface Characterization Laboratory is employing a number of different techniques to map the maze of plants structures enzymes must circumvent to access cellulose. Crystal clear three-dimensional images of plants structures have been developed using the newly-developed scanning confocal microscope. The largest biological computer model ever constructed is being built to examine the molecular dynamics of the cellulose-cellulase system. NREL has also discovered a hemicellulase enzyme that increased the yield of xylase by twelve percent. The increased breakdown of hemicellulose also increased the hydrolysis of cellulose, increasing glucose yields by six percent.

The formation of the Biomass Refining Consortium for Applied Fundamentals and Innovation also shows promise in creating better biomass pretreatment process. Each member is investigating a different pretreatment process in order to determine the best method for short-term and long-term biorefineries for a wide range of different feedstocks.

Research is also being conducted to develop microorganisms better suited to the fermentation of cellulose. As was stated before, the biomass mixture after the pretreatment process, known as a hydrolyzate, contains many byproducts that can be toxic to the microorganisms used for fermentation, resulting in reduced yields and efficiency. Examples include acetic acid, a high concentrations of solids, and of course, ethanol. Because baker's yeast is currently used for fermentation, five carbon ring sugars created from hemicellulase go to waste. NREL has recently inserted three genes from a bacterium into baker's yeast, allowing the yeast to ferment the five-carbon sugars. This “super yeast” would not only increase cellulosic ethanol yields, but corn ethanol yields as well.

NREL has also experimented with yeast alternatives in the fermentation process, such as the bacteria Zymomonas mobilis. In addition to its natural tolerance for high temperatures and ethanol concentrations, the laboratory engineered these bacteria to ferment five carbon sugars, like xylose. Not only has this bacteria been developed, but the species has been stabilized so that the population can be self-sustaining.

While research has been done on a number of different conversion technologies, research is also being done in creating biorefineries which incorporate many different conversion technologies and biomass sources to produce fuels. By integrating these processes in optimum ways, the most efficient biorefineries can be built. For example, residues leftover from the biochemical conversion process could be subjected to the thermochemical conversion process in the same biorefinery, thereby increasing energy yields and reducing wastes. Research laboratories are attempting to address numerous biorefinery issues. For example, the use of recycled water in ethanol plants has been shown to reduce yields. This problem is studied in the field of rheology, the science of the deformation and flow of materials. It is certain that cellulosic ethanol factories will need to recycle water, but how this process will occur is under investigation.

NREL is also pioneering advancements in the thermochemical conversion process, otherwise known as gasification, mentioned earlier in this paper. This process is particularly useful for converting lignin-rich plants, such as trees and mill residues, into ethanol. The products from this process are not clean, often containing large amounts of tar. NREL has developed a process that can remove up to ninety-seven percent of this tar and convert it into more syngas. The “tar-reforming” catalyst has also been engineered to be recovered and reused. It has been speculated that improvements in the use of the tar byproduct could reduce conversion process costs by up to thirty-three percent.

In addition to these more traditional practices, laboratories are developing new methods combining known processes to harness the energy within biomass. At Purdue University, scientists are touting the new hybrid hydrogen-carbon process (H_2CAR) as a way to use biomass and hydrogen, supplied from carbon free sources, to synthesize biofuels. This process treats energy in a different manner. Rather than looking to one single source of energy, biomass or fossil fuels are simply viewed as sources of carbon atoms for hydrocarbon synthesis. The hydrogen atoms are obtained from carbon-free sources such as nuclear energy, wind energy, or solar power. In addition to providing the hydrogen atoms, the alternative source will provide the energy to run the entire process. The land area needed to capture the hydrogen and run the hybrid hydrogen-carbon process from solar power would be much less than the land area needed to create an equal amount of fuel from current biomass conversion methods. There are a number of major benefits to this system. For one, the processing of coal creates no carbon dioxide emissions. Because there is no carbon dioxide waste, the amount of energy yielded from the energy sources is vastly increased.

The biomass land area required for this process is calculated to be less than forty percent of the biomass land area needed for other conversion processes that use solely biomass. Some scientists believe that this process could allow the entire United States transportation sector to use cellulosic ethanol, rather than the thirty percent predicted by other methods. This process can also be used to reduce emissions from coal to liquids conversions. Using the H_2CAR process, no additional carbon dioxide is emitted from the conversion from coal to petroleum.

In addition, the hydrogen used in the process could be part of a loop arising from the development of a hydrogen based economy. This conversion process also makes the best use of our current transportation infrastructure, requiring no reorganization. However, for this process to work long-term it will require a hydrogen economy and the development of a carbon-free hydrogen source.

There is also ongoing research to develop instruments to precisely measure the compositions of the biomass input as well as the final product. NREL has recently developed an instrument that uses near infrared spectroscopy to determine the composition of a sample. This involves reflecting light off the sample and examining the infrared spectrum. When subjected to infrared light, the chemical bonds in a sample "waggle" in response to the infrared energy. Different chemical functional groups, such as the hydroxyl group (OH), will "waggle" at different frequencies. These shifts show up on the infrared spectrum and allow for the identification of the unknown sample. This new technique is much more cost effective and results can be obtained much more quickly than with the traditional wet chemistry analysis. Through this process, biorefinery personnel can adjust the parameters of the conversion process to best suit the incoming biomass.

NREL is also employing techniques involving molecular beam mass spectroscopy and nuclear magnetic resonance spectroscopy to take measurements to predict adult plant composition when the sample is merely a sprout. In addition to this data, genetic information can be used to engineer the ultimate cellulosic ethanol feedstock.

There is also considerable research done to investigate unconventional sources of biomass. GreenFuel Technologies Corporation in Massachusetts is conducting experiments with algae farms, growing the algae in closed systems called algae-solar bioreactors. These plants are microalgae selected for their amazing growth rates and ability to grow in specific climate conditions. Algae plants have a number of advantages over other sources of biomass. Algae are the fastest growing plants in the world and can be grown year round. The plants don't require clean water or farm land, thereby eliminating competition with food crops. The entire biomass for algae can be used to produce energy, unlike current ethanol production with other fuel crops. Amazingly, these crops are naturally suited to these activities, completely free of genetic modification.

GreenFuel has determined that algae can be grown commercially on farms, starting with a 247 acre algae farm to be in operation within the next few years. GreenFuel's economic analysis indicates that algae farming will be an economically successful process. Algae also shows potential to be a crop with a negative carbon effect. GreenFuel does not currently use carbon dioxide from the atmosphere to grow algae, but rather from power plants and cement plants, thereby increasing algae yields as well as preventing carbon dioxide from escaping into the atmosphere. According to GreenFuel, a single acre of algae will consume 500 metric tons of carbon dioxide per year, consuming forty percent of the carbon dioxide emitted from a plant with round-the-clock operation. These systems also emit no nutrient run off, but instead the nutrients are recycled for maximum efficiency.

Hemp has also shown promise as a good source of biomass and has been touted as the most efficient source of biofuels. According to the American Society of Agronomy, hemp could serve as a valuable cover crop for vegetable producers. Biodiesel made from hemp can safely be used in any diesel engine with little or no modification. However, there are several barriers to the use of hemp as a commercial biofuel. Hemp yields are low and there is currently only limited production allowed. Especially in the United States, hemp is considered a niche crop and it is difficult for farmers to receive government approval. However, if United States farmers could gain permission to grow the crop, production numbers would receive the boost needed to make hemp a viable biomass source.

In addition to these advances, the renewable fuels standard has given cellulosic ethanol a boost. The standard, enacted in December, mandates the use of 100 million gallons of cellulosic ethanol by 2010. In addition to this, the mandate also demands an increase to one billion gallons by 2013. Automobile companies are also beginning to experiment with cellulosic ethanol. According to Agriculture Online, General Motors has announced plans for the construction of a high-tech cellulosic ethanol manufacturing facility.

In conclusion, advances are being made in every discipline of biofuels production. More efficient, cost-effective enzymes are being produced, more resistant “super yeast” are being engineered, and biorefinery designs are undergoing renovations for maximum efficiency. Better analytical tools and computer models are also being developed to convert every morsel of energy contained within biomass. Taking this information into account, as well as cellulosic ethanol production mandates, I feel that our nation is capable of developing a sustainable cellulosic ethanol industry. Mandates and subsidies may be required until technology improves, but it would be foolish to ignore the prospect of cellulosic ethanol when the world’s most abundant renewable fuel supply is only a few technological breakthroughs away from being available for commercial use. Among these breakthroughs, I feel that cellulosic ethanol processed through the hybrid hydrogen-carbon process shows the most potential to solve our energy woes in the long-term.

However, it is absurd not to invest considerable time and money in exploring unconventional sources of biomass. Algae especially has shown promise as an eco-friendly source of biomass. All of these advancements in conjunction with one another will result in a thriving cellulosic ethanol industry in the United States.

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