BIOETHANOL PRODUCTION BY USING MICROBIAL ENZYME

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ABSTRACT

Bioethanol has gained prominence as an alternative sources of energy due to the predictable exhaustion of fuel energy supply. The bio ethanol production during microbial fermentation provides with an inexpensively competitive source of energy. Today most of the ethanol manufactured in the important roles is produced from microbes. Existing technology is based on enzymes conversion to ethanol use marine microbes. To confirm the role of cellulase enzyme in ethanol production, carboxy methyl cellulase activity was assayed. Hydrolysis of cellulosic material by concentrated sulphuric or phosphoric acid is a relatively old process. In general, concentrated phosphoric acid is used followed by dilution with water to dissolve an hydrolyse or convert the substrate into sugar. In addition to ethanol could also be produced from lignocellulosic biomass (grasses, agriculture residues, such as cobs, stalks, and leaves, wood wastes, fast growing trees, sugar wastes, citrus and rice wastes, non-edible parts of plants. Due to the complex of nature of the carbohydrates present in lignocellulosic biomass, a significant amount of xylose and arabinose sugars derived from the hemicellulose portion of the lignocelluloses is also present in the hydrolysate. Hydrolysate obtained by acid pretreatment is used for fermentation by microorganisms. future large scale use of ethanol will most certainly have to be based on production from lignocellulosic materials. The new technologies required and the advanced in recent years to bring lignocellulosic ethanol towards industrial production.

Key words: Microorganism, Cellulase, hydrolysate.

INTRODUCTION

Biothanol production has gained prominence as an alternative sources of energy due to the predictable exhaustion of fuel energy supply (Zaldivar *et al*. 2001). The bio ethanol production during microbial fermentation provides with an inexpensively competitive source of energy (Yasuyuki *et al*. 2011). The bio ethanol production from renewable sources provides environmental protection by reducing global warming, cost effective development, and energy protection in the present context of raising emissions of green house gases with the rapidly exhausting oil resources (Kathiresan *et al*. 2011). Ethanol producing microbes has attracted much attention because of their growth rate, higher than that of the microbes conventionally used for commercial production of bioethanol. A microbial treatful conversion of cellulosic biomass is performed by mixtures of hydrolytic enzymes collectively known as cellulases (Intriago, (2012). Some of the exogluganaes initiate their action from the end of cellulose chains and liberate cellobioses along with the cellulose chains (Murashima *et al*. 2002). The transformation of cellulose into ethanol by means of cellulase is the recent drift in biofuel industries (Sun and Cheng 2002). Ethanol production from sugars derived from starch and sucrose has been commercially achieved using by the yeast, *sacchromyces cerevisae* (Lin and Tanaka 2006; Tian et al. 2009).

Utilization of cellulosic biomass is more complex than using the pure cellulose given that, lignocelluloses is multifarious structure, in which cellulose are encapsulated in lignin by hydrogen and covalent bonds, which construct the cellulose unapproachable for reaction with hydrolysis agent (Zinoviev *et al*. 2010). The conversation into existing fermentable sugers is the deciding element for the overall economization for the production of ethanol lignocelluloses (Chandal *et al*. 2011).

Various industries utilize huge volumes of cellulose wastes which provide a low-cost and sustainable resource for production of ethanol (Das and Singh 2004). Cellulose can be successfully hydrolyzed and depolymerized into fermentable sugars by the enzyme cellulose. This review is listening carefully on bioethanol production of cellulose, lignocellulose and hemicelluloses based on hydrolysed of enzymes and fermentation of the monomers into bioethanol. A further understanding of the ethanol fermentation needs to be reached and the current status of ethanol fermentation including biomass resources, microorganisms, technology, the practical examples, and especially the promising prospects of ethanol fermentation.

Sources and composition of microbial Bio-ethanol

Source of cellulose

Today most of the ethanol manufactured in the important roles is produced from microbes. Existing technology is based on enzymes conversion to ethanol use marine microbes. Ethanol production is carried out through a multistep process in a closed-loop bio-refinery. The major process for a fermentation of the microbial enzymes and the microorganisms was discovered in 1986 on the of Mediterranean sea floor feeding on carbohydrates in the super-heated, acidic ocean waters surrounding undersea volcanic vents. They genetically modified it so that the microbe could give food to straight on carbon dioxide gas, and do so at much lower temperatures than its original sea-floor habitat. The genetic modifications to the microbes allowed it to produce butanol, a biofuel that glow much like a conservative fossil fuel

Microbial enzymes involved from ethanol production

To confirm the role of cellulase enzyme in ethanol production, carboxy methyl cellulase activity was assayed. The amount of reducing sugar formed was measured by the method as (Miller,1987). One unit of CMC activity was defined as the amount of enzyme that liberated one µ mol equivalent of glucose under the assay condition. The cellulase activity was estimated after incubation for 24-120 hr. There are so many factors which can involve in the enzymatic activities and minimize that cost are required. In an ethanol production, the majority strictly parameters are temperature and pH. Fundamentally, the high temperatures present the higher productivity (Fig 1).

However, above a optimistic temperature, the enzymes starts down its activity. Also, an enzyme has an optimal pH. In the range, the enzymes shows the high production, the pH changes drastically from the range, the enzyme loses its activity again, this phenomenon is same as one with high temperature, that is to say, the extreme pH can break enzyme formation and it cannot be recovered.

Cellulosic microbial biomass composition

Cellulose

Hydrolysis of cellulosic material by concentrated sulphuric or phosphoric acid is a relatively old process. In general, concentrated phosphoric acid is used followed by dilution with water to dissolve an hydrolyse or convert the substrate into sugar. This process provides a complete and rapid conversion of

cellulose to glucose and hemicelluloses to 5- carbon sugars with little degradation (Kathiresan *et al*. 2011). Enzymatic hydrolysis can be applied at different levels of process integration: separate hydrolysis of fermentation, simultaneous saccharification and co-fermentation (Hamelinck *et al*. 2005).

The efficiency of enzymatic hydrolysis depends on the appropriate proportional ratio of the cellulose components. SHF process is carry out in two way special vessels and each step can be done under optimal conditions of pH and temperature. In the SSF, the enzymatic saccharification and fermentation process are run in the same vessels and glucose released by the action of cellulases converted directly into ethanol by the fermenting microorganisms. When the enzymatic system (cellulase) acts invitro on insoluble cellulose substrate, three significant process occur simultaneously: chemical and physical changes in cellulosic fraction: primary hydrolysis, which involves the release of soluble sugars from the surface of cellulosic molecules: secondary hydrolysis, which involves hydrolysis of soluble sugars to lower molecular weight sugars and finally to glucose (Mosier *et al*. 2005). Cellulosic ethanol is a new approach that may relieve land use and related concerns. The obvious advantage of cellulosic ethanol is its dependence on abundant and diverse raw materials rather than traditional feed stocks, and because humans cannot digest cellulose, it does not compete with food production.

Further more, exploiting the cellulose in corn plants or sugarcane rather than the kernels, could double corn's ethanol yield (Pimentel, 2001). The ethanol production from corn yields 25% more energy than invested in its production (Hill *et al*. 2006). Full enzymatic hydrolysis of crystalline cellulose requires synergistic action of three major types of enzymatic activities: endoglucanases (EGs) (1,4 – β- D-glucan 4-glucanohydrolases: EC 3.2.1.4): exoglucanases, including cellodextrinases (1,4- β- DD glucan glucanohydrolases: EC 3.2.1.74), and cellobiohydrolases (CBHs) (1,4- β- D- glucan cellobiohydrolases: EC 3.2.1.91) and β- glucosidase (BGL) (β-glucoside glucohydrolases: EC 3.2.1.21) (Zhang and Lynd 2004). Its has been suggested that the cellulosome provide anaerobic microorganisms with an advantages to degrade cellulose more efficiently, since cellulosomal cellulases degrade cellulose in a simultaneous manner rather than in a sequential manner (Murashima *et al* 2002).

The residual cellulosic fraction of biomass can then be enzymatically hydrolysed to glucose. Pretreatment method in biomass processing the lignin, hemicelluloses and cellulose can be simply separated. both the cellulose hemicelluloses hydrolyzates produced by this process can be fermented to ethanol by suitable yeast(Cao *et al*. 1996).one of the most capable ethanol producing yeast is saccharomyces cerevisiae which has a high tolerance to ethanol and other inhibitory compounds resulting from acid hydrolysis. Since wild strains of this yeast cannot ferment pentose such as xylose, arabinose and oligosaccharides, production of bioehanol from lignocellulosic hydrolysate is inadequate(Katahira *et al*. 2006).

Lignocellulase

In addition to ethanol could also be produced from lignocellulosic biomass (grasses, agriculture residues, such as cobs, stalks, and leaves, wood wastes, fast growing trees, sugar wastes, citrus and rice wastes, non-edible parts of plants and municipal wastes) (Lynd *et al*. 2005). Bioethanol production from cellulosic fraction of lignocellosic materials involves: hydrolysis of polysaccharides and fermentation of the monomers into bioethanol. Enzymatic hydrolysis is catalyzed by cellulolytic enzymes and fermentation is carried out by bacteria, yeasts or fungi. (Buruiana *et al*. 2013).

After pretreatment, the next step in the biochemical process of bioethanol production from lignocellulosic materials(LCM) are: enzymatic hydrolysis of polysaccharides and fermentation of

monosaccharides into bioethanol. They can be performed separately or simultaneously (Tomas-Pejo *et al*., 2008).

Microbial fermentation

Due to the complex of nature of the carbohydrates present in lignocellulosic biomass, a significant amount of xylose and arabinose sugars derived from the hemicellulose portion of the lignocelluloses is also present in the hydrolysate. For example, in the hydrolysate of corn stover, approximately 30% f the total fermentable sugar are xylose. Thus the ability of the fermenting microorganisms to utilize the whole range of sugars available from the hydrolysate is vital to increase the economic competitiveness of cellulosic ethanol. In recent years, metabolic engineering for microorganisms used in fuel ethanol production has shown significant progress. Besides saccharomyces, Bacteria such as Zymomonas mobilis and Escherchia coli have been targeted for metabolic engineering to improve their fermentation abilities, and thus improve cellulosic ethanol production (Jeffries and Jin, 2004). Microorganisms such as have been targeted through metabolic engineering for cellulosic engineering for cellulosic ethanol production. Recently, engineered yeast have been described efficiently fermenting sugars (Brat *et al*. 2009; Ohgren *et al*. 2006; Becker and Boles, 2003; Karhumaa *et al*. 2006). Yeast cells are especially attractive for cellulosic ethanol processes because they have been used in biotechnology for hundreds of and are tolerant to high ethanol, inhibitor concentratios and can grow at low pH values to reduce bacterial concentration.

Cellulose to ethanol fermentation

Hydrolysate obtained by acid pretreatment is used for fermentationby microorganisms. Because the hydrolysate include not only glucose, but also different monosaccharides such as xylose, galactose, mannose arabinose and oligosaccharides, microorganisms are compulsory to ferment these sugars (Katahira *et al*. 2006). These microorganisms can use carbohydrates with 6-carbon atoms, one of the most common being glucose. Cellulosic materials containing elevated stage of glucose or glucose precursors are most easily converted into bioethanol (Balat *et al*. 2008). There is a number of microorganisms that produce significant amounts of bioethanol (Steward and Russell, 1987). Xylose fermenting microorganisms are bacteria, yeast and filamentous fungi. (Hahn-Hagerdal *et al*. 2006). Reported that the adverse effects of lignin on cellulases can be surmounted by ammoniation and various N compounds. Moreover, theenzymatic treatment can be accomplished simultaneously with the engineered co-fermentation microbial process known as simultaneous saccharification and fermentation (SSF) (Bisaria and Ghose, 1981).

Production of biohydrogen and bioethanol through microbial fermentation are well known processes but thermophiles have many advantages compared to mesophilic microorganisms concerning fast growth rates and their ability to degrade a broad variety of substrates. Furthermore, many theromphiles produce fewer types of undesired and products compared to mesophiles (Sommer et al. 2004; Groenestijn *et al*. 2002).

Particular interest in targeting bioethanol production that can be derived from lignocellulosic biomass materials where both hexose and pentose sugars are available from the cervisiae is not able to ferment sugars other than hexose, an optimal fermentative microorganism should be tolerant to a high ethanol concentration and to chemical inhibitors formed during pretreatment and hydrolysis process. In response to this inability of S. cerevisiae to ferment pentose sugars, extensive efforts have been employed to develop genetically engineered microorganisms that are capable of fermenting pentose and hexose sugars simultaneously. An optimal fermentative microorganisms shoult be able to utilize both hexose and pentose simultaneously with minimal toxic end-products formation (Martinn *et al*. 2002). In an effort to summarize relevant advantages and major limitations of microbial fermentative species, potential microorganisms for

lignocellulosic-based biofuel fermentation including bacteria, yeast and fungi that could be optimized and become potential avenues to enhance alcohol yield and productivity in large-scale lignocellulosic-based ethanol fermentation (Ladisch *et al*. 2010).

Separation of ethanol

Bioethanol obtain from a fermentation conservation requires further separation and purification of ethanol from water through a distillation process. Fractional distillation is a process implemented to separate ethanol-water based on their different volatilities. This procedures consists purely of boiling the ethanol-water mixture. Because the boiling point of water (100°C).is higher than the ethanol-boiling point (78.3°C), ethanol will be converted to steam before water. Thus, water can be separated via a condensation procedure and ethanol distillate recaptured at a concentration of 95% (Cardona and sanchez, 2007). Liquid mixture are heated and allowed to flow continuously all along the column. At the top of thr column, volatiles are separated as a distillate and residue is recovered at the bottom of the column.

Economic Competitiveness and Net Social Benefits

Subsidies for otherwise economically uncompetitive biofuel are justified if their life-cycle environmental impacts are sufficiently less than for alternatives. In 2005, neither biofuel was cost competitive with petroleum-based fuels without subsidy, given then- current prices and technology. In 2005, ethanol net production cost was $0.46 per energy equivalent liter (EEL) of casoline (National Agricultural Statistics service 2005; Energy Information Administration 2006), while wholesale gasoline prices averaged $0.44 liter (Energy Information Administration 2006). Estimated soybean biodiesel production cost was $0.55 per diesel EEL (Fortenbery, 2005), whereas diesel wholesale prices averaged $0.46 liter (Energy Information Administration 2006). Further increase in petroleum prices above 2005 average prices improve the cost competitiveness for biofuels. Even when not cost competitive, however, biofuel production may be profitable because of large subsidies. In the U.S., the federal government provides subsidies of $0.20 per EEL for ethanol and $0.29 per EEL for biodiesel (shapouri and Gallagher, 2005). Demand, especially for ethanol, also comes from laws and regulations mandating blending biofuels in at least some specified proportion with petroleum. Ethanol and biodiesel producers also benefits from federal crop subsidies that lower corn prices (which are approximately half of ethanol production`s operating costs)and soybean prices.

Bio-ethanol-tomorrow from the residue of today

The increased concern for the security of the oil supply and the negative impact of bioethanol fuel on the environment, particularly greenhouse gas emissions, has put pressure on society to find renewable fuel alternatives. The most common renewable fuel today is ethanol produced from sugars or grains(starch). Consequently, future large scale use of ethanol will most certainly have to be based on production from lignocellulosic materials. The new technologies required and the advanced in recent years to bring lignocellulosic ethanol towards industrial production. One of the most challenges is to optimize the integration of process engineering, fermentation technology, enzyme engineering and metabolic engineering.

Conclusions future prospects

Cellulosic ethanol production from cellulosic biomass is a globally developing technology. One of the major issues for cellulosic ethanol production is enzyme hydrolysis by the naturally available strains to convert cellulose to glucose. Developing a single strain for efficient cellulosic ethanol production is the technical challenge. The cellulosic ethanol production could be increased by over expressing the genes and

optimizing the fermentation conditions for altering cellular metabolism for higher ethanol tolerance. The challenges for different process integration technologies for bioethanol production from cellulosic, lignocellulosic and hemicelluloses material are to obtain high degree of hydrolysis and high ethanol yields compared fermentation technology, where each stage takes place under optimal operating conditions(minimizing the interaction between hydrolysis and fermentation), the main fermentation advantages are as follows 1. Obtaining higher ethanol yield with small amount of enzymes; 2. Increasing the hydrolysis rate by sugars conversion ; and 3. Lower requirements under sterile conditions, because glucose is removed immediately by producing bioethanol. In fermentation process, enzymatic hydrolysis is releasing continuously hexose sugars, so that pentose sugars are fermented faster and with better yields. Our greatest challenge and the most essential are to find means for ethanol production. The bioethanol we use today is from petroleum. While the world`s petroleum reserves will decline gradually over the next 20-40 years.

Cellulosic- based biofuel is a potential alternative over food derived bioethaol originating mainly from microbes. Pretreatment, the most costly step is of particular concern due to the high recalcitrance of lingo-cellulosic raw materials. Given that lingo-cellulosic feedstock is a versatile material and bioethanol is a commodity products, it has been deemed imperative to design a general pretreatment combination that would be effective against a wide range of cellulosic material and hence deal with feedstock variability. For instance, researchers have shown that pretreatments involving steam explosion with either catalyst or lime are potential candidates to microbial residues. These processes are typically associated with thermophilic and and cellulolytic microorganisms including organisms such as T. reesei along with P.chrysosporium, K. marxianus and C.cellulolyticum with some of them possessing fermentative abilities in addition to their hydrolytic properties. Conjunction to rapid molecular biology techniques, mathematical modelling including MRA and biotechnology risk assessment (BRA) can be used to ensure greater predictability for limiting antibiotic resistant microflora and GMO dissemination during operation. While technological accomplishments and multiple research coalition efforts are still progressing, an efficient combination of the most advanced systems analysis and economical techniques designed to cope with feedstock versatility and commodity should emerge as the option of choice in an attempt to achieve optimal second- generation biofuel performance.

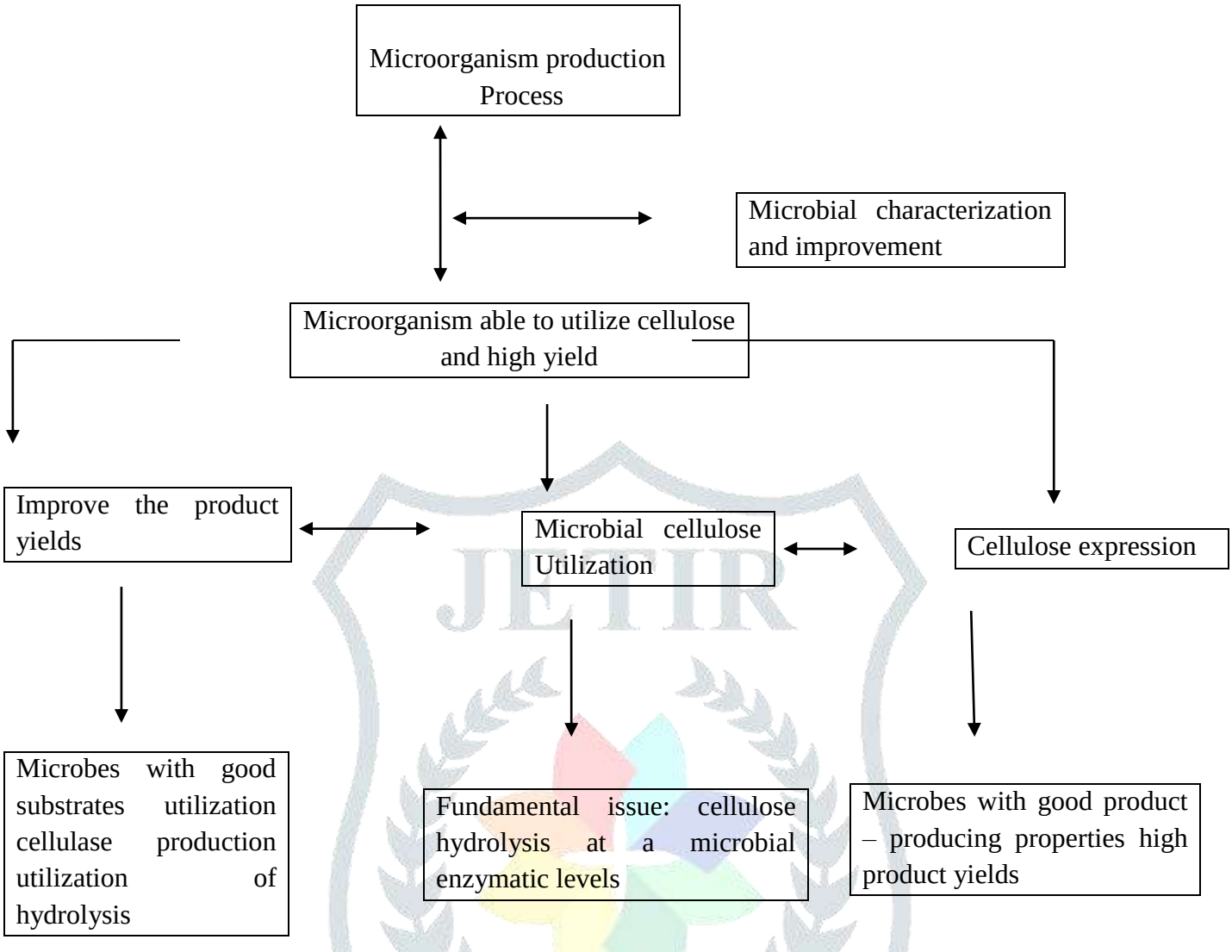
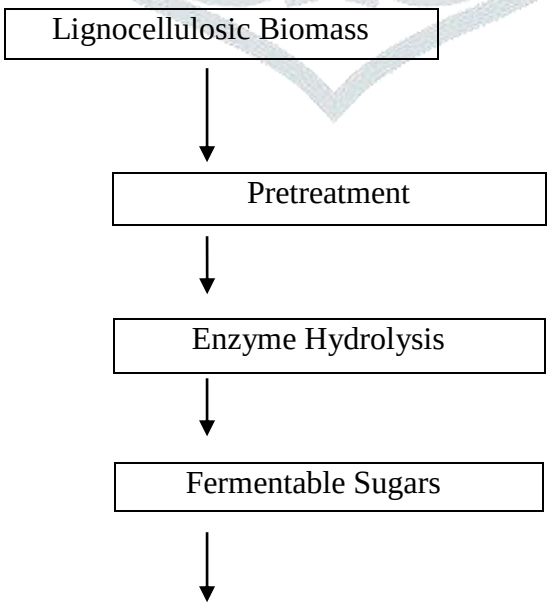


Figure:1 Microbial enzyme ethanol production



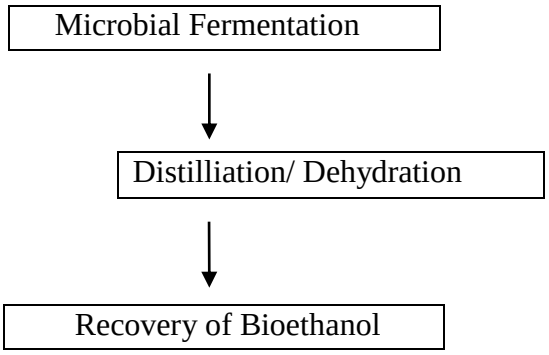


Figure 2. Schematic diagram of bioethanol production by fermentation process of cellulosic biomass

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