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Bioutilization of the distillery stillage of different grain species from bioethanol production

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Abstract. Wastewater from bioethanol plants is classified as highly concentrated in terms of organic pollution precisely due to distillery stillage. The main problem in the disposal of distillery stillage is the processing of the liquid phase, the volume of which is up to 92% of all wastewater from a bioethanol plant. The existing wastewater treatment technologies of a bioethanol plant can be conditionally divided into four types: evaporation, aerobic biological treatment with fodder yeast production, anaerobic stillage treatment with biogas production, combined schemes. The aim of our work was to study a combined method for cleaning grain stillage by the anaerobic-aerobic method with the immobilization of microorganisms on a fibrous carrier. Physicochemical parameters of grain stillage and purified methane mash were determined according to generally accepted methods for analyzing wastewater from distilleries.

Under anaerobic conditions, biogas was formed from distillery stillage, including low molecular weight organic compounds – methane, carbon dioxide, organic acids. After the first anaerobic stage of treatment, the pollution of wastewater decreased by 8-10 times, after which it was fed to the aerobic stage of post-treatment, which was carried out by microorganisms immobilized on a fixed carrier, which reduced the removal of biomass with the flow of purified water and improved treatment performance. The chemical oxygen demand (COD) of methane mash after the 1st stage of anaerobic fermentation was 1360 mg/dm^3 compared to the initial COD of grain stillage of 15800 mg/dm^3, which ensured a purification efficiency of 91.4%. The purification efficiency according to biochemical oxygen demand in five days (BOD_5) was 97.5%. After the aerobic stage, the purification efficiency was 98.2% in terms of COD and 99.8% in terms of BOD_5. The values of the content of total phosphorus also decreased by almost 20 times, nitrogen – by 9 times, sulfates – by 5 times. The advantages of the proposed method of wastewater treatment of bioethanol plants over existing ones are the ability to treat wastewater with any concentration of pollutants and additional obtaining of fuel – biogas, which can be used to replace natural gas, solving the problem of removing the biomass of microorganisms from the purification zone due to their fixation on a fibrous fixed carrier.

Keywords: grain stillage, wastewater treatment, anaerobic fermentation, aerobic stage, immobilization.

1. Introduction

Currently, in the alcohol industry there is a difficult environmental situation with the disposal of the main production waste – distillery stillage. In 2021, 103.3 billion liters of ethanol were produced by yeast-based fermentation of agriculture-derived carbohydrates (Renewable Fuels Association, 2022). Approximately 30% of this volume was produced from Brazilian cane sugar (mainly consisting of sucrose) and approximately 55% from corn starch derived glucose, mainly in the United States of America (Renewable Fuels Association, 2022). For a grain distillery with a capacity of 3000 dal of alcohol per day, the yield of raw post-alcohol stillage is 330-350 m^3 (Zielińska et al., 2021). Thus, the grain distilleries produce 625 million m^3 of grain stillage per year.

The lack of effective and economic methods for their purification leads to the accumulation of wastewater in artificial reservoirs, pollution of the environment, soil and groundwater with harmful substances and the formation of greenhouse gases in the amount of more than 6-7 million m^3 per year. In most countries of the world it is forbidden to dump stillage into water reservoirs or sewers without prior treatment (Directive, 1991). This is primarily due to the large amount of organic substances, proteins and ashes contained in the stillage (Potapova & Golub, 2018).

In the production of alcohol from grain raw materials, only one third of the dry matter of the grain is converted into alcohol, and the remainder of the unfermented substances passes into the stillage, the main part of which is proteins, ash substances and fats. The total content of proteins in the dry matter of stillage is two times higher than in the original grain raw material (Mikucka & Zielińska, 2020).

Fresh distillery stillage of grain has a light brown color with a characteristic bready odor. But after the stay in the septic tanks, it is gradually darkening and turns brown. Putrefactive processes begin to develop in it, caused by the presence of microorganisms, under the influence of which the enzymatic hydrolysis of nitrogen-containing organic compounds (proteins, amino acids) occurs with the formation of metabolic products – acetic, formic, isopropionic acids, ammonia, hydrogen sulfide. They are characterized by an unpleasant pungent odor. Organic pollution as a result of the discharge of stillage by a medium-capacity distillery is adequate to the pollution from a city with a population of 100,000 people. That is why one of the key issues of alcohol production is the comprehensive disposal of distillery stillage, which allows not only increasing the profitability of production, but also ensuring its environmental safety.

In the process of obtaining bioethanol, almost all nutrients remain in the stillage, with the exception of starch and sugars, so the stillage is a valuable feed product, regardless of the type of raw material having been used (Table 1) (Ghosh Ray & Ghangrekar, 2019). The yield of alcohol from a unit of raw materials and the amount of grain bard formed are given in Table 1 in decalitres. One decalitre (dal) corresponding to 10 litres.

Table 1. Consumption of raw materials for the production of bioethanol and its feed value (feed units) (Bukhkalo et al., 2019).

Type of raw material	**Yield of alcohol from a unit of raw material, dal/t**	**Feed value of raw material, feed units/1 kg**	**Yield of stillage, dal/dal of alcohol**	**Feed value of stillage, feed units/1 kg**
Potato	9.8	0.30	12.0	0.04
Sugar beet	9.0	0.26	12.0	0.04
Wheat	36.0	1.20	12.0	0.09
Corn	37.5	1.34	12.0	0.12
Rye	35.4	1.18	12.0	0.08
Barley	29.8	1.21	12.0	0.09
Molasses	31.0	0.77	11.0	–

The main problem in the disposal of distillery stillage is the processing of the liquid phase, the so-called centrate, the volume of which is up to 92% of all wastewater.

The existing technologies for processing distillery stillage can be divided into four main technological schemes (Shafiquzzaman et al., 2022; Umesha et al., 2023):

- evaporation at evaporation stations;
- biological wastewater treatment by aerobic processing of the liquid phase with the help of microorganisms and the production of fodder yeast;
- anaerobic method of stillage cleaning with biogas production in digesters (Shinde et al., 2020; Kang et al., 2022);
- combined schemes (Junior et al., 2019; Shvorov et al., 2019).

Currently, there are two main directions for the processing of distillery grain stillage: processing into a dry feed product with subsequent granulation, the so-called "dry stillage" and microbiological processing to obtain feed products using unicellular microorganisms.

Dry granulated stillage is commercially available as DDGS (Distiller's Dried Grain with Solubles). This product is a valuable protein feed and is highly rated on the world market. The sale of dry granulated stillage makes it possible to cover 30% of production costs and to reduce the cost of alcohol by 15% (Probst et al., 2013; Semenova et al., 2022).

According to option 2, the native stillage is divided into centrate and meal in a centrifuge. The centrate is evaporated on the evaporation plant from 2.6 to 30-35% of solids. Then the

concentrated centrate is mixed with grain and dried in dryers. The condensate formed during the evaporation of stillage centrate with other distillery wastewater is purified in biotanks with immobilized microorganisms. The output of dry stillage according to the 2nd disposal option at the distillery with a capacity of 3000 dal of alcohol is higher and amounts up to 30 tons per day.
But this method requires large capital costs. The cost of equipment according to the first method is about USD 80,000, and according to the second is USD 1,360,000.

The most well-known technology for processing stillage into biogas is based on anaerobic fermentation, when the stillage is fed into special containers along with anaerobic bacteria that convert nutrients into biogas. Zorg (Germany) has developed a technology for processing post-alcohol stillage into biogas according to the classical scheme or according to the technology of vertical reactors (https://zorg-biogas.com). Under anaerobic conditions, biogas is formed, including low molecular weight organic compounds – methane, carbon dioxide, organic acids and other minor impurities.

According to Pimentel et al. (2007); Filik İşçen & Ilhan (2008); Junior et al. (2019); Shvorov et al. (2019), the most promising and cost-effective method is the biological treatment of wastewater from alcohol production. For this, two-stage aerotanks are used, the technological characteristics of which can only be established experimentally, taking into account the specific conditions of the main production.

At bioethanol plants, the most favorable conditions for organizing biogas production are raw materials (waste) with a temperature of 40–50°C, as well as secondary heat sources (condensates, lutherwater, etc.). All this makes it possible to organize the production of biogas without the cost of the resulting bioenergy fuel for heating the medium in digesters.

The combination of anaerobic and aerobic methods makes it possible to purify wastewater to levels that allow it to be discharged into water bodies. Thus, several problems are solved simultaneously: energy, environmental and waste disposal (Bukhkalo et al., 2019; Okolie et al., 2022).

For treatment of high-strength (total chemical oxygen demand [CODtot] up to 20 g/L) industry wastewater combined biologic and chemical method was suggested. As a first step for COD elimination, wastewater is treated in anaerobic bioreactor, and then the chemical coagulation with Fe(III) (Sklyar et al., 2003).

At the current stage of development of technologies for the disposal of stillage residues of bioethanol plants, the intensification of the wastewater treatment process and the reduction of capital and operating costs due to this are of great importance. Traditional biological facilities are unstable due to the uneven supply of wastewater for treatment both in terms of quantity and quality of treated effluents with a significant fluctuation in COD values. We have developed a technology for stillage disposal and two-stage wastewater treatment with biogas production.

We have proposed a combination of an anaerobic method for purifying the liquid fraction of grain stillage with aerobic post-treatment of it in biotanks using an activated sludge biocenosis immobilized on a fixed inert carrier. In our opinion, this will increase the organic load of pollutants on the bioreactor, obtain a secondary product – biogas, which can be used as an additional type of fuel, solve the problem of excessive removal of activated sludge biomass from the reaction zone with a wastewater stream and reduce capital costs for the construction of treatment plants.

The aim of our work was to study a combined method for cleaning grain stillage by the anaerobic-aerobic method with the immobilization of microorganisms on a fibrous carrier. The uniqueness of the proposed technology lies in the combination of an anaerobic method for utilizing the liquid fraction of grain stillage and an aerobic method for its post-treatment with microorganisms immobilized on a fixed carrier in a mixture with other less polluted wastewater from the enterprise, which will reduce the load in the terms of organic pollution.

2. Material and Methods

The objects of research were corn post-alcohol stillage, methane mashes, anaerobic-aerobic processes treatment of wastewater, wastewater after treatment. Physicochemical indicators of grain stillage were determined according to generally accepted methods of analysis of wastewater from distilleries (APHA, 2005).

Chemical oxygen demand (COD) in wastewater was determined by the dichromate method (ISO 6060, 1989). Five-day biochemical oxygen demand (BOD_5) in wastewater was determined by the a bioassay procedure that measures the oxygen consumed by bacteria from the decomposition of organic matter (Delzer & McKenzie, 2003). The Kjeldahl method was used to determine total nitrogen in the post-alcohol stillage (ISO/TC 34/SC 4: ISO 20483, 2013), and phosphorus was determined by the ammonium molybdate spectrometric method (ISO 6878, 2004).

Chloride content was determined by the argentometric method (Harris, 2010). Determination of volatile fatty acids was carried out for the distillation method (Lie &Welander, 1997).

3. Results and Discussion

One of the most well-known and affordable methods of converting the products of photosynthesis into a technical form of energy is anaerobic fermentation with the production of methane. The developed technology was based on the vital activity of microorganisms that use the stillage and wastewater from distilleries as a nutrient medium.

The raw material for this process was grain stillage, selected after the distillation of alcohol from the fermentation column. It was fed to the filtration stage, where it was separated into a liquid fraction – stillage filtrate and a solid fraction – dry stillage residue. The solid fraction was pressed to remove excess moisture and a wet grain stillage concentrate was obtained. Wet concentrate can be sold as feed for farm animals, if direct sales are established with livestock farms. Otherwise, the wet concentrate was dried to a final moisture content of 10-12%, packaged and sold as ready-made dry feed.

Since the leachate of grain stillage has high indicators of chemical and biological oxygen demand, we proposed to mix it with domestic wastewater, which has a low index of COD and BOD_5 in order to reduce the organic load on the anaerobic bioreactor. An anaerobic-aerobic method of wastewater treatment was proposed.

Figure 1 shows the basic technological scheme of complex processing of distillery grain stillage. First, anaerobic treatment was carried out in an upflow anaerobic sludge blanket (UASB) reactor. The use of modern anaerobic bioreactors makes it possible to maintain a high concentration of biomass in the fermentation zone (60-80 g/dm^3), increase the load to 17-18 kg COD/m^3 of the bioreactor and reduce the treatment time to 1.5-2 days. Under the influence of methane bacteria, the indicators of chemical and biological oxygen demand decreased, that is, biological purification of the bard took place with the formation of biogas with a methane content of up to 70-72%. Biogas was sent for burning in the production boiler room. The formed excess amount of activated sludge was removed from the bioreactor and used as fertilizer.

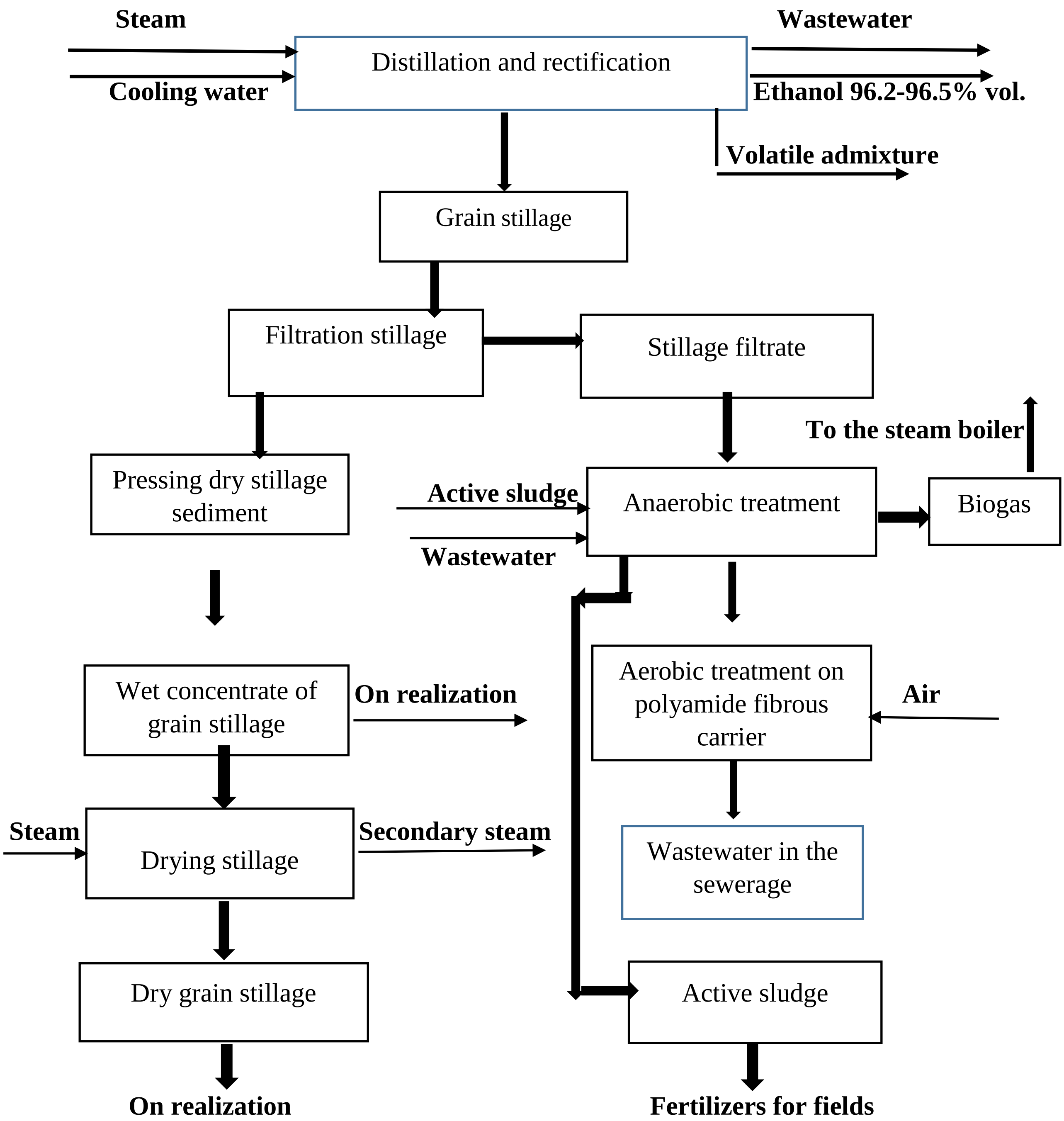

Figure 1. Basic technological scheme of complex processing of post-alcohol grain stillage

After the first anaerobic stage of treatment, the effluents had a pollution level 8-10 times lower and were fed to the aerobic stage of further purification in compact biotanks. Aerobic treatment was carried out by microorganisms immobilized on a stationary carrier. This technological technique makes it possible to fix a significant number of microorganisms-destructors of organic substances on the carrier, thanks to which the productivity of aerobic wastewater treatment increases, and there is no need disposing the excessive activated sludge, since there is practically no increase in it. A biologically inert polyamide fibrous carrier was used. After first anaerobic treatment, wastewater was further treated aerobically using microorganisms immobilized on a fixed carrier – a biologically inert polyamide fibrous carrier. The immobilization of aerobic microorganisms on the carrier makes it possible to reduce their entrainment with treated wastewater, to create a spatial succession, and thereby increase the efficiency of treatment.

Table 2 shows the physical and chemical indicators of anaerobic-aerobic treatment of distillery stillage. Corn was used as the raw material for alcohol production. The conditions of the experiment are mesophilic, continuous temperature mode of fermentation. In the start-up period, alkalization with sodium hydroxide was carried out to pH 7.95-8.

Table 2. Physical and chemical content of anaerobic-aerobic treatment of post-alcohol stillage from corn grain.

No.	**Parameters**	**Grain stillage**	**Methane mashes**	**Wastewaters after treatment**
1	pH	3.35±0.01	7.95±0.01	7.5±0.01
2	Biogas output, L/L stillage	-	12±0.1	-
3	Biomass carryover rate, l/day	-	7.8	1.56
4	COD, mg/L	15,800±1	1360±1	284±1
5	Efficiency of treatment for COD, %	-	91.4±0.1	98.2±0.1
6	BOD_5, mg/L	9,700±1	245±1	31.6±1
7	Efficiency of treatment for BOD_5, %	-	97.5±0.1	99.8±0.1
8	Dry residue, mg/L	24,340±1	2,980±1	185±1
9	Fried residue, mg/L	2,518±1	1,790±1	104±1
10	Content of volatile fatty acids, mg/L	200±0.01	720±0.01	-
11	Colloids, g/L	9.64±0.01	1.02±0.01	0.05±0.01
12	Nitrites, mg/L	0.284±0.005	0.009±0.005	0.001±0.005
13	Nitrates, mg/L	0.56±0.005	0.9±0.005	0.94±0.005
14	Total nitrogen for Kjeldahl, mg/L	2300±0.005	560±0.005	270±0.005
15	Ammonium nitrogen, mg/L	45±0.1	-	-
16	Total phosphorus, mg/L	250±0.005	65±0.005	12±0.005

17	Chloride content, mg/L	230±0.005	190±0.005	110±0.005
18	Sulphate content, mg/L	109±0.005	42±0.005	21±0.005
19	Potassium, mg/L	760±0.01	275±0.01	137±0.01
20	Sodium, mg/L	35±0.01	675±0.01	681±0.01
21	Calcium, mg/L	631±0.01	220±0.01	125±0.01
22	Amount of sulfides, hydrosulfides, hydrogen sulfide, mg/L	-	93.4±0.01	97±0.01

As can be seen from the data in Table 2, the methane mashes after the 1st stage of anaerobic fermentation had a COD value of 1,360 mg/L compared to the initial COD value of grain stillage being 15,800 mg/L, which provides a purification efficiency of 91.4%. The efficiency of treatment according to BOD was 97.5%. After the second stage of aerobic treatment, the wastewater had a COD value of 284.4 mg/L, and BOD_5 value – 31.6 mg/L. The treatment efficiency was 98.2% according to the indicator of chemical oxygen demand and 99.8% according to the indicator of biological oxygen demand for 5 days. Also, indicators of total phosphorus content decreased almost 20 times, total nitrogen content – almost 9 times, sulfates – 5 times. The increase in sodium content in the methane mashes was explained by the alkalinization of the medium to the optimal value pH 8. The increase in the content of sulfides, hydrosulfides, and hydrogen sulfide and the content of volatile fatty acids in the methane mashes was explained by the formation of these substances at the stage of anaerobic fermentation.

The results obtained indicate that the technology of anaerobic-aerobic purification proposed by us makes it possible to reduce the contamination of methane brew by 91.4% in terms of COD, and by 97.5% in terms of BOD_5 after the first anaerobic purification stage. The yield of biogas after the first stage of purification according to the proposed technology is 12 liters from 1 liter of stillage. The use of biogas in the boiler house of a distillery saves natural gas. Additional purification of methane brew at the second aerobic stage reduces wastewater pollution by almost 7% in terms of COD and by 2.3% in terms of BOD_5. The decrease in the BOD_5 of methane mash from 1,360 mg/L to 284 mg/L as a result of aerobic post-treatment was achieved by mixing methane mash with less polluted wastewater from the plant, which had COD values of 100-150 mg/L, which significantly reduced the load for organic pollution on the biotank and thus contributed to an increase in its productivity. The indicators of wastewater obtained as a result of anaerobic-aerobic treatment meet the requirements for their discharge into open waters or city sewers.

The immobilization of aerobic microorganisms on a biologically inert fixed carrier makes it possible to reduce their carryover with treated wastewater, thereby increasing the efficiency of treatment and reducing its duration. The fixation of microorganisms reduced the removal of activated sludge biomass by almost 5 times (Table 2). Thus, according to the main indicators of COD and BOD_5, the method of anaerobic-aerobic treatment of the liquid fraction of grain stillage mixed with less polluted wastewater of the plant, developed by us, makes it possible to reduce wastewater pollution by 98.2 and 99.8%, respectively.

4. Conclusions

It is possible to reduce the negative impact of bioethanol production on the environment by processing waste through anaerobic-aerobic fermentation of distillery stillage and wastewater.

The developed technology makes it possible to purify wastewater with any concentration of pollutants by mixing it with less polluted wastewater at the stage of aerobic post-treatment, to obtain additional fuel – biogas, which saves natural gas. At the first stage, the biodegradation of organic compounds was carried out by methane fermentation in anaerobic bioreactors with a high specific load and a rate of substrate decomposition. At the second stage, the stillage was purified under aerobic conditions with immobilized microorganisms on a fixed biologically inert carrier with high adhesion to bioreactors. This method reduced the removal of activated sludge biomass with treated wastewater by almost 5 times and created conditions under which each type of microorganism occupied its ecological cell, thereby increasing the purification efficiency.

According to the main indicators of chemical oxygen demand COD and biological oxygen demand BOD_5, the method of anaerobic-aerobic treatment developed by us made it possible to reduce wastewater pollution by 98.2 and 99.8%, respectively.

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