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THE ALCOHOLIC FERMENTATION*

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THE ALCOHOLIC FERMENTATION

for the production of POWER ALCOHOL, what means the alcohol for the use as fuel in motor cars, or dehydrated alcohol as feedstock in chemical industry.

The production of such alcohol, based on the use of renewable sources like whole plants, fruits of plants or wastes of plants in industrial scale is to be done with special emphasis to energy saving during the process in order to justify such undertaking.

The energy which is characterizing the POWER ALCOHOL as a liquid energy carrier has been accumulated by photosynthesis in various raw materials as sugar, starch, cellulose, pentosanes and the like, accompanied by proteins, fats, organic acids and inorganic compounds.

For the fermentative transformation of sugar and starch to ethanol, the main part of solids, available in the raw materials, is used. The remaining organic and inorganic compounds are to be found in the so-called slops or stillage, a heavy polluted liquid, running of the bottom of the distilling column.

In principle, we have to distinguish three different groups of raw materials, as there are:

Sugar containing solutions
starch containing suspensions and solutions from the cellulose industry.

Examples for the first group are

cane molasses,
beet molasses and
juices from sugar cane or sugar beet.

Beside the main product ethanol the following by-products can be obtained from raw materials belonging to the first group:

- pure carbon dioxide, which is free from carcinogenic substances
- fodder yeast of the *Saccharomyces* strain, containing at least 50 % of valuable proteins
- concentrated slops as an animal feed additive or as liquid fertilizer.

The second group of starch containing raw materials comprises

- corn and sorghum
- barley, wheat and rye
- potatoes and cassava, finally also
- starch containing wastes.

The by-product of this group is a valuable supplement for animal feeding, containing 20-30 % crude protein and available either as concentrated slupe or dried feed stuff.

To the last group of raw materials are belonging

- sulfite waste liquor of the cellulose factories and
- hydrolyzate of cellulose containing products.

Besides hexoses, which are responsible for the formation of ethanol, also a considerable part of pentoses and other organic compounds are present in such solutions.

The attached table 1 gives a general view over the energy repartition.

Figure 1a shows this repartition after processing of corn, meanwhile figure 2 shows it after processing of molasses.

Detailed data have already been given in one of the preceding reports.

The losses are caused by

- transformation into fermentation heat
- material losses through - preparation of raw materials
 - alcohol losses through escaping gases
- and - cleaning losses.

As far as the preparation of raw material is concerned, the three groups differ considerably in the proceedings.

Sugar containing solutions like diluted molasses or juices of cane or beet are treated in a continuous way:

After preparation of a well determined concentration, either by dilution or by pre-concentration, the solution is to be clarified by sedimentation or centrifugation in order to separate some sludge which might influence the subsequent fermentation in a negative sense.

Heating up of the sugar containing substrate follows, in order to minimize the risk of undesired salvage fermentations, which are occurring without sterilization. Some metabolites of such irregular fermentations are toxic for our yeast and therefore responsible for longer fermentation times and for reduced yields.

Regarding the utilization of starch containing raw materials, the preparation of a fermentable substrate is done after adequate cleaning or peeling, grinding or reaping of the cereals or tubers. Next step is the liquification of the starch particles by heat, assisted by the activity of alpha-amylolytic enzymes. The resulting suspension is now treated by amyloglucosidic enzymes to continue the degradation of starch to soluble and fermentable sugars.

As for the third group of raw materials sulfite waste liquor is the main representative. Its application is prepared by stripping off the inhibiting substances such as SO_2 , before inoculation of yeast strains resistant to low pH-values.

The enzymatic decomposition of cellulose is considered as a future way in the exploitation of cellulose. Nowadays, the process is not sufficiently mature for an economic application in industrial scale. For reasons of energy economy the

the known processes with acid hydrolysis are not feasible enough for the industrial production of ethanol.

The enclosed table 2 gives a rough survey of the consumption figures for utilities for the preparation.

The data refer to 1.000 l ethanol calculated at 100 % end corresponding to 794 kg).

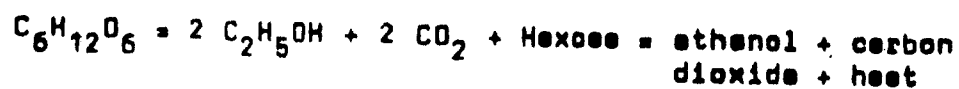
The attached table 3 shows the utilization of raw material for the obtained quantities of fermentable substance during the preparation.

1.000 l ethanol produced in the fermentation result after distillation and dehydration in:

- 972 l "power alcohol" with 99,8 % vol
- 20 l technical alcohol with 93 % vol
- approx. 7 l fuel oil.

The fermentation is a microbiological conversion of hexose to ethanol and carbon dioxide.

The basic equation is:



Inevitably different secondary products originate from this conversion:

- Yeast dry substance
- glycerin, organic acids, esters
- aldehydes and
- fuel oil

It is evident that the production of alcohol as a fuel additive asks for advanced techniques in order to meet the economic requirements of POWER-ALCOHOL-PROGRAMS.

Batchwise processing has been used till now in the most cases, as the size of the equipment was in the range of obtaining 25.000 - 30.000 liters a day.

As the plants to produce POWER ALCOHOL are to be built in the range of 100.000 to 500.000 liters of alcohol production per day, it is inevitable for economic reasons to use as much as possible the most advanced scientific and biotechnological knowledge for the engineering of such installations.

In the sphere of enzymatic conversion taking place by the activity of microorganisms during the fermentation it is advisable therefore to provide optimal conditions for the selected strain of *Saccharomyces cerevisiae*, as far as the offer of sugars and assimilable nitrogen, nutrient salts like phosphates and sources of potassium, sodium, calcium and magnesium are concerned. Further care should be taken to the environmental conditions for the yeast cells in the mesh, as there are the pH, the temperature, the offer of oxygen for better multiplication and a certain mixing intensity, responsible for homogenic uptake of nutrients and for quick elimination of the carbon dioxide, formed by the anaerobic metabolism.

Last but not least it is to mention that the design of equipment and the process itself should be planned in consideration of the fact that the full success of such plants

is only given, if the risk of undesired and detrimental fermentations, caused by bacteria and molds is kept under complete control.

Depending on the raw materials and the possibility of extracting valuable by-products, the following processes are used:

- continuous fermentation process or
- semi continuous fermentation process

for the purpose of providing

- low investment costs
- low consumption of energy and
- low running costs

The processing of sugar containing solutions for fermentative conversion of carbohydrates into alcohol in the large scale of POWER ALCOHOL production is done in a continuous way. The yeast is separated at the end of the fermentation by centrifuges and its main part is recycled to the first vat of the fermenter line.

Vigorous yeast cells are the result of this proceeding, and a relatively high concentration of bio-mass can be kept in suspension. The residential time of the mash is kept short and in the range of 10 - 12 hours; on the other hand, high concentrations of alcohol are obtained easily, as the " mash " or " beer " is leaving the last fermenter with 8,5 - 10,5 % in volume.

The yeast cream, obtained by centrifugation of the alcoholic mash, is passing a treatment tank before re-entering the process in the first fermenter, in order to keep the risk of bacterial infections at a minimum.

A small part of the yeast is harvested and used as a valuable protein source, either for human consumption or for animal feed purposes. Such harvesting of bio-mass makes it necessary to aerate the mash slightly in the first fermenter, just as much as to multiply the yeast cells in the range of the crop. Such proceeding can be made responsible for keeping all yeast cells in a young state, giving no possibilities for too long a stay in the system ; the number of worn out cells is kept therefore at a minimum.

By the presence of such a high number of vigorous yeast cells, as it is given by at least 15 grams of yeast dry matter per liter, the conversion of sugars to alcohol is fast and finished within 12 hours. The gross fermenter volume of the whole system is therefore kept small and in the range of 6 - 7,5 m³ per each 1000 liters of daily production.

Providence against disturbing infections is made by the fact that the alcohol itself is chosen as a very effective inhibiting factor against growth of bacteria. Different to batchwise processing the continuous fermentation process is avoiding the initial period of each batch, when the alcohol concentration is between zero and about 3,5 %. The residential time of the sugar containing mash in the first fermenter is dimensioned in a range of about 3 hours, sufficient for the yeast in its considerable concentration to maintain the alcohol concentration to maintain the

alcohol concentration constant and at a minimum level of 3,5 - 4,5 %. Such a level can be kept only by recycling of sufficient yeast cells from the last fermenter to the first one in order to compensate the washing-off effect.

Another important feature of a well-guided continuous process is the fact that the provided multiplication of yeast cells in the first part of the cycle is not only compensating the harvesting of a certain percentage of bio-mass, but also keeping the yeast cells in a vigorous state of high fermenting activity. No chance is given to the always renewing bio-mass to grow old and worn out, which is connected with a stop of metabolism, settling down to the bottom of the fermenter and offering an excellent substrate for bacterial growth by losing the proteinaceous cell content after breaking up of cell walls.

The alcoholic yield varies between 91,5 - 92,5 % of the theoretical yield according to the equation found by GAY - LUSSAC, and referred to the amount of available sugar. Resulting are 62 to 63 liters of ethanol 100 % per each 100 kg fermentable sucrose, or 105,3 kg fermentable mono-saccharide of hexose structure.

Depending on the over-all capacity of the plant 6 to 10 closed fermenting tanks are operated in a subsequent line, overflowing one to the other. The formation of alcohol is depending on the presence of vigorous yeast cells and temperature of the mash, coming to an end by lack of sugar and by the inhibiting effects of higher

alcohol concentrations and increased influence of osmotic pressure by accumulated fermentable substances.

To keep the whole system in a fast fermenting state, not only the first fermenter is receiving a supply of sugar containing solution. On the other hand, the last three vessels receive nothing but the overflow of the preceding one to convert the remaining sugar as far as possible to alcohol and to avoid losses of yield by content of unfermented sugar in the substrate leaving the last fermenter.

The leaving liquid is pumped to a battery of centrifuges; meanwhile the alcoholic mash, free of yeast cells, is transferred to a intermediate tank before distilling, the yeast suspension is conducted to a special treatment at lowered pH, before it reenters the first fermenter in a continuous flow.

Without additional provisions 30 - 40 kgs of yeast dry matter are gained per each 1000 liters of alcohol; in case of harvesting this amount of bio-mass, approximately 1 metric ton of dried fodder yeast can be collected per 30.000 liters of alcohol and day.

A certain part of the yeast suspension, coming continuously from the centrifuges, will be subjected an additional step of centrifugation in order to reduce the amount of accompanying unfermented substances and to give the possibility to recover the alcohol present in the environmental liquid of the yeast cells.

The yeast cream will have now a dry substance content of about 18 % and is ready for a thermal treatment to crack open the cell walls before drying. The drying itself is performed by drum dryers or spray dryers.

As an example a plant for the production of 10.000 liters of alcohol (100 %) per hour shall be described:

The flow of mash will be to 100 m³ per hour, if the final alcohol concentration could be kept at the level of 10 %.

The equipment necessary to provide the fermentative conversion of sugar from molasses will consist of the following units:

- 1 pure culture vessel of 1 m gross volume,
- 2 pre-fermenters of 25 m³ gross volume, used as vessels for intermediate treatment of yeast suspension during the time of continuous processing. Engagement of the two vessels as pre-fermenter will be necessary only, when the process is started after a longer period without production,
- 7 main fermenters of 200 m³ gross volume
- 2 nozzle-centrifuges for a throughput of 60 m³ of mash per hour each.

For the harvesting of bio mass in abundance the following equipment is necessary:

- 2 nozzle-centrifuges for 10 m³ throughput per hour,
- 1 unit for thermolyzing yeast by steam,

- 3 drum dryers for 150 kg of dried yeast per hour.

The production of dried fodder yeast will be between 7200 and 8400 kgs per day.

A simplified flow-sheet of such a fermentation department, as just described, is reproduced in a diagram 1.

The process itself can be dominated by 2 men in a shift easily, one for supervision of the fermenters, the other to operate the centrifuges.

The processing of starch containing raw materials is to be done in a different way, as the prepared substrate, ready for fermentation, will contain a considerable amount of solids, such as husks, hulls, fibres and the like. A proper separation of such ingredients would cause to high a consumption of energy on one hand, and considerable losses of yield on the other hand, as considerable quantities of starch is still linked to such particles. Finally it is to be mentioned that the very last step of saccharification happens in the fermenter itself, after the present sugars have been partially converted into alcohol, giving space for further enzymatic degradation of starch.

The time of fermentation is considerably longer, therefore, and kept in the range of 40 - 50 hours; the second reason besides post-saccharification in the fermenter is the fact that yeast cannot be recycled after centrifugation of the mash. The high content of solids is prohibitive to such proceeding, as the nozzles of the centrifuges will be clogged within seconds.

To finish the transformation of sugars to alcohol in the mentioned period, special emphasis is to be given to the steady propagation of new yeast and to really uniform distribution of cells in the broth.

Yeast propagation is provided by a sufficiently high offer of oxygen, adequate offer of nutrients like nitrogen and phosphate, and low pH as protective measurement against bacterial growth during the period of pre-fermentation.

In the main fermenters further aeration takes place to continue the growth of yeast population and to homogenize the broth, in the first stage of fermentation.

As soon as the concentration of the alcohol has reached a certain level, the aeration is stopped and the same installation is used for the even distribution of carbon dioxide in the liquid instead of air. Thus the recuperation of CO_2 as a by-product can be done without any perturbation by nitrogen and oxygen.

A final concentration of alcohol in the range of 8,0 to 8,5 % by volume can be reached. The alcoholic yield varies between 90,5 and 91 % of the theoretical value, according to the equation of GAY-LUSSAC, and referred to the amount of applied starch (see also table No.4). Resulting are 64,5 to 65,0 liters of ethanol (100 %) per each kgs of starch, converted by enzymes into fermentable sugar.

In accordance with the capacity of the plant the process of fermentation is conducted in a batchwise manner, if small or middle-sized, and in a continuous way, if the plant is of a large scale of more than 180.000 liters per day.

To submit a basic impression of such a batchwise processing of starch containing raw materials to alcohol, a short description of manipulations will follow:

Two pre-fermentations are operated in an alternating way, charging 10 % of main fermenter net volume within 12 hours with an adequate amount of yeast. Some of the yeast, gained by such proceeding, is left in the pre-fermenter to start the subsequent step; every six hours, a main fermentation can be started. The first 12 hours are utilized for slow intake of substrate and aeration, in order to continue the propagation of yeast. From the time, the main fermenter has got its complete filling at net volume level, another 28 to 38 hours of fermentation will follow. For medium sized plants a special "starting" - fermenter inserted between pre-fermentation and main-fermentation, will be advisable.

All the individual steps can be linked together by some efforts in automatization to an "almost"- continuous process.

Now it is quite simple to explain the continuous process.

Pre-fermentation is made in continuous way, discharging its overflow into the first vessel of a line of 6 main-fermenters; here the substrate, coming from the saccharification department, enters as well. The last main-fermenter of such a line delivers the alcoholic mash and all its yeast to the distilling column.

A plant in large scale of producing 240.000 liters of alcohol per day, under the conditions of using starch containing raw material, as for example corn, will consist in its fermentation department of

- 1 starting fermenter (to start the process)
- 2 x 1 pre-fermenter of 50 m³ gross volume
- 2 x 6 main-fermenters of 500 m³ gross volume each.

the average in residential time in the main fermenters at 90 % net filling will be about 45 hours.

A simplified flow-sheet of a department for continuous fermentation, based on starch containing raw materials and as just described, is reproduced in diagram 2. The process can be supervised by 1 man per shift only as no centrifugation of the alcoholic mash takes place.

For biotechnological reasons the processing is divided into two distinct lines, making cleaning and sterilisation of the equipment easier and without stopping completely.

The " stillage ", remaining after distilling off the alcohol in the first column, still contains valuable substances for animal feeding besides the protein and vitamins of yeast. Subsequent concentration of acid liquid and final drying of the concentrate makes it possible to obtain a animal feed additive of about 30 % of crude protein. The quantity will be in the range of 800 - 820 kge dry matter per each 1000 l of ethenol, providing a considerable revenue and making the whole process more feasible and free of waste water troubles.

As a summary it can be concluded that bio-technology of today is prepared to provide processes for economic conversion of renewable sources. We are allowed to be convinced that each step forward in the realization will bring better proof of man's knowledge and further progress.

Now the time has come when bio-technology should become one of the leaders in progress, as in the last decades and especially in the field of energy provision, the progress in our world was not always a real one.

Why should the developing countries not use their chance to recover a certain part of the gap to developed countries by utilizing their richness in sunshine and the productivity of photosynthesis? Manpower is available as well and as fermentation processes can be traced back to antiquity their nature has more transparency than other techniques.

Its close connection to agriculture offers one of the best pathways to accelerate the formation of trained technologists and skilled operators, forming a pool to select in near future the tripulations for plants running more sophisticated processes.

from molasses

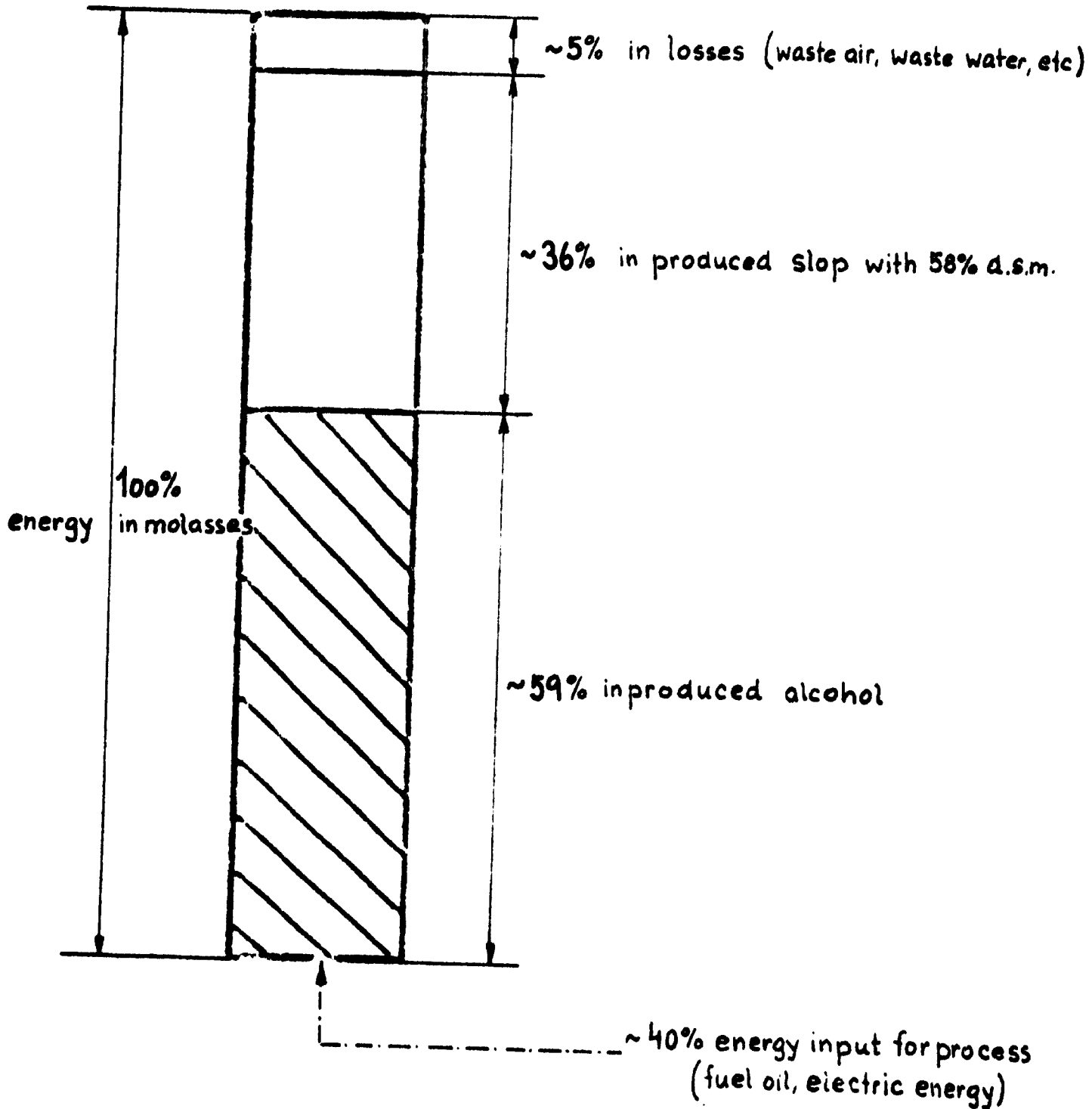


table 1 fig. 1a

from corn

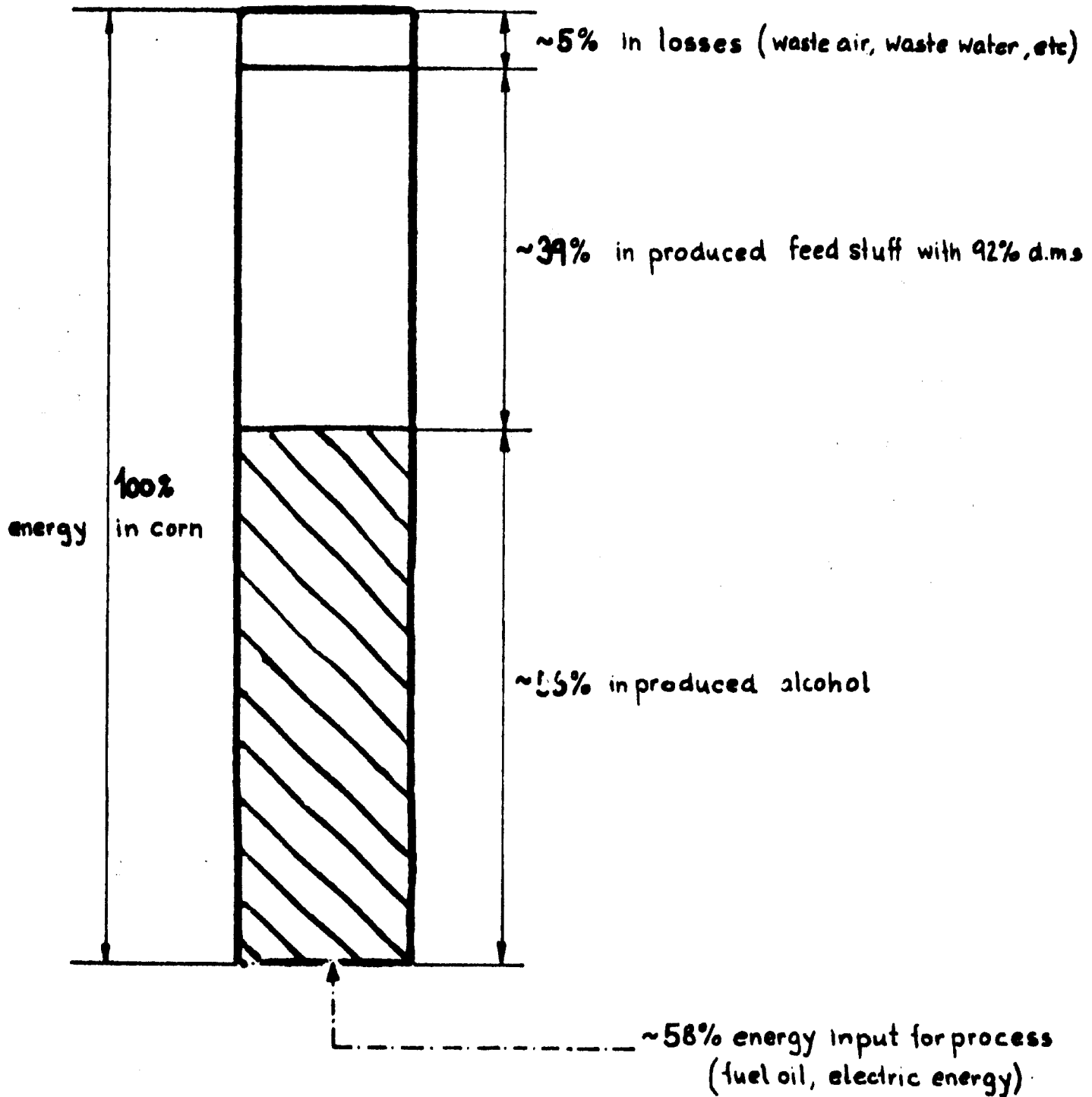


table 1 fig. 1b

auxiliaries for preperation of raw material

for production of 1000 l alcohol 100% in fermentation

raw material process		molasses contian.	sugar cane contian.	Corn	
				contian. "Convert."	discont. "Henze"
steam (with 9bar abs.)	kgs	400 + 450	1100 + 1200	1300 + 1350	2000 + 2150
electrical energy	kWh	30 ÷ 35	ca. 180	ca. 120	ca. 70
process water	m ³	8,0	3,5	9,0	9,0
cooling water, 20°C	m ³	0,5	12,0	30,0	45,0

table 2

Input of raw material

for production of 1000l alcohol 100% in ferment.

raw material .	molasses	sugar cane	corn
kgs raw material	3270	14100	2530
% ferm. sugar in raw material	50,0 ¹⁾	12,8 ¹⁾	
% starch in raw material			62,5
kgs fermentable sugar	1635 ¹⁾	1805 ¹⁾	
kgs total starch			1581
- kgs not utilizable starch			27
= kgs utilizable starch			1554
- kgs losses	19	189 ³⁾	6
= kgs to fermentacion	1616 ¹⁾	1616 ¹⁾	1548 ²⁾

¹⁾ as fermentable disaccharid (saccharose)

²⁾ as utilizable starch

³⁾ in bagasse, torta

table 3

alcohol-yield from raw material starch

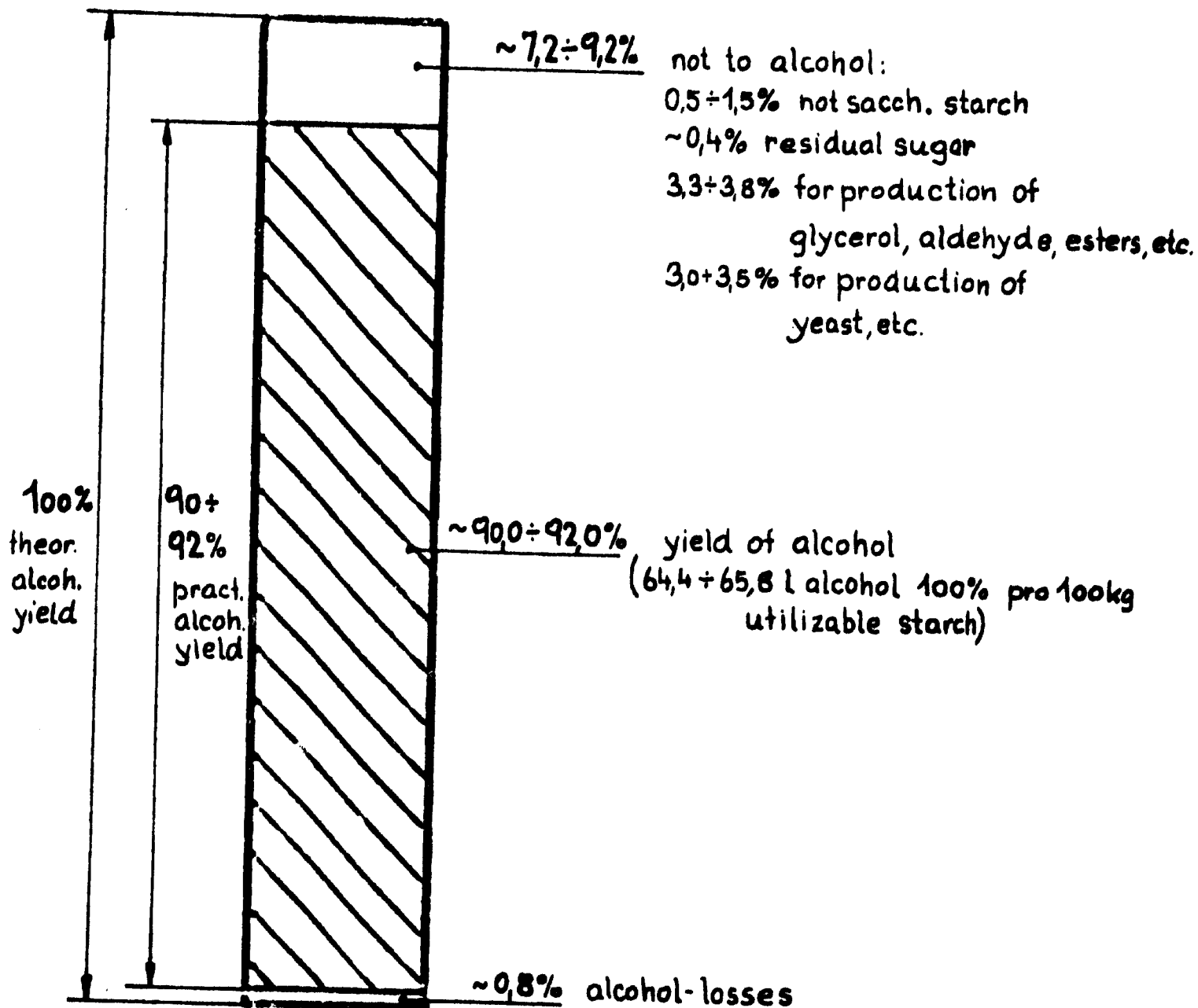


table 4

alcohol-yield from sugar-solution

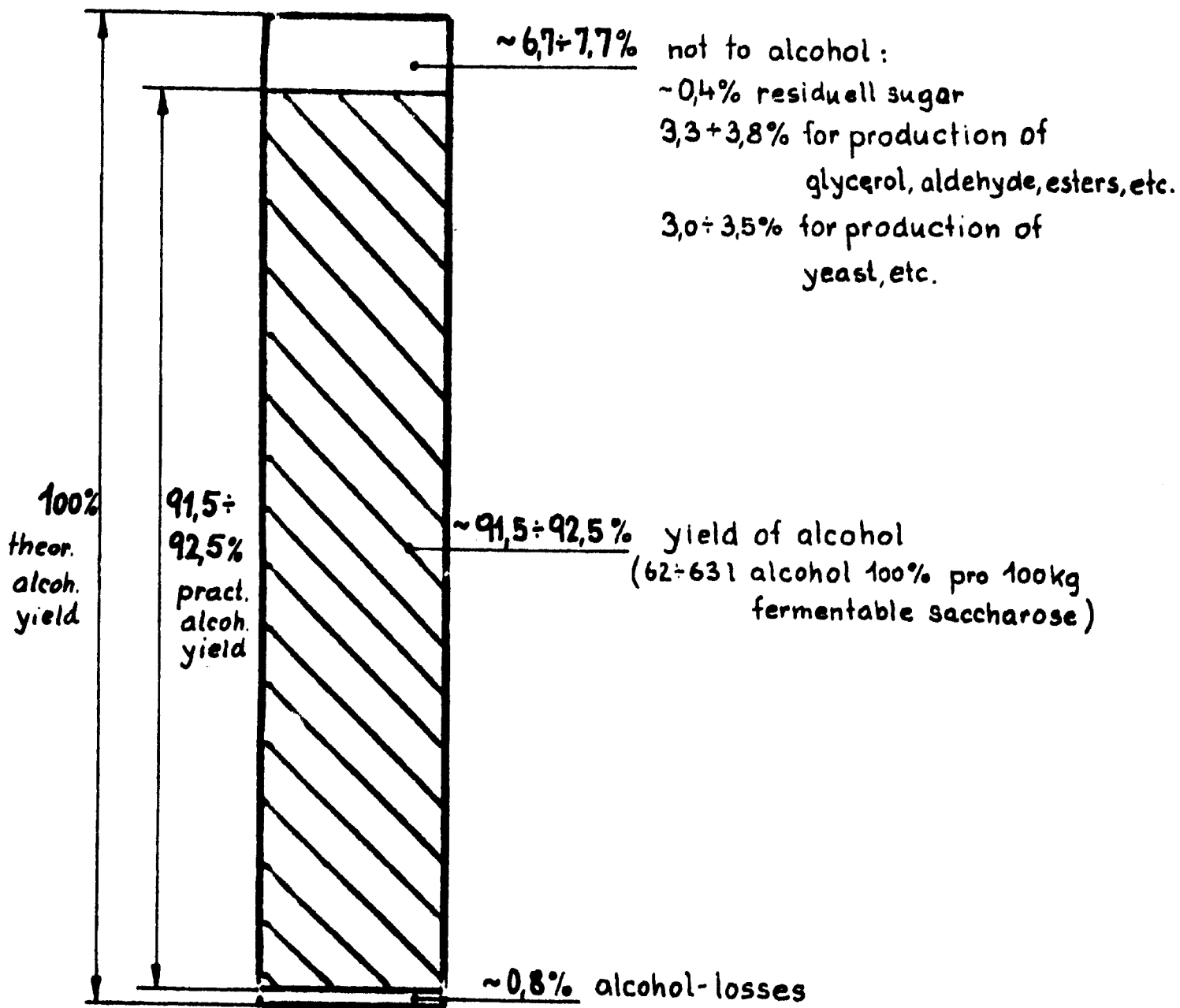


table 5

Wine yeast cream

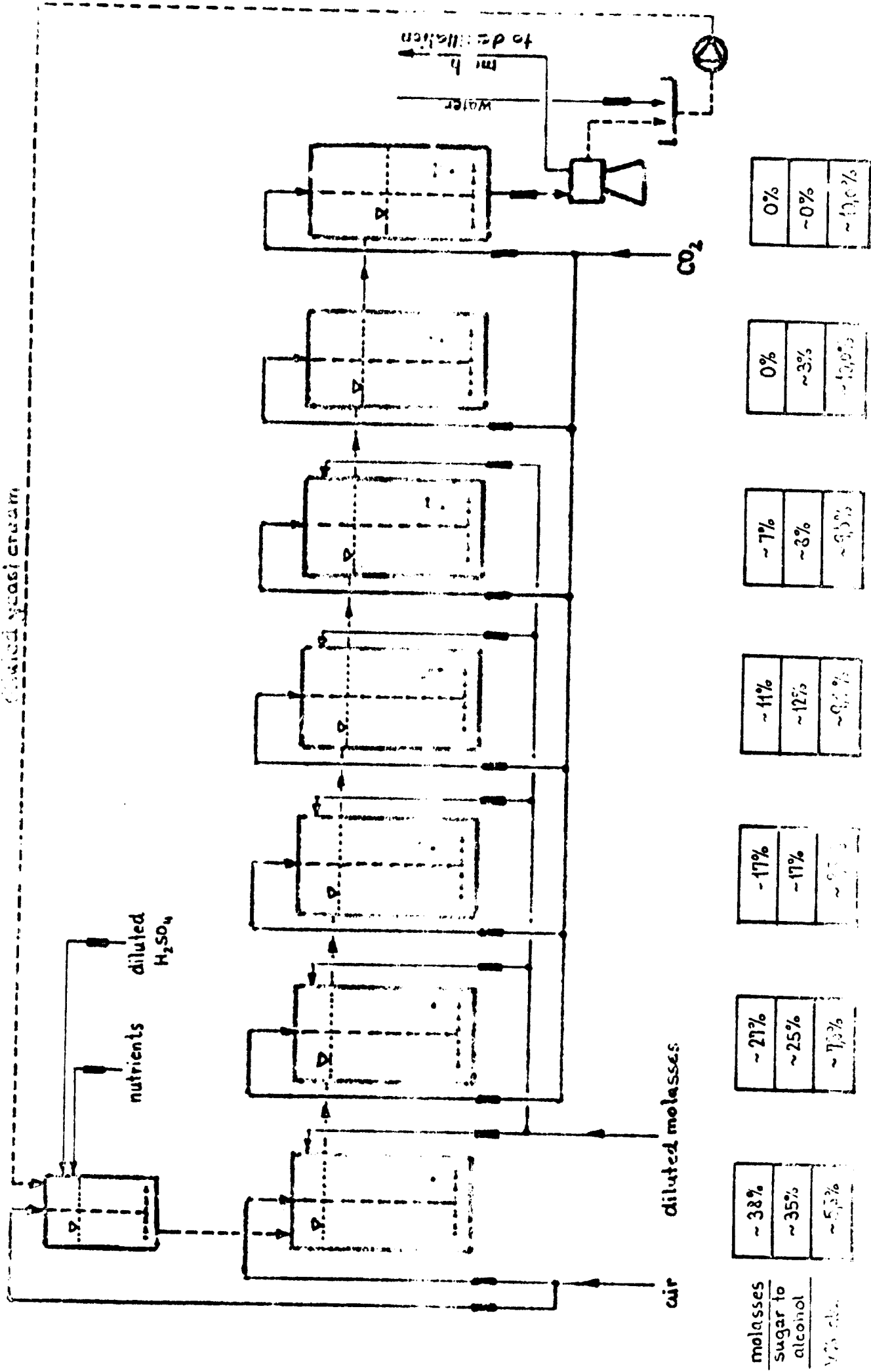
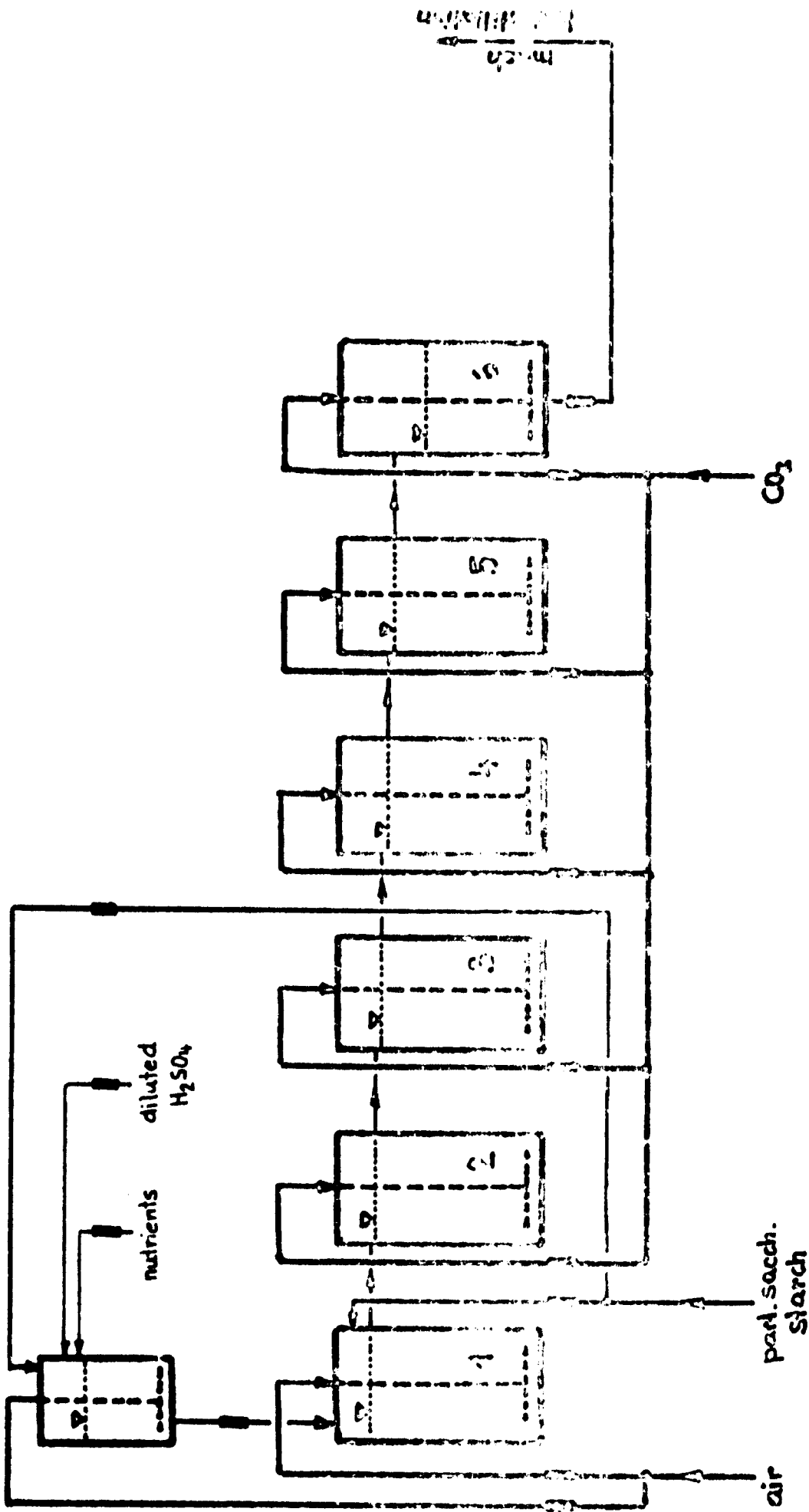


diagram 1

Pre ferment.



100%	0%	0%	0%	0%	0%
~50%	~23%	~9%	~3%	~1%	~0%
~10%	~4%	~2%	~1%	~0%	~0%

100% alcohol

G - 83



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