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Biotechnology in Food Industry

Seminar I

Model solution of a task

UNIVERSITY OF
CHEMISTRY AND TECHNOLOGY
PRAGUE

Batch cultivation of *Clostridium* bacteria to produce solvents

Introduction:

Some species of Clostridium bacteria are capable of forming solvents in anaerobic fermentation, namely acetone, butanol and ethanol (ABE). The industrial use of this technology was first tested during the First World War in England, and then successfully operated in many races around the world. Together with the decreasing price of oil and its derivatives, however, the production of solvents has ceased to be economically viable and individual production plants have been phased out. At the moment, however, this process is starting to be attractive in the context of the oil crisis, which has begun in recent decades to find ways to produce alternative energy sources. In 2008, the first new factory for microbial solvent production in Brazil was opened. Solvent-producing bacteria have the ability to utilize different mono-, di- and polysaccharides by the glycolysis pathway, and in the first stage batch culture (lag phase and exponential growth phase) usually form acetic acid and butyric acid. After accumulation of acids in the culture medium, accompanied by a slight pH decrease, the metabolism reversal, the induction of solvent-forming enzymes and butane and acetone are formed instead of acids. This phenomenon should occur during the batch culture at the end of the exponential growth phase and is associated with the beginning of sporulation in most strains. In the so-called solvent-forming phase of the fermentation, the already-produced acetic and butyric acids are partially re-used, but they still use the sugar substrate. At the same time both fermentation - acid - forming and solvent - producing fermentation gases - hydrogen and carbon dioxide and, to a lesser extent, other products - ethanol, acetoin and lactic acid. A simplified scheme of metabolic pathways for acenton-butanol fermentation is shown in Fig 1.

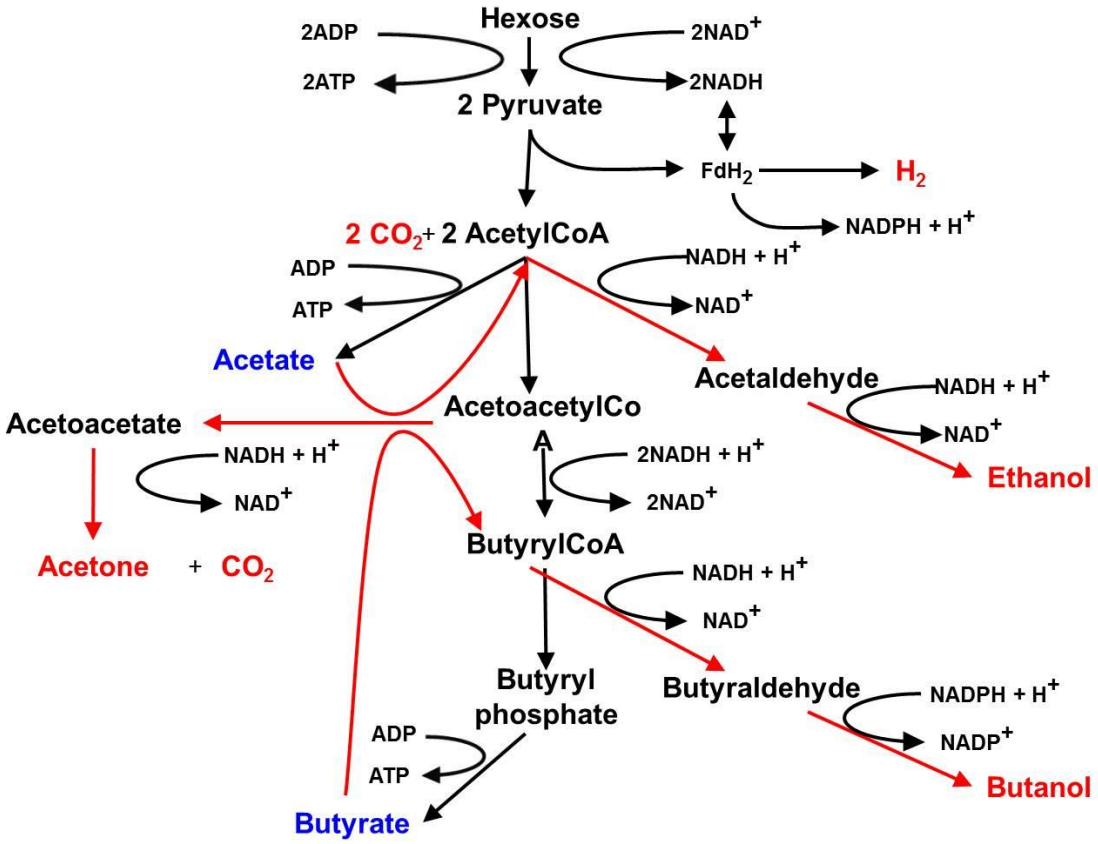


Fig.1 **Simplified metabolic scheme of ABE fermentation**

Input data:

All data presented in the following text as the basis for the task assignment are specific culture data obtained at the Department of Biotechnology in batch culture of *Clostridium pasteurianum* in a 5 liter laboratory bioreactor BIOSTAT B-DCU, B. Braun Biotech International, Melsungen Germany).

Production strain: *Clostridium pasteurianum*

Inoculum - 200 ml culture in exponential growth phase

Production medium TYA

1 liter of media contains:

glucose 40.0 g

yeast extract 2.0 g

tryptone 6.0 g

KH_2PO_4 0.5 g

ammonium acetate 3.0 g

$MgSO_4.7H_2O$ 0.3 g

$FeSO_4.7H_2O$ 0.01g

The pH was adjusted to 6.8

Bioreactor working volume: 3 l

Cultivation conditions:

pH before inoculation 6.8, not modified, temperature 37°C, stirring 300 rpm, at the beginning of cultivation the content of the bioreactor was nitrogen-induced - induction of the anaerobic atmosphere.

Course of cultivation:

Samples were sampled during cultivation, a total of 13 samples of 20 ml were taken. Samples were centrifuged, biomass was used for gravimetric determination of solids, substrate (glucose) and products (acetic and butyric, ethanol, acetone and butanol) were determined in the supernatant by high performance liquid chromatography. The fermentation gases were collected in the sampling bag and the hydrogen and carbon dioxide content was determined by gas chromatography. In total, 43.35 l of outgoing bioreactor gas with an average concentration of 37.1% by volume of hydrogen was collected in the bag.

The values measured during the course of the batch culture are shown in Table I. The time dependencies of the measured values are plotted in the graphs of Figures 2-5.

Table I - Concentration of substrate, biomass dry matter and products during cultivation

Time (h)	glucose	Acetic acid	ethanol	acetone	Butyric acid	buthanol	Dry mass	Hydrogen*	CO_2*
	(g/l)	(g/l)	(g/l)	(g/l)	(g/l)	(g/l)	(g/l)	(% vol)	(% vol)
0.0	38.56	2.36	0.02	0	0.09	0.03	0.171	3.0	53
3.0	34.78	2.46	0.02	0	0.14	0.03	0.129	13.2	42
6.5	33.72	2.81	0.03	0	0.29	0.03	0.186	25.8	60
9.5	33.11	2.44	0.03	0.12	0.34	0.19	0.271	34.5	49
13.0	31.51	2.24	0.03	0.46	0.33	0.6	0.471	40.9	44
16.5	27.6	1.83	0.06	1.35	0.34	1.71	1.114	42.7	50
19.7	19.87	1.49	0.09	2.29	0.7	3.06	2.457	40.9	32
22.8	14.65	1.72	0.13	3.12	0.92	4.73	3.114	ND	29
25.6	10.28	1.63	0.14	3.4	0.84	5.47	3.114	40.9	8
28.5	7.55	1.62	0.15	3.75	0.86	6.48	3.2	44.2	0
31.7	5.29	1.55	0.16	4.18	0.94	7.04	3.071	43.4	ND
47.1	3.95	1.51	0.16	4.29	1.13	7.25	2.386	44.2	ND
50.6	3.83	1.49	0.16	4.26	1.13	7.21	1.071	42.4	ND

In total, 43.35 l of outgoing bioreactor gas with an average concentration of 37.1% by volume of hydrogen was collected in the bag.

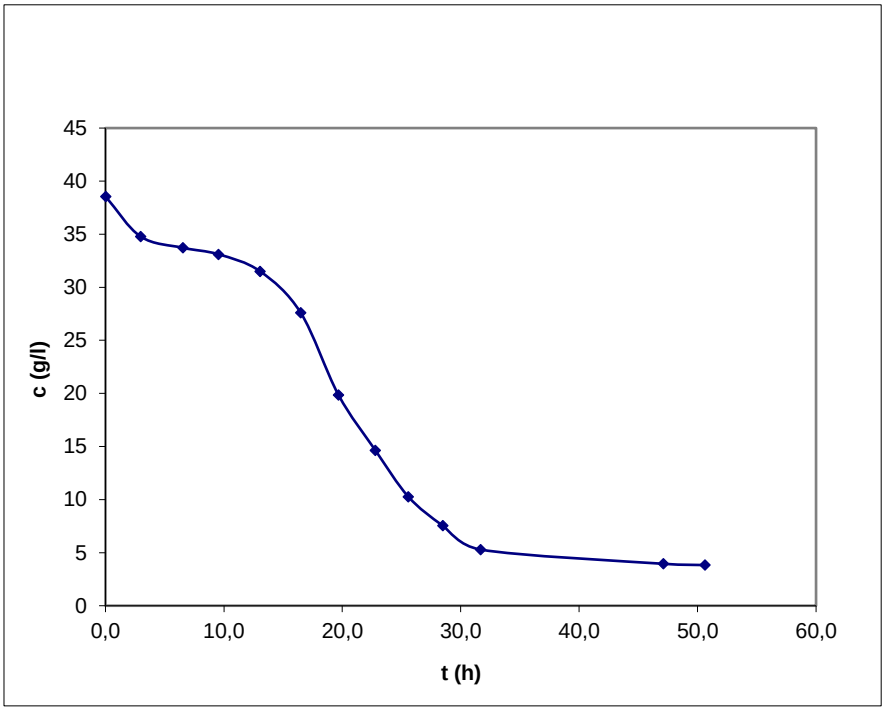


Figure 2 - Glucose consumption during cultivation

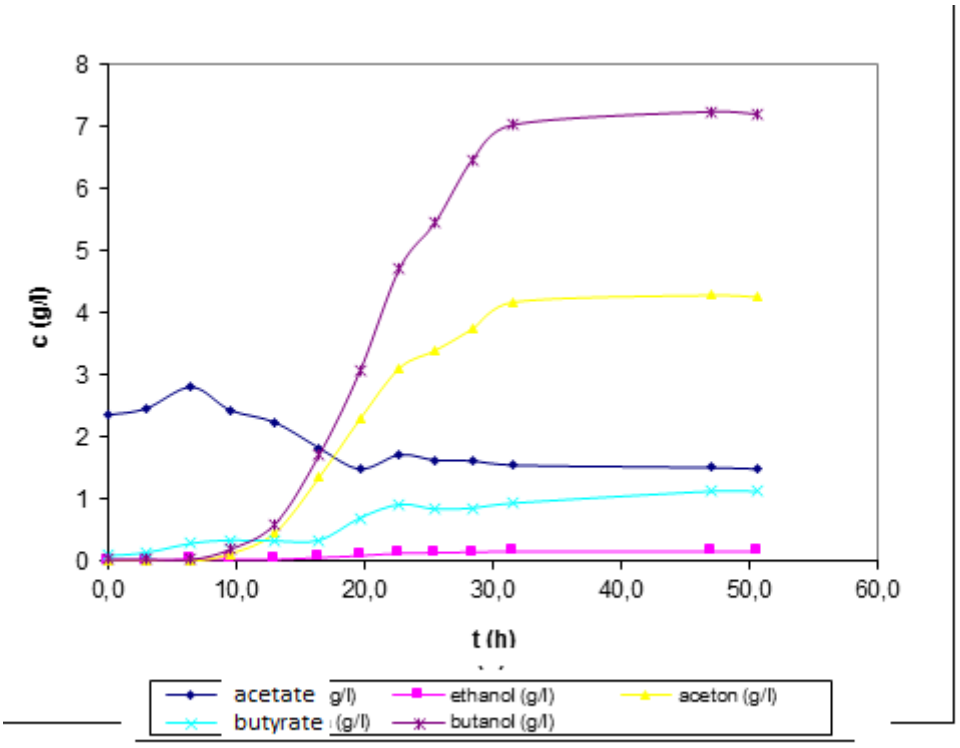


Figure 3 – Formation of liquid products during cultivation

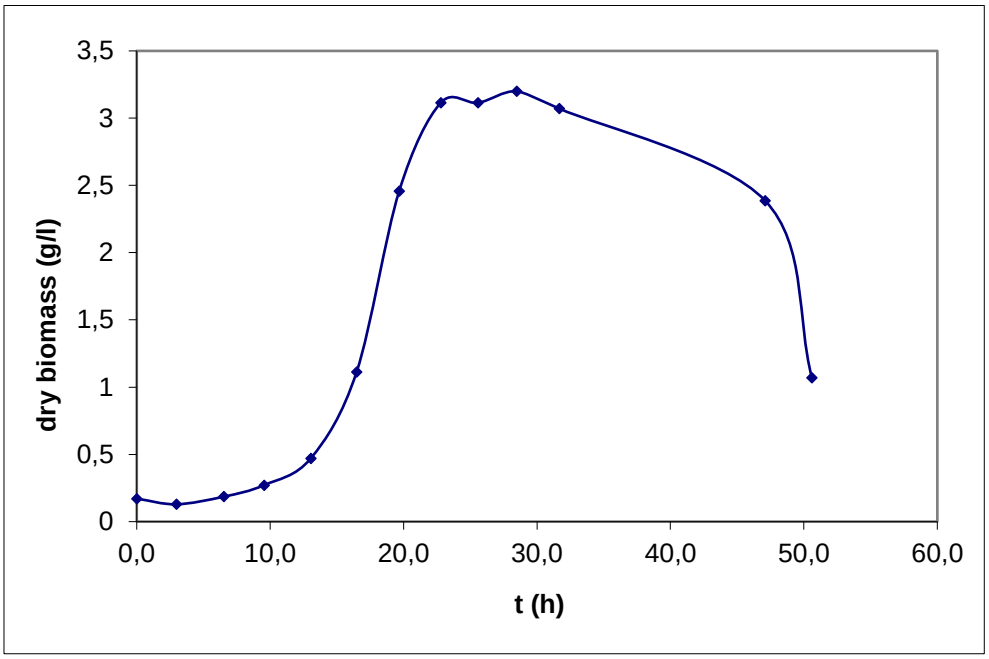


Figure 4 - Growth during cultivation

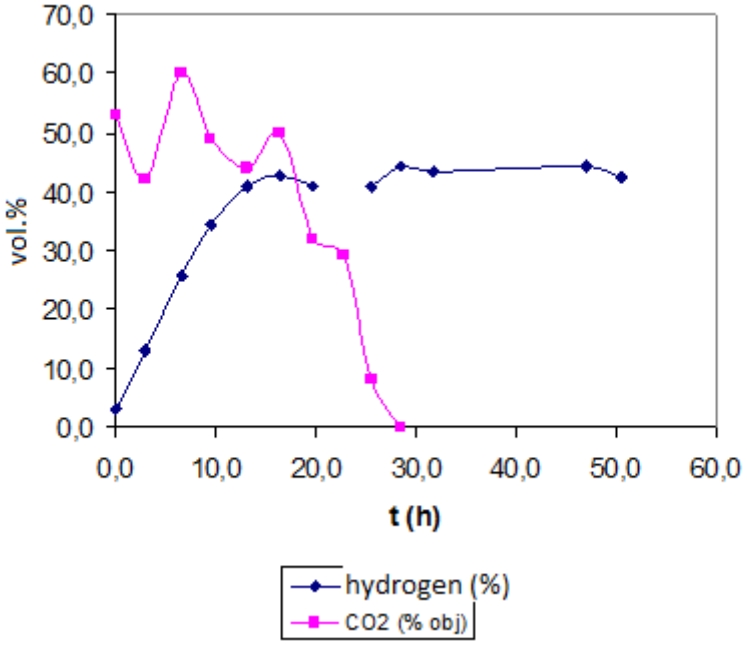


Figure 5 - Concentration of hydrogen and carbon dioxide in the off-gases

Tasks:

Based on the balance of the whole batch culture process, calculate the following data (carbon sources supplied as yeast hydrolyzate and tryptone, neglected):

1. How much glucose was consumed during batch cultivation (include sample sampling in the volume of the bioreactor). How many percent of substrate was not consumed? What was the average glucose consumption rate from the beginning of cultivation to the end of the exponential growth phase?
2. What was the volume production of butanol and the total volume production of solvents (acetone + butanol + ethanol)? Express the result in milliliters. The density of acetone is 0.7899 g / cm^3, butanol 0.8063 g / cm^3, ethanol 0.789 g / cm^3
3. In what proportion do the solvents form? Express the result as a ratio of the weight parts of butanol to acetone and to ethanol. Compare the calculated data with the data reported in the literature.
4. What is the recovery of butanol and total solvents (acetone + butanol + ethanol) on the spent substrate? Express the result in $g.g^{-1}$.
5. What was the total volume production of hydrogen during batch cultivation? Express the result in liters
6. What is the yield of biomass on the consumed substrate at the end of the exponential growth phase? Express the result in $g.g^{-1}$.
7. What is the specific growth rate of biomass in the exponential growth phase? Express the result in h^{-1}.
8. Calculate the total production of butanol and all solvents (acetone + butanol + ethanol) for the exponential growth phase and for the final stage of cultivation (beginning of the stationary phase until the cultivation is complete). Express the result in $g.l^{-1}h^{-1}$ for the individual cultivation phases.
9. Compare and discuss the absolute values of concentrations, yields and productivity for butanol and total solvents (acetone + butanol + ethanol) with literature data.

Solution:

Process Balance:

Volume at start of batch: 3 l

Volume at end of batch: 3- (13 * 0.02) = 2.74 l

Initial concentration of glucose: 38.56 g / l

Residual glucose concentration: 3.83 g / l

Beginning of cultivation - substrate available: 3 * 38.56 = 115.68 g

End of culturing - residual substrate: 2.74 * 3.83 = 10.49 g

Final butanol: 7.21 g / l

Final concentration of acetone: 4.26 g / l

Final ethanol concentration: 0.16 g / l

Total volume of fermentation gas: 43.35 l

Average hydrogen content in fermentation gas: 37.1%

Question 1

Consumption of glucose during cultivation: 115.68-10.49 = 105.19 g

Unused substrate: (10.49 / 115.68) * 100 = 9.1%

Glucose consumption rate to the end of the exponential growth phase: (115.68-5.29) / 31.7 = 3.65 g / h

Question 2

Butanol produced: 7.21 * 2.74 = 19.76 g, i.e. 24.41 ml

Acetone produced: 4.26 * 2.74 = 11.67 g, i.e. 14.75 ml

Ethanol produced: 0.16 * 2.74 l = 0.44 g, i.e. 0.56 ml

Total solvent volume: 24.41 + 14.75 + 0.56 = 39.72 ml, ie 31.87 g

Question 3

Total solvent production: butanol: 7.21 * 2.74 = 19.76 g, acetone: 4.26 * 2.74 = 11.67 g, ethanol 0.16 * 2.54 = 0.41 g, Together 31.84 g of solvents

The ratio between individual solvents: B: A: E = (19.76 / 31.84) :(11.67 / 31.84) :(0.41 / 31.84) = 6.2: 3.7: 0 , 01

Question 4

Yield of butanol on the spent substrate - Y_B / S = 19.76 / 105.19 = 18.8%

$Y_{ABE / S}$ = 31.84 / 105.19 = 30.3% Yields of total solvents per consumed substrate

Question 5

Volume of hydrogen produced: (43.35 / 100) * 37.1 = 16.1 l

Question 6

At 22 h - end. biomass dry matter 3.114 g / l, 8 samples taken over 20 ml ie 0.16l, bioreactor volume – 2.84l – i.e. biomass 8.84 g

Initial Biomass: 3 * 0.171 = 0.513 g

Glucose concentration at 22 h 14.65 g / l

Unused glucose: (3-8 * 0.02) * 14.65 = 41.61 g

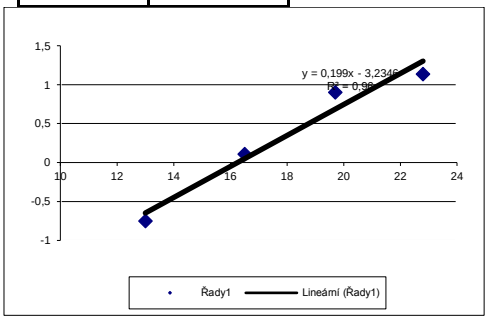
$Y_{X / S}$ = [((3-8 * 0.02) * 2,814) - (3 * 0,171)] / [(3 * 38,56)] = 8.41 / 74.07 = 0.11 gg-1

Question 7

μ = (ln3,14) - (ln0,471) / (22,8-13,0) = (1,14 + 0,75) / 9,8 = 0,19 h-1

Graphical determination of μ from the exponential growth phase (13-22 hours):

Time (h)	ln X
13	-0.7529
16.5	0.107957
19.7	0.898941
22.8	1.135908



Question 8

Total production of butanol: 7.21 / 50.6 = 0.14 g.l-1h-1

Productivity of Butanol Production in Exponential Growth (13 - 22.8):

[(4,73-0,6)] / (22,8-13) = 0,42 g.l-1h-1

Productivity of butanol formation in part 2 of cultivation (22.8-50.6 h)

(7.21-4.73) / (50.6-22.8) = 0.11 g.l-1h-1

Total productivity of ABE formation: (7.21 + 4.26 + 0.16) / 50.6 = 0.23 g.l-1h-1

Productivity of ABE in the exponential growth phase

[(4,73 + 3,12 + 0,13) - (0,6 + 0,46 + 0,03)] / (22,8-13) = 0,70 g.l-1h-1

Productivity of ABE in 2nd part of cultivation (22.8- 50.6 h)

(7,21 + 4,26 + 0,16) - (4,73 + 3,12 + 0,13) / (50,6-22,8) = 0,13 g.l-1h-1

Discussion of results:

Very different results obtained under different cultivation conditions with different production strains on different substrates of varying concentration are reported in the literature. Comparing the results is therefore not a simple matter, but in principle it can be said that the results achieved correspond to already published results.

Cultivation using four different types of clostridium (*C.acetobutylicum*, *C. saccharobutylicum, C.sacharoperbutylacetonicum* and *C.beijerinckii*) in a 4% glucose medium resulted in the total concentration (butanol, acetone and ethanol) of the average solvent concentration of 12, (Sheheen et al. 2000), which totally corresponds to our results - the total solvent output is 11.63 g / l and the yield is 30.3%. In the case of the same type of C.pasteurianum, there was a worse result in cultivation in 5% glucose medium - the concentration of butanol at the end of cultivation, after 27h, was 4.45 g / l, but at the same time a high concentration of butyric acid was determined in the medium - 5.02 g / l (Harris et al., 1986). The concentration of butyric acid in our case was 1.13 g / l and the highest butanol concentration achieved was 7.21 g / l.

Generally, batch cultivation typically achieves a total solvent concentration of 12-20 g / l, yields between 30-40% and productivity (probably related to the whole process) of 0.3-1.2 g / l / h (Ezeji and others 2005) . At the lower boundary of these values are also the results from this cultivation.

Literature:

Shaheen R., Shirley M., Jones D.T.: Comparative fermentation sudies of industrial strains belonging to four species of solvent-producing clostridia. J.Mol. Microbiol. Biotechnol. (2000), 2(1), 115-12

Harris J., Mulder R., Kell D.B., Walter R.P., Morris J.G.: Solvent production by *Clostridium pasteurianum* in media of high sugar content. Biotechnol. Lett. (1986), 8(12), 889-892.

Ezeji T.C., Qureshi N., Blaschek H.P.: Industrially relevant fermentations pp. 797-812 v Handbook on Clostridia ed. P. Dürre (2005), CRC Press, Boca Raton.

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Biotechnology in Food Industry

Seminar II

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PRODUCTION OF ETHANOL OF THE FIRST GENERATION FROM STARCH MATERIALS

Group task 1

Design the size and layout of biotechnology processing of maize for bioethanol to be used as a gasoline additive. Prepare the operation to ensure the daily production of 54 tons of pure anhydrous alcohol. As a production microorganism, use the *Saccharomyces cerevisiae*, counting that the operation will work continuously with an annual 2-month shutdown required for plant sanitation and maintenance. Collaborate in a group and together design all the technological steps of this operation and their continuity, follow the tasks described below. Remember that each operation must be linked to each other, so when solving some tasks, you will have to count the data that results from the previous step. In order to solve the task, draw a block diagram of the whole technology and balance all the inputs and outputs of the process including the emerging gaseous and liquid "waste", suggest where possible how these outputs could be recycled or otherwise used. Calculate the consumption and price of raw materials in this operation, please indicate their cultivation in the Czech Republic and hectare yields. Design the use of waste materials generated in this operation (distillation effluents, carbon dioxide). Part of the solution is also the selection of suitable equipment for this operation, including the design of individual container sizes. In the report, list all the equations and formulas you used in the calculations, including the explanation of all the symbols. For calculations, use the data entered in this entry in the "input data" box. If you need additional data for the calculations, find them in professional literature, quote the source and comment on the calculation. If you need to consult some parts of the technology, please order the consultation in advance. Just send an email to the teacher and we can discuss your calculations together.

I hope you will enjoy the solution and I wish you a successful mastery!

Input data:

• The input raw material has 87% dry matter and the starch content in dry matter is 63% by weight.

• Before sacrificing, fermentation is required, 95% of the starch hydrolysis is considered glucose.

• When starch is decomposed, water is added to the glucagon molecule, so be sure to apply a glucose-glucose correction factor.

• The broth (culture medium produced from corn and water) will be prepared in proportion of 1 part corn to 2 parts water.

• The inoculum will be produced from dried distillery yeast, using a dose of 0.5 kg dried yeast per m3 of working volume of the bioreactor. The inoculum will be produced in two stages, growth will take place at each stage of 18 h. The inoculation ratio to the fermentation step will be 10% vol.

• The expected ethanol content of the hazel will be 10% vol.

• Ethanol recovery from glucose will reach 85% of the theoretical yield.

• The working volume of the reactors represents 80% of the total volume of the vessels in this technology.

• The daily production of distilleries is about 650 m3 of sparse burns with a density of 1028-1048 kg / m3.

• Calculate the energy intensity of processing 1 t corn for bioethanol to 3.03 GJ.

• Calculate that the normal consumption of cooling water to produce 1 m3 of anhydrous alcohol is 100 t.

Subtasks:

1. Draw a flow diagram of inoculum preparation including all input and output streams and their weight balance. Draw a biochemical schematic of inoculum production and make a balance of inputs and outputs. On the basis of the literature search and the given data, suggest the appropriate composition of the inoculation and production media. Consider that the production of the inoculum will take place in batch in two steps (see input data), suggest the arrangement and duration of yeast cultivation to keep the specified inoculum ratio in all stages and deliver the desired amount of inoculum to the main fermentation. Use commercially available dried yeast distilleries as a batch and calculate their initial dosing to batch volume. Consider the usual biomass yields reported in the literature for this type of process.

2. Calculate the amount of dried yeast you need to prepare the inoculum, choose a suitable supplier, and calculate what yeast costs you have to calculate if you make a one-year contract.

3. Draw a biochemical scheme of starch hydrolysis and make a balance of inputs and outputs. Draw a technological diagram of mash preparation, including all input and output streams and mass balance, calculate the amount of corn you need to prepare the mash. Do not forget to include the cooling temperature at the weighing temperature in the flow chart and balance. Calculate the amount of process water you need to prepare a sponge and design its sources. If necessary, suggest adding nutrients (the type of nutrients and their amount to the desired volume of the mash), drawing from the literature and explaining it. Suggest which commercial enzyme preparations are used for starch saccharification, calculate the dosage of enzyme preparations and state the enzyme hydrolysis conditions. Choose a suitable enzyme supplier and calculate how many enzyme preparations you will need to order if you contract your supplier once a year. Estimate the price you would be able to accept in this contract and justify it. To estimate the price of enzymes, use the web sites of companies that supply the enzymes in larger quantities, not Sigma Aldrich web.

4. Draw a biochemical scheme of fermentation and make a balance of inputs and outputs. To draw up a master fermentation scheme, consider batch production of ethanol in multiple tanks at once to keep a continuous spill on the distillation column (use the specified daily amount of ethanol to do so). Do not forget to take into account the unproductive times required for draining, cleaning and replenishing tanks, and also the times required to pump the mash from one tank to another. Perform the mass balance of input and output currents. Calculate the productivity of ethanol production in this process.

Calculate the total size of the bioreactors and containers, calculate the filling to 80% of the total volume. Do the engineering design of the bioreactor, draw on the Bioengineering of Yeast Processes or Aiba, Humprey, Millis: Bioengineering (Academia, 1971). Calculate the hourly consumption of cooling water for this operation, consider re-use of the process water for cooling / heating in the individual technological steps.

5. Calculate the amount of corn processed by your plant in one year. Calculate how much cropping area you have to make with your corn vendor and what you have to calculate for the annual volume of maize you can count (you can count the price of the raw material on the average selling price of corn over the last 5 years, quote the source and quote the source) to conclude an annual contract. Estimate how much of the total sowing area used in the Czech Republic for maize growing consumes your crop (please refer to the source of the data on the size of the crops used for growing maize and

its yield per hectare). Estimate what price you will pay to Czech Railways for transportation of raw material from the place of cultivation to your factory, consider only the number and price of the containers you will have to rent, neglect the transport distance (refer to the volume of the offered containers and their rental price, quote source).

Next, calculate the total economic costs of all input raw materials (yeast, corn, enzymes, alcohol drainage material). From the energy intensity of the operation (steam production, water and electricity consumption - see input data), calculate the operating costs, investments in the plant and their depreciation, and the wage costs of factory workers at this time are negligible. From the total volume of alcohol produced and its average purchase price and the price you receive for the sale of the evaluated waste (tailings, CO2), calculate the annual operating revenues. Then evaluate whether the calculated cost of raw materials and transportation will be economical at the given purchase price of alcohol.

6. Design a distillation scheme for this operation so that you keep a continuous feed of the ethanol produced on the distillation column. Assume that the fermented broth is fed to the boiling column, ethanol is extracted from the top of the column, and the distillation flap is dropped. Make a simple balance, neglect the losses on individual devices. Suggest the possibility of obtaining anhydrous ethanol and how to regenerate the reagent. Estimate the consumption of the material you use for ethanol drainage, choose a suitable supplier, and calculate how much material you need to order if you run the annual production run and at what cost you have to calculate. find out the supplier and the price. Calculate the energy intensity of steam preparation used for distillation. 1. Wastes from alcohol production are distillates containing non-volatile components of the fermentation medium. Characterize distilleries and calculate the annual production of distilleries corresponding to the proposed size. Specify the average composition of the distillate frogs (dry matter content, protein content), suggest their further processing and end use. Draw a technology schema of burnout processing including mass balance of inputs and outputs. If possible, find out the average purchase price of distilleries and calculate the economic profit from their processing. Another by-product of the fermentation is carbon dioxide. Calculate the annual production of carbon dioxide in this operation and suggest its use. Draw a technological scheme for its processing, including input and output balances. If possible, calculate the economic profit from its processing.

Final task for the whole group: Put together the entire process flow diagram including all input and output streams and their mass balance

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Seminar III

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PROJECT OF PRODUCTION OF BAKERY YEAST

The task of the project is to produce 20 t of pressed baker's yeast (27% dry). The assignment is designed for a group of students (see individual tasks below). However, some tasks need to be solved in a sequence of operations. The process is multi-stage and one technological stage lasts around 18 h. Detailed processing of the final two stages (2nd generation and shipping yeast). However, it is necessary to process the whole process from the laboratory to the final stage. Nutrition Calculation, Flow Diagram and Calculation of Hourly Dose and Reactor Size, including Centrifugal Flow estimates for II. the nascent generation and for expedition yeast. In the end, you will not be able to solve filtration and liberation. The amount of the final yeast "milk" must correspond to the specification, ie 20 t D27. Molasses: Dry weight (saccharification): 80%, sucrose content 50%, total N: 1.2%, assimilable N: 0.5%, total P: 0.04%

Study the basic principle of making yeast from molasses. For all calculations, it is necessary to provide the calculation formulas and the origin of the values obtained.

Solution Sequence (Individual Tasks):

- Overall production scheme: laboratory, operational promotion, I., II. generation, shipping yeast. Calculate the amount of molasses required for each stage. The task is the starting point for all other solutions. (Task 1)
- Nutrition calculation for individual generations and expedition stages. Consider the 1: 1 standard molasses dilution, adhere to a 1: 5 promotional ratio. In the first generation, the final yeast concentration should be 25 kg / m3 (as D100), 35 kg / m3 in the second generation, and in the case of shipping yeast concentration 60 kg / m3. Just consider nutrients N (liquid ammonia) and P (phosphoric acid) as nutrients. When computing, respect the quality requirements for yeast. Yeast yield for 1st generation is 35%, for 2nd generation 40% and for expedition 45%. (Task 2)
- Propose molasses for the second generation and shipment. Under the assumption that in both stages the molasses medium will be fed, suggest the total amount of water required to produce (= the sum of the water added to the medium + the amount of water added at the beginning), respect the conditions that could cause the Crabtree effect. (Task 3).
- From the previous calculations (from the quantity and composition of inflow medium for the second generation and the amount of water needed to add to the start of cultivation) for the second generation, suggest an 18-hour cultivation of second generation yeast. The target yeast concentration (such as D100) is 35 kg / m3. The specific yeast growth rate (μ) at this stage will be 0.08 h-1. Part of the cultivation design will be a flowchart (16 h of inflows, 2 hours without inflow). 8h exponentially, 8h linear, when you have to deliver the rest of the media from the template. Calculate the total reactor volume (effective volume) for a given stage. What performance must centrifuges have? (Task 4)
- From the previous calculations (from the quantity and composition of inflow medium for shipping yeast and from the amount of water needed to add to the start of cultivation) to cultivate shipping yeast, suggest 18 hours cultivation of the shipping yeast. The target yeast concentration (such as D100) is 60 kg / m3. The specific yeast growth rate (μ) at this stage will be 0.15 h -1. Part of the cultivation design will be a flowchart (16 h of inflows, 2 hours without inflow). 8h exponentially, 8h linear, when you have to deliver the rest of the media from the template. Calculate the total reactor volume (effective volume) for a given stage. What performance must centrifuges have? (Task 5)
- The whole production must be processed into a detailed scheme showing the inputs and outputs of the medium, its composition, the amount of water added, the reactor size and the

number / performance of the centrifuges. The values given must be consistent with the results. The technological scheme to be considered must be drawn into individual blocks. (Task 6).

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Seminar IV

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Production of 1-butanol from straw

Input data:

Design a pilot production batch with a capacity of 1000 t solvents per year. Production should be planned so that the distillation column can operate in a continuous mode ie in several production bioreactors. The main product will be 1-butanol, which is produced in acetone-butanol-ethanol fermentation, as by-products of production consider acetone, ethanol, hydrogen and burns. Assume that the production microorganism will be a spore strain of *Clostridium beijerinckii* and the main raw material will be straw. Consider straw containing 36% cellulose and 17% hemicellulose in dry matter (hemicellulose consists of 80% xylose and arabinose residue). Straw is pretreated by milling and alkaline and enzymatic hydrolyses; the yield of hydrolysis is 93% for cellulose and 80% for hemicellulose. Glucose, xylose and arabinose are usable by the producer. Upon completion of fermentation in the production bioreactor, the total solvent yield will be 40%. The minimum residence time in the production reactor is 36h. The solvents will be obtained by distillation. Disstillation slops will be concentrated and used either to produce biogas or other purposes, the evaporated and condensed water will return to production.

1. Draw a master fermentation scheme. Consider the inflow to the distillation column at 24 tons / h. Provide the necessary number of containers if you suspect continuous distillation. When designing, take into account the fermentation time and the required operating times (distillation column distillation, cleaning and sanitation (CIP), filling of sterile medium, inoculation) 6h. Consider filling the bioreactor vessel up to 80% capacity. Suggest the composition of the inoculation and production media. Suggest a three-step inoculation with a 1% inoculum ratio and the inoculum growth time of each 18h step. Consider the first two steps as laboratory and last in the inoculation bioreactor. For the last step, consider the necessary 4h operating times. Consider filling the bioreactor vessel up to 80% capacity. Design equipment for operational and control laboratories.

2. Design a distillation scheme. Assume that the fermented broth is fed to the boiling column, the solvent head is extracted from the head of the column, and the condensate is condensed from the bottom. The solvents are fed to a concentration column headed off with acetone and ethanol, while 1-butanol and water are removed from the heel as unstable azeotrope. Azeotrope of butanol with water is taken to a decantant where the mixture is separated and anhydrous 1-butanol is obtained. Acetone with water is fed to another column to give anhydrous acetone (from the head) and an azeotrope of ethanol with tap water. The ethanol / water azeotrope is passed to a zeolite column in which water is collected. Describe the composition of the individual distillation streams and make a simple balance, neglect the losses on individual devices, consider that 15% of the water from the mash is distilled along with solvents on the flask.

3. Processing ofthe slops. The slops are thickened on a multistage evaporator of about 2% by weight. dry matter to 15% by weight. dry matter. Estimate the composition of the fragments (based on the composition of the culture medium and the course of the ABE fermentation) and estimate the amount of biogas produced. Suggest other options for burnout usage. Consider using lignin for chemical production or heat production (consider hydrolysis about 30% of straw delignification). Refer to the literature for information on the possibility of producing Group B vitamins with *Clostridium* and consider the possibility that these slops will contain these vitamins.

4. Evaluation of hydrogen as a by-product. Based on the ABE fermentation process balance, estimate the amount of hydrogen produced in the fermentation gas. Consider that the hydrogen concentration in the fermentation gas is 40%. Please indicate how hydrogen could be separated from the fermentation gas and what it could be used for. Consider combustion of the fermentation gas

and the use of fermentation gas to produce heat, indicate how much heat you get by burning 1 m3 of fermentation gas.

5. Overall economic appreciation. Please indicate how many tonnes of wheat straw you need for the production (indicate also the amount of straw in the packages) and how large the wheat area will correspond to this amount of straw. Find out the sales prices of manufactured products and raw materials. Consider that for 1t of solvents you have to spend 9,000 CZK for steam production, for water and electricity consumption. Based on the estimated operating costs, decide on what conditions this production will be economically beneficial.

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Biotechnology in Food Industry

Seminar IX

UNIVERSITY OF
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Process design for ABE production from sugar beet juice

Model case

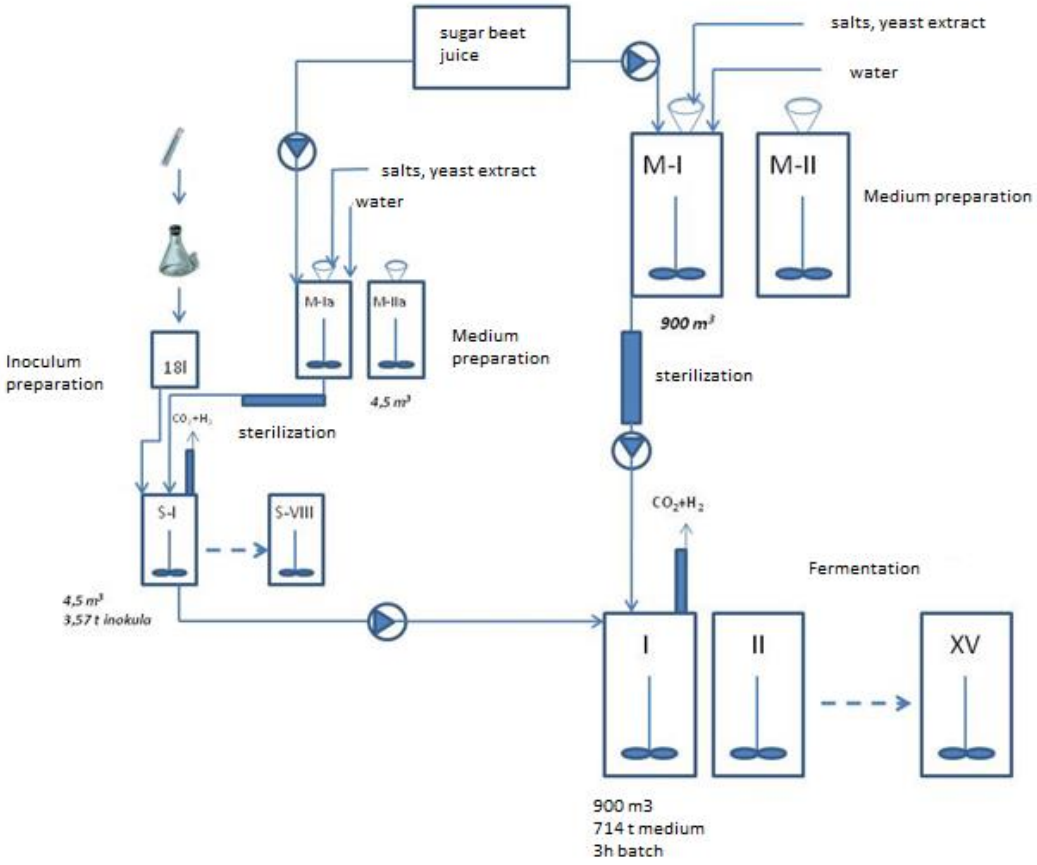

Figure 1 - Batch Scheme ABE Fermentation:

M-I, M-II, M-Ia, M-Ib - medium mixers, PS - flow sterilizer, S-I to S-VIII to I-XV fermenters.

Initial Assumptions:

Feed for distillation column: 238 tons / h
I.e:
at 8000 working hours (ie 333 days) per year ... .1.9.106 t / year (density of fermented mash ~ 1 kg / m3)
concentration of solvents in the medium (sum of ABE) ... 20g / l, of which 13g / l butanol
of 1,9.106 of the mash yields 38 kt of solvents, of which ~ 25 kt of butanol per year
The fermentation time in the production fermenter (I to XV in Fig. 1) at the ideal course is 36 hours from inoculation ... i.e., the productivity of the fermentation itself is 0.55 g / l / h

Filling of mixers, inoculants and production fermenters is assumed to be about 80% of their volume.

Preparation of inoculum:

The inoculation ratio, ie the amount of bacterial sprout on the seeding, is about 0.5% vol.

The preparation of the inoculum is three-step, of which the first two steps (from the spore culture to a flask and from a flask to a 25 liter barrel) are performed in the laboratory (see Fig. there is also a microscopic control and culture selection for the next experiment. The laboratory output is an 18 L inoculum which is inoculated into an inoculating fermenter (vessels S-I to S-VIII, each ~ 4.5 m3). (*Note: I would estimate the cost of equipping a 24-hour laboratory, providing the activities necessary for the operation of the plant, to about CZK 10 million -.)*

The growth time of the inoculum in the seed fermentor (vessels S-I to S-VIII) is 18 h after inoculation; the output of each seed fermentor is an inoculum of 3.6 m3 (~ 3.6t).

The number of containers is given by the number of fermenters (see section of fermentation)

Requirements for the technical solution of the container: see the section of the fermentation itself.

The cycle of preparation of the inoculum in one fermenter, including the operating times, is about 23h (the schedule is similar to the fermenter and is shown in brackets in Table I).

Fermentation:

The actual fermentation takes place in 15 fermenters (I to XV, see Fig. 1), where the fermentation parameters in one fermentor are as follows:

Container volume: 900 m3

Amount of fermented mash (ABE 20 g / l): 714 t

Requirements for the technical solution of the container: a duplicator jacket, allowing temperature control to within ± 1 ° C, temperature and pH probe, mixer, exhaust cooler, sterile inoculum inlet, CIP (cleaning-in-place) (sterilization-in-place) operations

Table I - Fermentation cycle, including necessary operating times, for one fermenter

	Process time (h)	End of process after (h)
Fermentation time from inoculation	36 *(18)*	36 *(18)*
Pumping the fermented mash onto the distillation column (into the production fermenter)	3 *(1)*	39 *(19)*
Cleaning (CIP)	1 *(1)*	40 *(20)*
Sterilization of the fermentor (SIP)	1 *(1)*	41 *(21)*

Pumping sterile medium to bioreactor	3 *(1)*	44 *(22)*
Inoculation, pH adjustment	1 *(1)*	45 *(23)*

The data in brackets are for one inoculating fermenter of 4.5 m3.

To ensure the continuous feed of the fermented mash on the distillation column, 15 fermenters are required; their number is given by the length of one whole fermentation cycle (see Table I) and the size of the container. The size of the container was chosen having regard to the fact that after fermentation (36 h) the good fermentation of the fermentation was not allowed to stand for a long time; The 3h pump per column therefore appears to be optimal.

Process Balance

The achievable solvent yield of added non-consumed sucrose is 40%.
A concentration of 20 g / l of solvents is obtainable from a medium containing 50 g / l of sucrose.

Table II - Raw materials consumption

variable	**Annual amount**
saccharose	$9{,}5\ .10^4$ t
Sugar beet (saccharose conc. 17.5 %weight.)	$5{,}4.10^6$ t
Concentrated sugar juice (70% saccharose)	$1{,}4.10^5$ t
Sugar bbet area if yield is 65 t/ha	87 000 ha*
Yeast extract (conc.5 g/l)	9500 t
Ammonium sulphate (conc. 2 g/l)	3800 t
KH_2PO_4 (conc. 1 g/l)	1900 t
$FeSO_4$ (conc. 0,1 g/l)	190 t

+ H_2SO_4 andNaOHpH adjustment–ě 1000 t/rok

**** This year (2011), sugar beet is grown on an area of 58,000 ha for the production of sugar and ethanol in total.***

+ water

Table III – Products

variable	**Annual amount**
Fermented medium	$1{,}9.10^6$ t
solvents (A+B+E)	$38\ .10^3$ t
1-butanol	25.10^3 t
acetone	$9{,}7.10^3$ t
ethanol	$3{,}3.10^3$ t
CO_2	20.10^3 t tj. 1.10^{10} l*
H_2	500 t tj. $5{,}6.10^9$ l*
slops	~ $1{,}86\ .10^6$ t

*1 mol gas –volume 22.4 l

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Seminar V

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Production of ethanol from syngas

Input data:

Design a pilot production of ethanol with a capacity of 1000 liters / year from syngas using *Clostridium ljungdahlii*. Syngas will be made from municipal waste by gasification in a plasma reactor at 1000 ° C and will be used as a substrate for ethanol production after cooling. Consider the model composition of syngas 25% CO, 20% H2, 5% CO2, remainder N2. Consider that production will work in a continuous mode and will run in two stages, biomass growth at a pH of 5.5 will occur in the first stage, and in second stage ethanol production at pH 4.5 will be performed. Consider for the case of simplicity that in the first stage only acetate from syngas will be formed and in second stage only ethanol from both acetate and syngas will be formed as products. At the output of the second stage, the biomass will be separated by microfiltration and its portion / total will be returned to the bioreactor. The biomass dry matter concentration in the second bioreactor should be about 5 g / l at a steady state. Recycle of biomass will be set so that initially 100% of the biomass is returned to the reactor and after a month of running, only part of the biomass will be returned to the bioreactor so that the biomass concentration remains constant. (Recycle therefore prevents the bioreactor from flooding off the biomass). Both streams will carry liquid streams with mineral medium (2x concentrated) and gas will be fed to both stages, which will be cooled down (condensation of water) and then incinerated. In both steps the pH will be adjusted to the desired values. The liquid output from the first stage will be led to the second stage; the doubling time of biomass in the first stage is 16 h, in the second 24 h.

The fulfillment of the task presupposes the submission and approval of a written study of the solution and preparation of the presentation of the individual parts of the task.

Tasks to solve:

1. The microorganism uses Wood-Ljungdahl's metabolic pathway to produce autotrophic growth under anaerobic conditions; however, it can grow even heterotrophically, ie on carbohydrates. Provide an autotrophic path diagram and try to build / find the appropriate culture medium for autotrophic growth.

2. Preparation of the first stage of the inoculum and storing the strain will take place heterotrophically, suggest appropriate conditions and laboratory equipment for this step. Consider anaerobic cultivation in bottles by the so-called Hungat technique. The second step of the inoculum will be semi-heterotrophic / autotrophic in the bioreactor where a small amount of yeast extract will be added to the defined medium. The second stage inoculum will be inoculated into the first stage of cultivation. The inoculation ratio will be 1:10. Design the entire preparation of the inoculum and the equipment of the control laboratory.

3. Draw the master scheme of the main fermentation and specify the required container volumes. Considering that you need to produce 1000 liters of ethanol per year, the ethanol production rate is 0.3 g / l / h, the ethanol yield of this synthesis is 40%, and only half of the total CO supplied is used as the substrate. The gas leaves the bioreactor saturated with water at a given temperature (37 ° C), condensing water in the condenser returns to the reactor. The residual CO and hydrogen are used in a cogeneration unit to generate electricity / heat. The concentration of ethanol at the exit of the second fermentation step is 20 g / l. Count on the device going to steady continuous mode for 1 month. Consider filling the bioreactor container up to 70% capacity. Consider the first reactor as mechanically stirred, second as a bubbling column with biomass recycling or as a biomass immobilization / reactor filling / membrane reactor. Supply gas to both stages at level 1 VVM.

4. Design a distillation scheme for this operation to keep a continuous feed of the ethanol produced on the distillation column. Assume that the fermented broth is fed to the boiling column, ethanol is extracted from the top of the column, and the distillation flap is dropped. Make a simple balance, neglect the losses on individual devices. Suggest the possibility of obtaining anhydrous ethanol and how to regenerate the reagent. Calculate the energy intensity of steam preparation used for distillation. The waste from alcohol production is distillation effluents containing non-volatile components of the fermentation medium, in which case they will be drained, the water will be recycled and the remainder taken to the sewage treatment plant.

5. Find out what amount of heat or electricity you can get after using the remaining syngas after passing through the bioreactor in the cogeneration unit. Consider that the combustion heat of this gas is about 3 MJ / m3. Calculate how much gas you need to produce for a given amount of ethanol. Find out in the literature how many raw materials (biomass) you need to get the amount of gas - specify for different examples of biomass (wood chips, average municipal waste ...).

Final task for the whole group:

Specify the conditions under which production could be profitable, considering that you will have the input material (waste) for gasification free of charge; to gasify will be plasma at 1000 ° C and excess heat from the cogeneration gasification will be used for distillation.

Default recommended literature (not limited to it):

- Kopke M., Held C., Hujer S. et al. *Clostridium ljungdahlii* represents a microbial production platform based on syngas. PNAS 107, 13087-13092, 2010.
- Martin ME, Richter H, Saha S, Angenent LT. Traits of selected *Clostridium* strains for syngas fermentation to ethanol. Biotechnol. Bioeng. 113, 531-539, 2016.
- Bengelsdorf FK, Straub M, Durre P. Bacterial synthesis gas (syngas) fermentation. Environmental Technol. 34, 1639-1651, 2013.
- http://www.lanzatech.com/innovation/technical-overview/

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Seminar VI

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SUCCINIC ACID - DESIGN OF TECHNOLOGY AND EQUIPMENT

Input data

The task of this credit project is to design biotechnology for succinic acid and equipment for its production. There will not be any major traffic, but just a verification operation, where 100kg of pure acid should be produced daily. Succinic acid is an aliphatic, saturated C4 organic compound that can be a precursor for various chemical reagents, synthetic resins, biodegradable polymers, herbicides, fungicides, detergents, and others. The microbe producer can be a number of microorganisms ranging from yeast, mold to bacteria. Recently, it has been shown that the most advantageous microorganisms for its production are the kapinophilic bacteria of the species *Actinobacillus succinogenes, Anaerobiospirillum succiniciproducens, Mannheimia succiniciproducens.* Work entirely on *Actinobacillus succinogenes* or *Mannheimia succiniciproducens*. Your choice is based on the number of publications available and mainly available and useful for your work. In addition to microbial technology, it is critical for the whole production and its economy to get the product clean. In the project, you will deal with the "downstream" process only in crude amber acid with a purity of about 90% (dry product). However, it is necessary to state what impurities are present in the product.

Because you will be working in a group, you need to discuss the lessons learned. It should be remembered that individual operations must be linked to each other, even in terms of the quantity of intermediates. Build a block diagram of the solution and choose one project manager (manager) to coordinate the solution.

In order to solve the task, draw a block diagram of the whole pilot technology including the balance of all the inputs and outputs of the process, ie the incoming and emerging gaseous and liquid substrates and "waste".

Calculate the consumption and cost of raw materials for this semi-run. Suggest also which raw materials of synthetic medium could be replaced and what.

In the report, list all the equations and formulas you used in the calculations, including the explanation of all the symbols. For calculations, use the data found in the professional literature, including all the resources used. You probably do not find a comprehensive survey and technology-focused article, so you have to carefully control the results and compare them in the whole context. Do not mix results achieved for different microorganisms and different cultivation conditions

Tasks:

1. Task

Research work on succinic acid and its production by *Actinobacillus succinogenes* and **Mannheimia succiniciproducens**

Focus on the origin of bacteria, their metabolism (metabolic pathways) in relation to the production of succinic acid. Look for the composition of the cultivation media and make a choice for your solution. Learn how individual investigators have done cultivation, look for kinetic data and other technological parameters to help you solve the project. It is very important to find a graphical representation of cultivation (growth and production curves) with the greatest amount of monitoring (graphical dependence).

Kinetic data and all important parameters are shown in the table and try to calculate the derived quantities.

Indicate the possible use of recombinant bacteria and with what results.

Work with the genetically engineered yeast of *Saccharomyces cerevisiae* and *E. coli* is also emerging. What results have been achieved?

2. Task

Design of media preparation, inoculum and selection of cultivation mode.

When designing media, go out of the literature, judge a few cases, and finally select one, or you can combine designs appropriately. Both bacterial strains are fairly sensitive to compliance and composition of the medium. In this solution, you must address the most appropriate way of preserving the culture. This information should also be sought in the literature. To determine how many promotional steps you will need before inoculating the last reactor, determine the size of the reactor first (see Task 3). Laboratory promotion will only be conducted at a volume of about 10 liters. Other promotions will take place in a series of small bioreactors. Design the size, shape and equipment of promotional containers. Specify how the cultivation of the inoculum will be controlled and when the volume from one container will be transferred to the other. Draw a technological diagram of inoculum preparation (design number and size of containers including design of process conditions - mixing, aeration, temperature, pH) including all input and output streams and their mass balance. Make the production schedule of the inoculum from the beginning to the first production stage if the operations are continuously continuous (take into account the times required for manipulation - eg over-pumping) and a sufficient amount of inoculum was delivered to the main fermentation. Draw the inoculum production diagram and make the input and output balance of the fabric.

3. Task

Design of the bioreactor for semi-operational amber production

When designing a bioreactor, first come out of the data that 100 kg of succinic acid is to be produced per day, which will be in the form of a crude product of 90% purity (ca 111 kg). Then proceed to process the following points:

- The type and specificity of the fermentation
- The media properties (viscosity)
- The necessity of the additives
- Absorption
- The pH profiles adjusting the pH
- Sensitivity of the culture to oxygen, the shear forces
- How many streams the medium and auxiliary compounds
- Gas consumption and production
- Concentration of carbon source
- Some redox potential of the medium is maintained
- Under all conditions, you will bring CO_2 to the medium, introduce the equation for the transfer of carbon dioxide to the medium and then to the cells. Calculation for KLa (CO_2). What is the literature? Based on the analysis of individual points, design the geometry and size of the reactor, the effective volume of the medium, the fittings, the agitators, the sampling, the sterilization of the medium - the method of heating and cooling, the medium sterilization (in situ or outside the reactor) carbonate,

draw the reactor diagram, state the way of its sanitation (in more detail). Try to design the mixer and calculate the engine power.

4. Task

Design of batch /fed-batch culture of *Actinobacillus succinogenes* and *Mannheimia succiniciproducens*

The aim of this part of the project is to give a complete overview of the production of succinic acid. You can use the following points to solve.

- Choose from the literature as much information as possible about the course of cultivation, provide charts showing the time dependencies of the main and by-products.
- Which cultivation methods are most commonly used in the laboratory, in operation (if you find the appropriate literature)
- Write the requirements for the inoculum (size, age, physiological criteria). How to evaluate the properties of the inoculum for its selection?
- Based on the analysis of the available materials, suggest the fermentation method (batch or inflow) and the choice to justify
- What analyzes should be performed and which analyzes would only illustrate the course of the fermentation
- Which bioengineering quantities will be measured
- Pay special attention to the composition of the media. It is desirable that the productivity of biosynthesis be as high as the main product concentration. How can this be achieved?
–How to evaluate the inhibitory effect of the product, succinic acid, on the fermentation process.
- What kind of mixing you recommend for this fermentation and why,
- Draw the time course of one fermentation that you consider the best. What micro-organism would you use? The graph will contain a larger number of variables, the values of the individual variables must be checked on the basis of the balances made,
- Complete this monitoring with a table of the resulting quantities (concentration, growth rate, yield, productivity ..)
- Draw and describe the whole technological scheme (mass and fabric balances) inputs (including inoculation) to the outlet (gas, medium).
- Indicate how the system would sterilize and how the plant would be sanitized
- It is possible to design two reactors instead of one reactor if you know it would be more beneficial for the process
- How will you defy the fermenting medium?

5.Task

Proposal for isolation of succinic acid from the fermentation medium, its concentration on a dry raw product

This part is very important for the economy of production. Use the following points to resolve. It is not necessary to keep them exactly, but definitely do not miss them.

- Make an overview of the methods described in the literature for isolating organic acids from the fermentation medium (citric, malic, fumaric acid).
- Indicate by which methods the best results have been obtained (according to total yield)
- For example, methods of precipitation, chromatographic, electrochemical, extraction, membrane (eg pertractions), etc., are available
- Analyze the results of these isolation methods, For each given method, please provide a list of necessary equipment and chemicals.

-Finally, choose one that best describes you and describe it in detail, provide calculations, schematics, charts.

6. Task

Preparate the individual results into a detailed technological scheme (it contains the described individual devices, their capacities, the streams of the media and their material and mass balances.) Waste balance (mass, fabric)

This chapter is synthetic and will use the information of other solvers. connected with the technology Put together the entire process flow diagram, including all input and output streams and their weight balances, try to make a total economic evaluation of the process (mainly the cost of chemicals) Consider the possibilities of cheaper raw materials if the literature mentions it. water, their expected load (organic / inorganic) not only after fermentation but also after isolation. Is anything recyclable? I would recommend finding and using a reference to the industrial production of succinic acid.

recommended literature

- I.B. Gunnarsson et al. / Industrial Crops and Products 62 (2014) 125–129
- International Journal of Systematic Bacteriology (1 999), 49,207-2 1 6
- MSc Thesis: A.J.Hepburn: Queen's University Kingston, Ontario, Canada, (April, 2011) – izolace
- Zou et al. Microbial Cell Factories 2011, 10:87, http://www.microbialcellfactories.com/content/10/1/87
- Li-Wen Zhu,1 Cheng-ChengWang,Journal of Biomedicine and Biotechnology, Volume 2012, Article ID 626137, 9 pages
- James B McKinlay et al. A genomic perspective on the potential of *Actinobacillus succinogenes* for industrial succinate production
- B. H. Davison et al. Applied Biochemistry and Biotechnology Vol. 113–116, 2004, p. 653
- Hyohak Songa, Sang Yup Lee: Production of succinic acid by bacterial Fermentation. Enzyme and Microbial Technology 39 (2006) 352–361

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Seminar VII

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MANUFACTURE OF MICROBIAL BIOMASS FROM CASSAVA (MANIOK)

Design complete biomass production from dried subtropical / tropical cassava crops (tapioca, manioc) batch cultivation of *Candida utilis* yeasts for daily production of 500 kg of dry matter. The final draft will include, in particular, the balancing schemes for all inputs to semi-operational production and all outputs (including solid and liquid "waste"). In addition to the necessary calculations (give the complete calculation with the word suggestion for calculation), the final result will be the flow diagram of the whole process, indicating the weight and material balance of the individual operations. Water and air balance is also important. When designing each operation and the required equipment, give the reasons for your proposal. Your solution will include the choice of equipment needed to design the technology and the capacity it needs. Select the device to be slightly over-sized (about 20%). Pay close attention to your own bioreactor, where you need to design its design parameters (H / D, agitators, engine power, compressor capacity, etc.). In the introductory part of the report, discuss the raw material about its cultivation (hectare yields) in the selected country and, above all, its composition in relation to technology. Furthermore, the specified microorganism, its growth abilities, bioengineering parameters when cultivated on carbohydrate substrates. For the growth of biomass, it will be necessary to supply nutrients that are not in sufficient quantity in the raw material.

Input data:

- Input raw material will be 85% w / w. dry matter, starch content on dry weight basis - 60% by weight.

- Achievable dry biomass concentration in the medium at the end of cultivation of 35-40 kg / m3

- Cultivation time will depend on reactor aeration capacity (up to 24 h)

- The resulting biomass should have a min. 60% crude protein in dry matter

- Tapioca is a starchy material, and due to the absence of amylolytic enzymes in the production micro-organism, scraping will have to be done.

- An important parameter for the optimal process will be the design of the V / S ratio - the ratio of added water to the feedstock delivered. This ratio is chosen so that the final glucose concentration in the resulting suspension is 8-10% by weight.

- Data to calculate mixing: specific gravity of the suspension at the beginning of the fermentation - 1100 kg / m3, dynamic viscosity - 0.008 Pa.s, gas retardation - 25%, ventilation 1 VVM

Tasks

1. Discuss cassava crops (composition, cultivation, ha-yields, find references to a cyanide-containing crop, world cassava production, world price, its total utilization, and biotechnological uses). Next, discuss enzymes suitable for the distribution of starch to fermentable sugars (product information, liquefaction / squeeze feed, use, durability, storage, very useful information on the price of enzymes)

2. Processing the dry cassava into a medium containing glucose (grinding, enzymes used), removing the solid phase from the resulting suspension such as solid phase solids, solid phase separation devices, nutrient addition calculations into clear media, and total treatment. Cooling to the weeding temperature.

3. Preparation of *Candida utilis* yeast inoculum, composition of inoculant medium, inoculation ratio, number of inoculation tanks, size, tanks equipment. Cultivation time of inoculum. Critical criteria for suitability for further transfer of inoculum.

4. Engineering design of the bioreactor (basics from Bioengineering of Fermentation Process, from Aiba, Humphrey, Millis: Bioengineering

5. Fermentation process - description, kinetic process data, graphical representation of batch fermentation (biomass, glucose, ammoniacal nitrogen, CL, specific growth rate, ethanol as a function of time). Design of optimal ventilation depending on cell growth

6. Separation of yeast from the medium (centrifuge type, centrifuge hourly rate), yeast milk wash, yeast milk concentration and its amount. Supernatant volume, dry matter concentration, or COD. Yeast thickening and thermolysis, drying. Amount of evaporated water, type of evaporator and dryer. The final dry weight of the product - 93% w / w.

7. Utilization of solid and liquid wastes (feed production, biogas production). Indicate the composition of these wastes, this is important for further decisions on how to conduct further processing. For biogas production, consider that 1 kg of organic matter is produced ca. 500 liters of biogas (under normal operating conditions), then design the gas tank size and further biogas processing.

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Seminar VIII

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Production of feed biomass (*Saccharomyces cerevisiae*) from agricultural crops

Model design

Requirement: 160 kg yeast / day, dry matter 20%

Raw material: wheat, topinambury

Design of technology:

Prerequisites: Yield of biomass during aerobic cultivation: $Y_{X/S}$ = 0.45 g / g

Starch content in wheat: 67% (Dvořáček et al., 2010)

Topinambur inulin content: 16% (Čepl et al., 1997)

Enzyme hydrolysis yield: 90%

Consumption of raw materials per day:

Daily production of 100% biomass: X = 0.2 · 160 = 32 (kg / day)

Daily Sugar Consumption: S = ΔX / Y_ (X / S) = 32 / 0.45 = 71 (kg)

Daily wheat: m = wheat = 71 / (0.9 * 0.67) = 118 (kg)

Daily amount of topinambur: m_topinambur = 71 / (0.9 * 0.16) = 493 (kg)

Annual consumption of raw materials during operation 300 days / year: 54 t of wheat or 148 t of topinambur with production of about 10 t of dry biomass (50 t of biomass with dry matter 20%)

Overview of batch process:

sugar content in medium 45 g / l, yeast growth 20 g / l, cultivation time 15 h, average biomass productivity 1.3 g / lh.

Batch volume: V = (32,000) / 20 = 1,600 l = 1,6 m ^ 3

The volume of the inoculum at the 1:10 inoculation ratio will be 160 l

Preparation of the inoculum: 1st stage - laboratory, volume 150 ml, inoculation from malt agar, 18 h (to be tested)

2nd Grade - Laboratory, Volume 1.6 L, Vaccination from the previous stage, 12 h

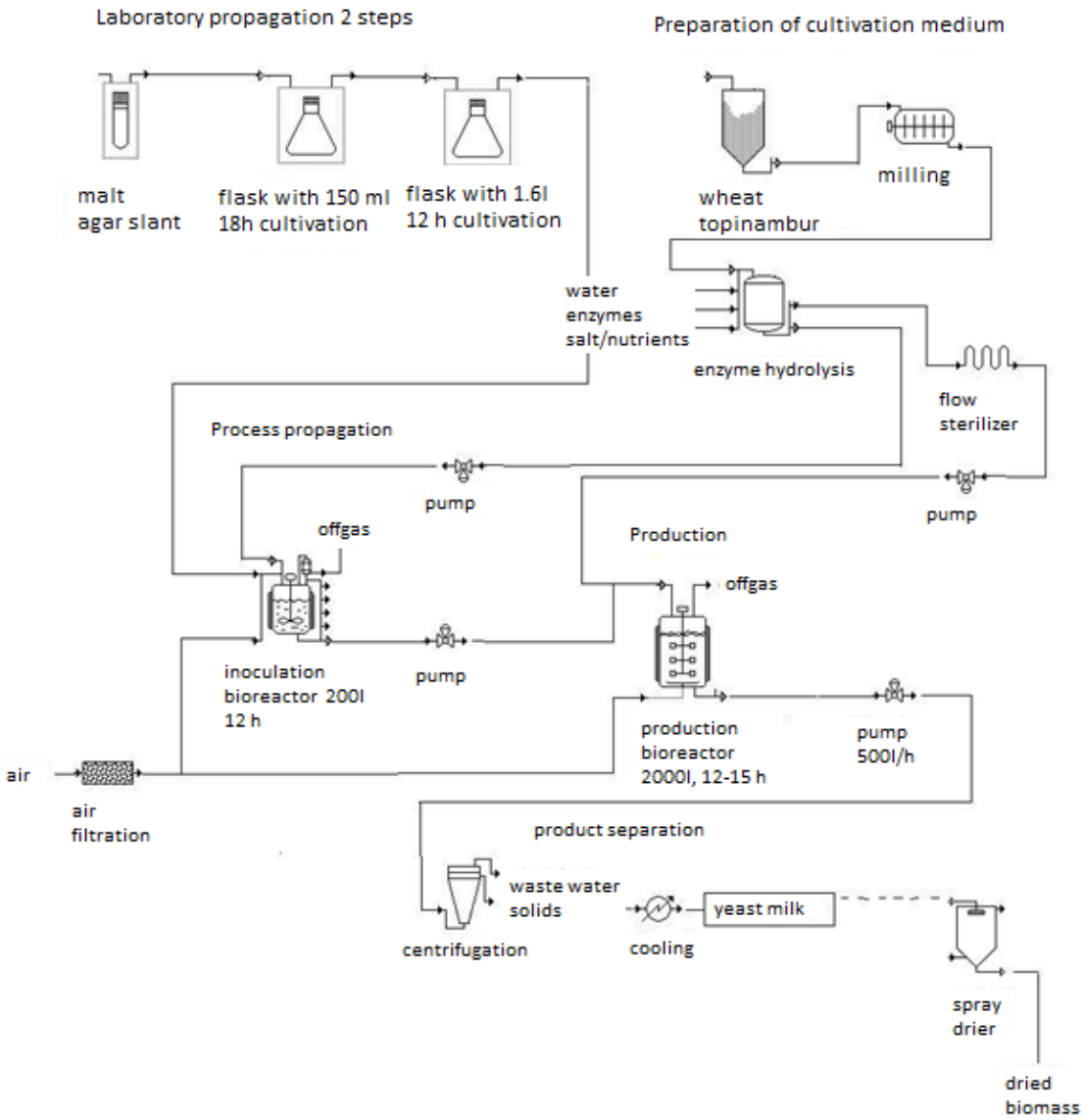
3rd stage - operational bioreactor, volume 160 l, vaccination from the previous stage, 12 h

Biomass production: operating bioreactor, 12-15 h, volume 1 600 l

Bioreactor volume: 2 m3 production, 0.2 m3 inoculation

Note: Even in the aerobic cultivation of *S. cerevisiae* a small amount of ethanol (~ 0.5-1%) can be produced !!!

The process diagram is shown in Figure 1.



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Seminar X

Food hygiene topics for group work

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1. Suggest the main points that should include training for workers who will work with larger packs of enzyme preparations in food production.

2. You are a member of the Czech Agriculture and Food Inspection Authority and your task is to take a sample of milk from a tank in a food factory and check its microbiological safety. How will you proceed? Also suggest what you will determine and how.

3. You want to produce protein with a genetically modified microorganism. What legal requirements do you have to meet which documents you have to process and what you need to ask for?

4. You work in a bakery. What kind of papers you will require from the flour supplier and what you will randomly check the flour.

5. You should check the efficiency of CIP cleaning in the brewery. How do you stand up to the challenge?

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Seminar XI

Topics for short presentations

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Edible algae cultivation

Find information on the growing of edible algae in the literature, especially so called microalgae such as *Chlorella, Desmodesmus (*formerly *Scenedesmus) or Artrospira (*formerly *Spirulina*). Focus on obtaining information about the difference between cyanobacteria and algae, about the way of life of micro-algae (metabolism, reproduction, cultivation requirements - light, mineral resources), about their cultivation (cultivation in open, outdoor and closed systems) and their processing for human nutrition (dietary supplements).

Microbial systems for biological support of life in the cosmos

Find information on microbial systems, or on algae systems, designed to maintain the right living conditions for the human organisms in the universe in the literature. Focus on the recycling of waste (especially the decomposition of organic matter and nitrogen cycles) and the exchange of CO_2 / O_2 gases mediated by these microbial systems at space stations or planned human flight crews on Mars. Emphasize the advantages and disadvantages of biological systems to support life compared to the currently used physicochemical ways of regenerating the atmosphere in space missions.

Microbial production of carotenoid pigments

Find information on the formation of carotenoid pigments by bacteria and yeasts in the literature. Focus on the brief characterization of microbial carotenoid pigment producers, the mechanism of carotenoid pigment formation in microbial cells, the cultivation conditions under which carotenoids are formed and the possibility of further utilization of carotenoid pigments.

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Seminar XII

Topics for short presentations

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Topics for Short Oral Presentation - Fermentation Technology

1. What is shii-take-and how it is grown

2. How to make a spirits - gin

3. How to make a spirits (distillate) – „borovicka“

4. How to make liqueur wine - Madeira

5. How to make sake

6. How to obtain propionic acid by fermentation

7. How ethanol is metabolized in microbial cells

9. Give the complete taxonomic classification of *Saccharomyces cerevisiae* and relative yeast

10. How to make tequila

11. How to make apple vinegar

12. Why should not drink alcohol after eating the edible fungus from *Coprinus* genera

13. How to make Malaga liqueur wine

14. How to make dextran

15. How to make xanthan

16. What is fermentation of tea

17. How to produce fat and fatty acids by fermentation

19. How is fermented gluconic acid

20. Which mycotoxin decomposes by ethanol fermentation under what conditions this mycotoxin forms

21. How to make truffles

22. How to make quick-chew cucumbers what kind of fermentation is it?

23. What happens when fermenting cocoa

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Seminar XIII

Convenient topics for ethics discussion

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Convenient topics for discussion of ethics issues in Biotechnology in Food Industry

- GMO crops as feedstock for biotechnology
- GMO microorganisms to be used in Food industry (ethanol production, winemaking, organic acids..) versus GMO microorgnaism used in Pharmaceutical biotechnology for vaccines, hormones, therapeutic proteins…
- Irradiation to reduce food contamination
- Biofuels of first, second and third generation
- Burning of food crops under over-production
- Planting of corn for biogas production

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Seminar XIV

HACCP design

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Based on the following scheme, prepare a brief design of main HACCP principles necessary to maintain during winemaking.