Complex utilization of dairy waste (whey) in Biorefinery

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Abstract: - Whey is one of the most important industrial wastes. The annual worldwide milk collection and processing is around 622 Million tons [1], and the rest of the process is whey, which content and quality is determined by the dairy technology: while after cheese production sweet whey is the byproduct, skim and cottage cheese production results sour whey (poor in lactose). While pure sweet whey have several high added value containing commodities (for example for infants) with increasing demand and production, sour whey is usually combined, stored and handled together with primary washing waters of the facility and is transferred for final degradation. Since the latter one is poor in lactose and further diluted with washing streams, it is very difficult to utilize it. However, it still has high COD and TOC content resulting high cost of wastewater treatment and even high risk to the environment. For this reason we developed a complex utilization of such dairy waste streams for providing more products and less environment risk with less costs for the production site.
We set up with the help of experiment supported process simulation (i.e. flowsheeting) a biorefinery concept, in which the low lactose content of this waste is converted into yeast biomass for ergosterol (i.e. previtamin D_2) production, and the residual yeast debris of ergosterol section is combined with the lactic acid containing residual organic part of the used whey for application to propionic acid and vitamin B_{12} production.
Our simulation study revealed, that with appropriate ratio between production lines "zero landfill" theory can be reached and valuable products with profit can be generated at the same time.

Key-Words: - dairy waste, whey, process simulation, biorefinery, propionic acid, ergosterol

1 Introduction

According to recent trends the annual milk production in the EU is slightly increasing [1]. Gira's report for European Commission calculated with special potential of whey and its increased production, consequently. Meanwhile basically the main valuable components of milk are transferred into its commodities (like cheese, butter, whole-milk-protein:WMP, skim-milk-powder:SMP, whey etc.), the resting solution have still some lactose, protein and lactic acid content resulting high chemical oxygen demand (COD) in wastewater treating and high costs as well.
The problem of whey is not new: it has more decade long history to develop different utilization processes and strategies. However, the existence of the problem can be well indicated with the numerous recent publication in that field: *www.sciencedirect.com* gave 7.613 results for the term "whey utilization" and among them 1.711 was published in the last 5 years!
In Hungary the milk market is around 1,7 million tons/yr [2], and its 65% is connected to the top 4 companies [3], thus their whey processing strategy is determining the quality and quantity of this waste.
Among the 4 market leaders one is situated in Transdanube, and has a dairy facility with butter, milk, yoghurt, skim and cottage-cheese production. Thus it produces sour whey as byproduct, which has low carbohydrate content but relatively high lactic acid content. This makes it more difficult to utilize it in comparison to cheese manufacturer's whey (sweet whey), which can easily refined to infants nutrients.
While "whey utilization" resulted more than 7.000 results at www.sciencedirect.com, "whey biorefinery" only gave 196 results, but the most of them were only applying whey as a supplement to different biorefineries such as poly-

hydroxyalcanoate production [4], succinic acid fermentation [5], nisin and lactic acid [6], propionic acid fermentation [7], or xanthan gum [8] without giving a full list.
Gilson et al [9] presented a sweet whey processing biorefinery, which only separate and purify the components of sweet whey.
The main object of this research was to set up on partly literature and partly experimental basis a complex whey utilization process converting the available components of sour whey into high value added derivatives, providing economic and environmental benefits at the same time.

2 Problem Formulation

The fourth largest dairy facility in Hungary produces 20m3 waste liquid in every two days with whey basis. As the facility produces butter, milk, yoghurt, skim and cottage cheese, its whey was expected as poor organic matter content. However we found 7g/L protein, 9g/L lactose, and 15g/L lactic acid content. This organic content can be evaluated as too high from environmental aspects, (27.000COD/L) but too low for biotechnological utilization. To overcome this contradiction, we aimed to convert this useful compounds into high value added products like vitamins, of which can be partly utilized *in site* for dairy products. Thus our main goal was to examine both experimentally and *in silico* ergosterol production on this waste. Furthermore, there are only a few microorganisms able to utilize lactate as carbon source. Among them we found *Propionibacteria* interesting, since they produce not only the valuable propionic acid but probiotic biomass as well. To combine and study these processes together we built up a flowsheet, and used process simulation as an effective tool for determining the optimal stream ratio and feasible size.

3 Problem Solution

We bought yeast for studying ergosterol fermentation and *Propionibacterium* for propionic acid and B12 fermentation.

3.1 Experimental part

Every fermentation process were studied in shaking flask experiments, but also checked in a bench top fermenter (Biostat Q, B.Braun). Every tools, container, fermenter and media solution were sterilized in Tuttnauer ELV3870 autoclave for 121°C 1bar 20 min. While cell growth were monitored by off-line spectrophotometric determination of OD_{600} with triplicates, substrate and product concentration were determined by Waters Breeze HPLC system on BioRad Aminex HPX87H column at 65°C and 0,5ml/min eluent rate with RI detection at 40°C.

3.1.1 Ergosterol fermentation

We started this research with looking for appropriate strain: which is able to utilize lactose, and can produce as high as possible of ergosterol content. According to several literature sources, *S.cerevisiae* is one of the best ergosterol producers [10], but it can not consume lactose (or only after hydrolysis) and furthermore have Crabtree (i.e. inverse Pasteur effect limiting the maximal carbon-source level), thus we have chosen *Cryptococcus terricola* according to Pasanen et al. [11].
After strain selection (*Cryptococcus albidus sp. aerius* NCAIM $Y.01319^T$), we characterized the strain through applying different carbon sources (glucose, galactose, lactose, saccharose) and different nitrogen sources (yeast extract, tryptone, NH_4Cl) in a "reference" media according to Hansson et. al. [12], composed from 20g/L carbon source, 1g/L yeast extract (YE), 1g/L tryptone, 3g/L KH_2PO_4, 1g/L $MgSO_4*7H_2O$, 15mg/L $FeCl_3*6H_2O$, 7,5mg/L $ZnSO_4*7H_2O$, 0,5mg/L $CuSO_4*5H_2O$, and 1g/L NH_4Cl. Other parameters were 250rpm, pH=5.5 (controlled manually once pre day with 5% NaOH solution), and 25°C. These examined parameters had the following effects on cell growth (supposed to correlate with ergosterol content, which is cell-membrane component):
-we could not detect any significant differences among the tested carbon sources in terms of final cell concentration, but lactose utilization seemed to be somewhat slower, than the consumption of monosaccharides (Fig.1A.)
-among the tested nitrogen sources lower level of inorganic N-source seemed to be more effective (Fig.1B.)
-among the tested pH we could not observe significant differences, more acidic pH seemed to be slightly more effective (Fig.1C.)

Furthermore, we also checked, wether the poor whey sample from the dairy plant should be supplemented with different part of the reference media (first whey with salts+YE (no tryptone), then only with salts (nor YE, nor tryptone) and finally with salts and YE supplemented with extra sugar, but without tryptone) (Fig.2.).

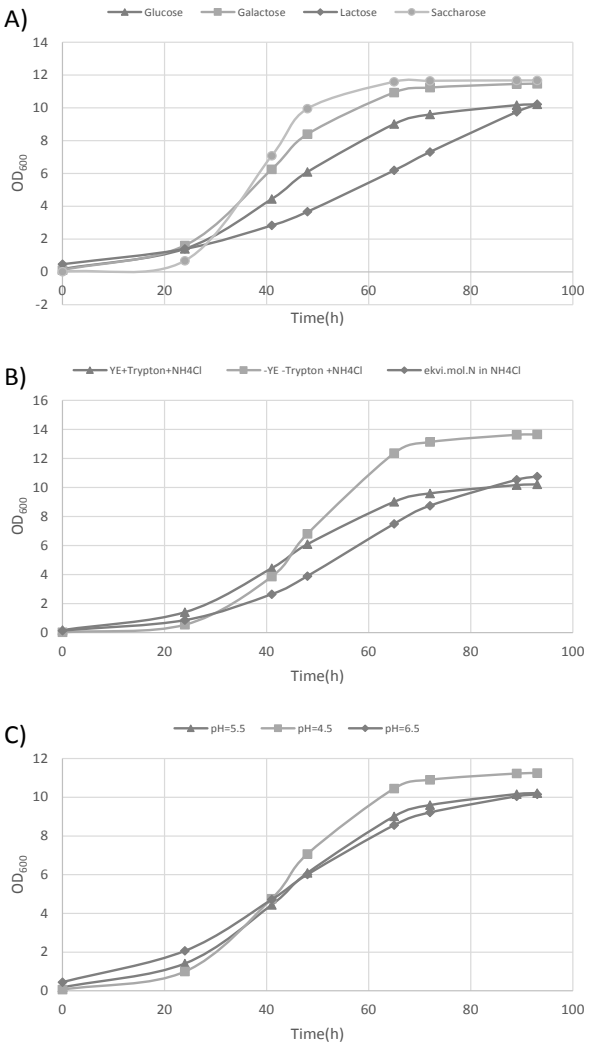

Figure 1.: Characterization of C.albidus ergosterol fermentation on reference media

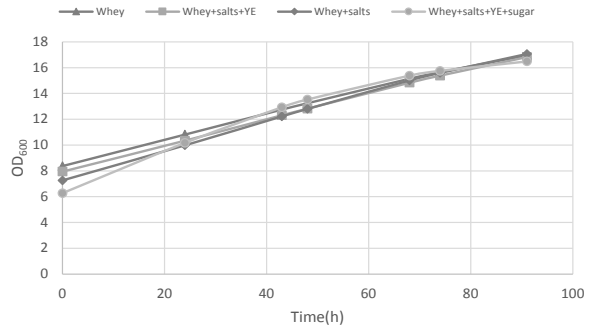

Figure 2.:C.albidus cell growth on natural and supplemented whey

Finally, we "scaled up" the process to bench-top fermenter. We controlled the temperature at 25°C, pH=5.5 350rpm with magnetic stirrer and 0.4vvm aeration in 0,7/1L volume. For inoculation 100ml overnight culture was used grown on the above described Hansson media. (Fig.3.)

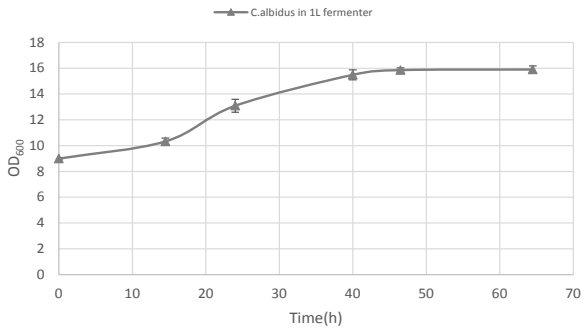

Figure 3.: C.albidus fermentation on pure whey in bench-top fermenter

This experiment was really successful, since the residual lactose was 0,1g/L according to HPLC analysis, and remarkable cell growth occurred.

3.1.2 Ergosterol isolation

Parallel to fermentation improvements, ergosterol content were always monitored after disruption of cells by 8% NaOH solution at 85°C for 2h followed by centrifugation and resuspension in extractant solvent 96% ethanol (once with same volume of NaOH, and twice with 66% volume of NaOH) [13]. The determination was carried out by Shimadzu Prominence UFLC system equipped with DAD detector and Kinetex 2,6µ XB-C18 100x4,6 mm column. On the basis of this measurements ergosterol isolation was also improved, and specific ergosterol content of different fermentations as well as different extraction methods were compared.

The results showed 2-4mg/g cell dry weight ergosterol content which is far from the value in the reports (2-4%, i.e. 20-40mg/g), but since the utilization of the whey was successful, and ergosterol is administrated in relative low amount (µg/body kg), we were interested in its feasibility, but before presentation of it, we introduce the preliminary results of *Propionibacterium* fermentations.

3.1.3 *Propionibacterium* fermentation

Propionibacteria are obligate or facultative anaerobic strains. We preferred the later one, thus *Propionibacterium acidipropionici* (DSM20273) has been chosen. Since these bacteria occur in dairy products like cheese,

whey is one of their favourite and natural media. This fact was also confirmed by our experiments (Fig.4.), where the lactate content of whole whey was consumed preferably, while lactose content was utilized secondly.

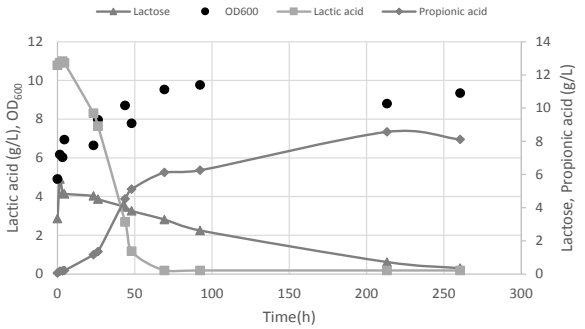

Figure 4.: Propionic acid fermentation on sour whey (30°C, oxygen rare conditions, pH=6.5, 300rpm, 10%inoculation)

These results also reinforced our plans, namely to use lactose-less whey obtained after ergosterol fermentation for propionic acid fermentation.

3.1.4 Recovery of propionic acid

In bioprocesses the downstream section often has higher cost than upstream part, because the most of the bioprocesses are in aqueous environment, and are really dilute systems.

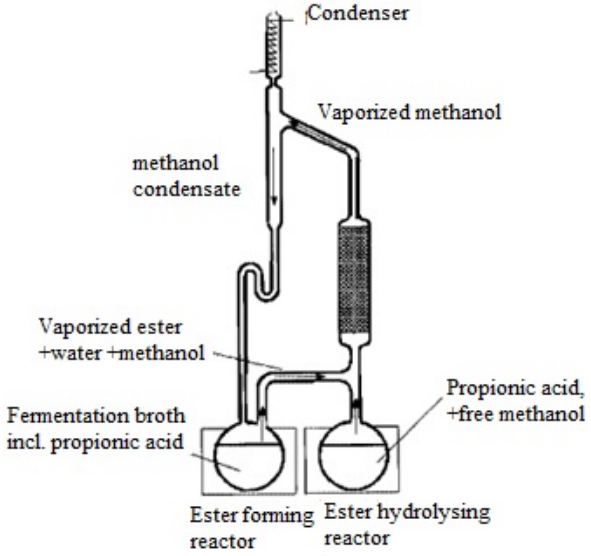

Figure 5: Propionic acid recovery via reactive extraction

Additionally, fermentations have very complex matrix in the fermentation broth, thus the purification of any products is both difficult and costly. Product recovery commonly has at least 3 major steps: cell removal, product recovery and concentration. Therefore, any innovative processes reducing the number of necessary processing steps should be considered.

We previously adapted the method of Kumar et al [14] for lactic acid recovery directly from lactic *Lactobacillus* fermentation broth through reactive extraction with methanol. Recently, we also adapted it successfully for recovery of propionic acid, since this method is based on formation of volatile methyl ester from the organic acid, and after homofermentative acid production only the desired product escaped from the fermentation broth containing even the cells. The modified and used apparatus is shown on *Fig.5*.

While the esterification reactor was heated to 95°C (pH=1,5), the hydrolysis reactor was kept at 100°C, and furthermore both reactors was set to pH=1,5 with sulphuric acid. Since the fermentation broth is a dilute aquatic media, ester formation with water escape is inhibited, thus we applied 2:1 mol/mol ratio in term of MeOH:to Propionic Acid ratio to force the ester formation. We have taken regularly samples from both reactors, and analysed them with HPLC (described in 3.1.Section). Results are presented on *Fig. 6.* which indicated, that propionic acid was successfully purified in one step, but further optimization is essential for real application.

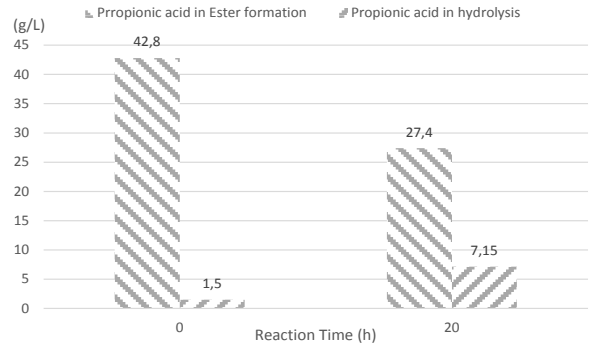

Figure 6.: Preliminary results of one step propionic acid purification

3.2 Process simulation part

Most of the above presented experiments need further optimization, but we were interested in synthesizing a new technology for complex sour whey utilization, thus we integrated these results in their present form, and make simulation studies with *SuperPro Designer v.8.5* (Intelligen Inc. US) flowsheeting software. This software is a very useful tool for calculating the overall mass and energy balances for the flowsheet, and for generating a rough economic feasibility and environmental impact.
Fig.7. represents the simulated flowsheet.
The simulated Biorefinery has three main sections: 1) Ergosterol production (with *C.albidus* fermentation) and isolation, 2)Propionic fermentation and products recovery (i.e. production of probiotic, propionic acid, vitamin B12, and pepton), 3) partly digested whey powder.

3.2.1 Ergosterol production section

This section involves a 2,5m3 fermentation (96h, 25°C, 1vvm, lactose utilization with 46% biomass yield) of *C.albidus* yeast followed by bowl centrifugation, and cell disruption with 8% NaOH, and finally after separation of supernatant by the same bowl centrifuge debris is extracted with ethanol for obtaining ergosterol (4mg *pre*vitamin D2/g CDW), which is converted by UV light into *pro*vitamin D2 concentrated in the last operation. The used ethanol for extraction is recycled, thus beside the product vitamin D2 only the extracted debris is a by-product, which can be suggested to market as animal feed.

3.2.2 Propionic fermentation section

This section involves two scale of fermentation. The first stage utilize the lactose-less whey residue provided by ergosterol line for inoculation (one 3m3 seed fermentor in staged mode with one more 3m3 fermenter) of 23m3 fermenter. The seed culture can only grow on residual lactate, but the main fermentation utilizes both lactose and lactate of fresh whey. While the production scale is running for 96h, inoculation fermentation is only 48h long, thus it can provide exponentially growing culture of *P.acidipropionici* at 37°C, 0,001vvm nitrogen flow, with 44% biomass and 33% propionic acid yield, respectively.

3.2.3 Propionic broth processing section

Fermentation broth of *P. acidipropionici* is separated on bowl centrifuge, and the supernatant is processed by reactive extraction for obtaining propionic acid as described in 3.1.4. section, with additional peptone production line on the basis of the protein rich residue of extraction. The separated biomass can either market itself or further processed via cell disruption and after re-suspension in condensate of propionic acid concentration, pre B12 (4mg/g CDW) is converted to active cyano-cobalamine which is precipitated and separated then concentrated for vitamin B12 production. The debris containing residue is drawn back to peptonisation.

3.2.4 Whey powder section

Whey powder is commonly produced from sweet whey having high nutritional values coming from high lactose and protein content. In our case, sour whey is more lower-grade, but after neutralization (of lactate) it can also be powdered by spray drying. This section provide a “buffer capacity” of the flowsheet, since the demand for whey powder is very high, thus this product has rarely marketing problem, thus it can balance the fluctuation of the market demands of the above products.

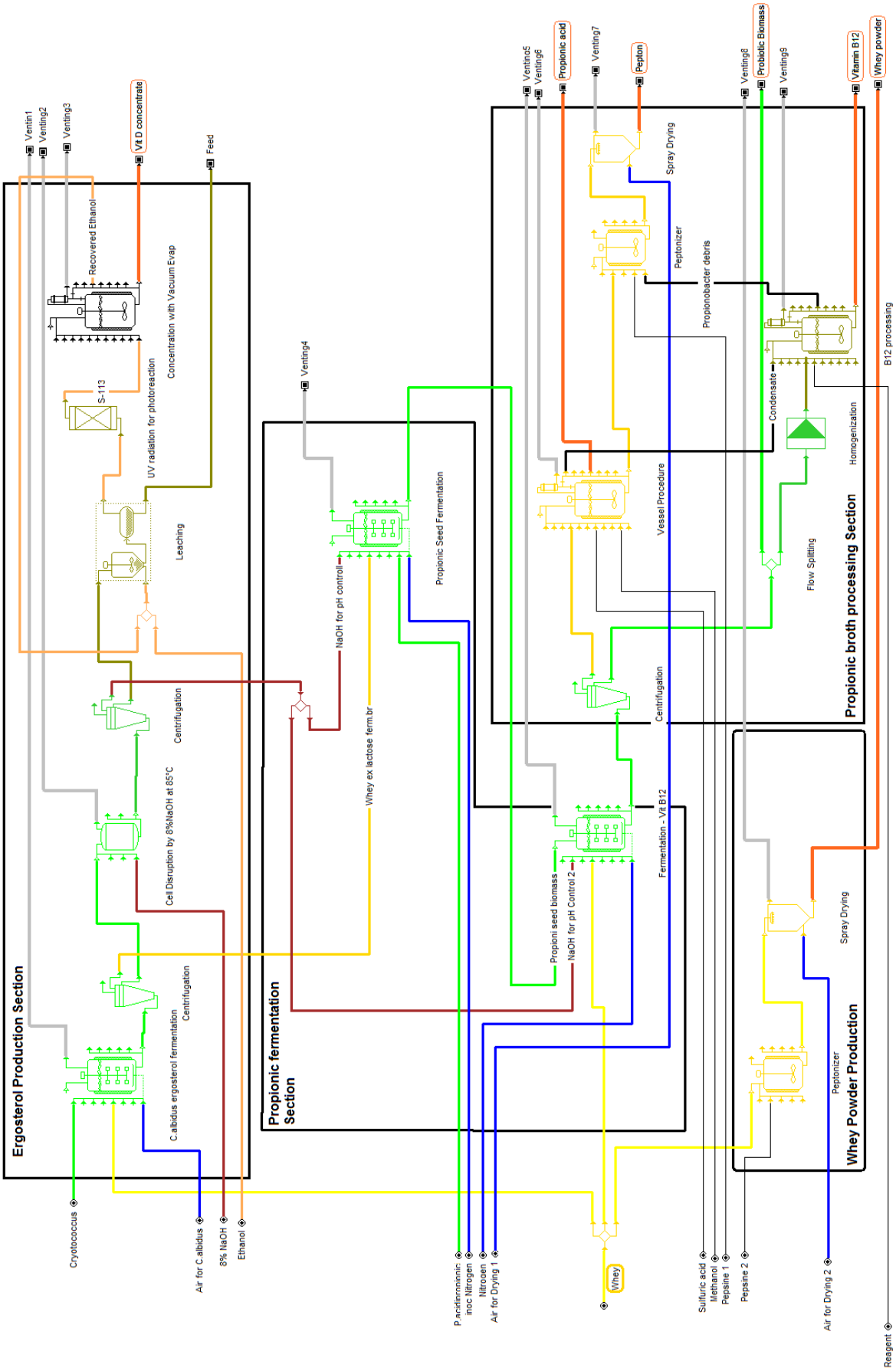

Figure 7. Simulated flowsheet for complex utilization of sour whey due conversion into ergosterol (previtamin-D2), propionic acid, probiotic, and vitamin B12

3.3 Economic evaluation of the simulation

The cost dispersion is summarized in *Table .1.*

Table 1.: Cost dispersion between sections (in 1000$/yr)

Section	Materials	Facility	Labor	Utilities	TOTAL	%
Main Section	108.00	1025.00	38.00	5.00	1175.00	10.97
Ergosterol	731,00	1339,00	423,00	25,00	2518,00	23,52
Whey	258.00	233.00	109.00	7.00	607.00	5.67
Propioni Fermentation	386,00	2017,00	1304,00	164,00	3871,00	36,15
Propionic broth processing	572,00	1466,00	451,00	47,00	2536,00	23,69
TOTAL	2055.00	6079.00	2325.00	247.00	10706.00	100.00

For further economic evaluation the ratio of revenues to operating costs (called feasibility factor) was selected as the indicator. If this is higher than 1, cash flow is positive, if it is between 0 and 1, than cash flow is negative.

3.3.1 Sensitivity of the simulation

Sensitivity of the simulation can be demonstrated due simulating changes in the raw material whey (*Fig.8.).*

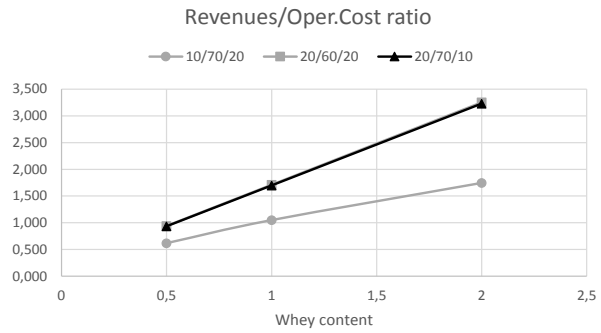

Figure 8.: Sensitivity of simulation to changes in raw-material

This sensitivity analysis resulted linear correlation between the concentration of whey and feasibility factor, and also indicated, that the present whey content is the edge of feasibility: the product revenues just cover the operating costs, but more dilute whey wouldn't provide positive cash flow in the base case (in which 10% of whey is directed to ergosterol production, 70% to propionic branch, and 20% to whey powder). If the ratio to ergosterol is increased to 20%, with either the decrease of propionic branch or with decrease of whey powder branch, the same higher slope (i.e. sensitivity to whey concentration) and feasibility can be reached.

3.3.1 Robustness of the simulation

Robustness of the simulation was examined by changes of the ratio of the different sections. *Fig.9.* shows the effect of changes in whey utilisation applying different ratio (i.e. distribution) among product lines.

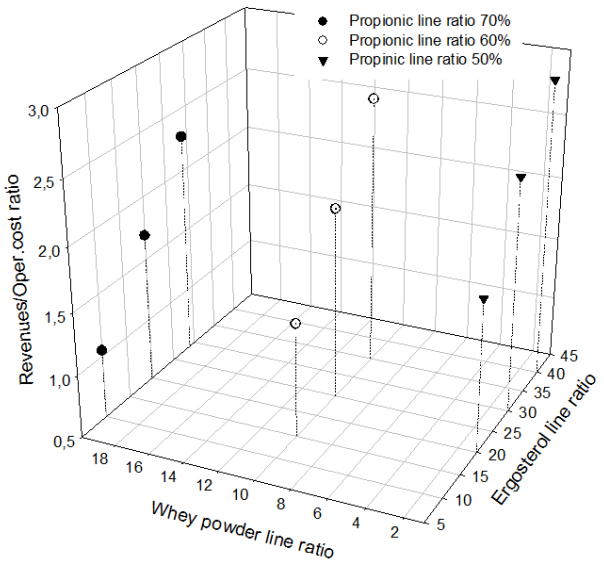

Figure 9.: Changes in feasibility depending on the distribution of production lines

This conclude to the fact, that the ratio of ergosterol should be increased either on the expense of whey powder or of propionic acid to reach higher feasibility

3.3.3 Reliability of the simulation

Since only preliminary experimental results were considered, reliability is strongly influenced by such data like biomass yields, and product yields or even agent content of biomass. To evaluate the effect of these factors on feasibility factor (=revenues/operating costs) we performed a 3^5 Taguchi design of experiments with 27 runs (with MINITAB 14 software), and its results are presented on *Fig.10.* Factors, having significant effects are biomass yield of *C.albidus*, and ergosterol content of its cells.

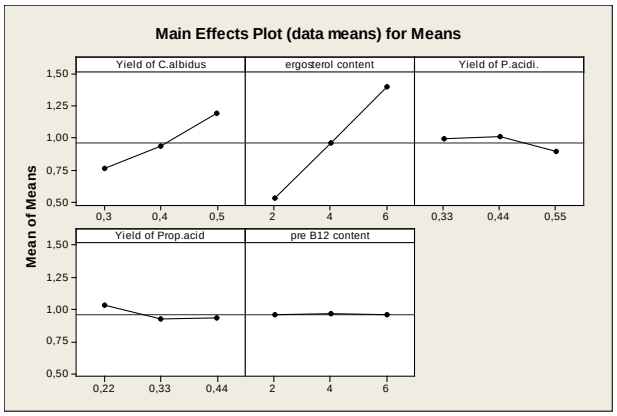

Figure 10. Statistical analysis (Taguchi 3^5) for evaluation of yields on feasibility

On the other hand, reliability of the cost estimation is influenced very strongly by the equipment costs (which is also the basis of the estimation of the other cost of investment (like building, yard improvement, insulation, piping etc.) thus its error can be amplified. To examine the sensitivity of the model to the equipment prices we ran simulation also with ±10% changes in equipment purchase cost (*Fig.11.)*

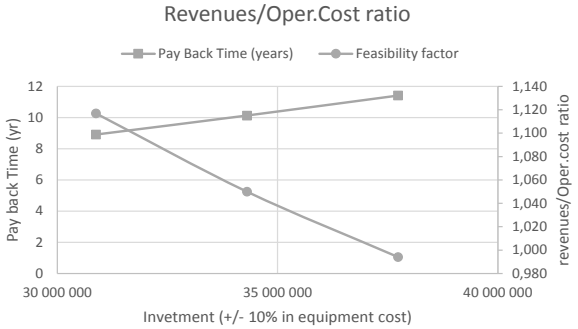

Figure 11.: The effect of equipment cost estimation with +/- 10% differences

These simulations showed, that +/- 10% deviation in equipment purchase costs can cause c.a. 2 years difference in payback time.
Additionally, cost estimation of the process is also strongly affected by the simulated selling price of the products, of which estimation is difficult since their quality is not really known, and relevant prices (i.e. manufacturer prices in high amount) are hardly available. Thus the changes in product prices were also studied in simulations with ±10% changes in the product prices (*Fig. 12.).*

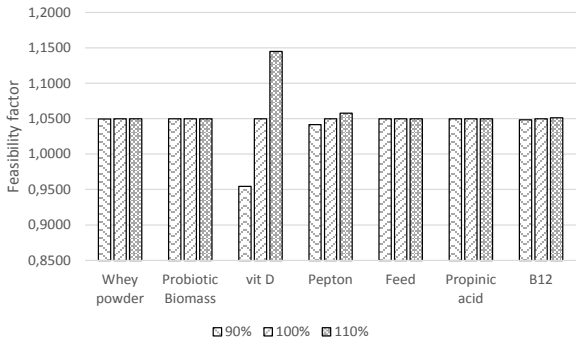

Figure 12.: Simulation of product price changes (+/-10%)

It can be observed, that significant effect could only be measured with vitamin D2 price changes, and a little effect was detected with changes of peptone price.

3.3.4 Environmental impact of the simulated biorefinery

Thoroughly reviewing the flowsheet (*Fig.7.)* one can consider, that the original goal was reached: there is no liquid waste coming out from the technology, thus we can say, that the original waste whey was fully utilized, and its initial COD (22.900mg/L for 20.000L is 458000kg COD) and TOC (7.227mg/L) content was diminished.
On the flowsheet 8 different "venting" port is indicated which should be considered from emission point of view. The *Environmental Impact Report* of SuperPro Designer contained only two emitted compounds: 42,391kg CO_2/batch and 0,966kg methanol/batch is emitted out of the used and emitted ca. 7000kg air/batch.

4 Conclusion

We set up a construction of different biotechnological operations to convert the valuable content of waste sour whey of an operating dairy facility in Hungary. We tested our hypothesis experimentally, and on that basis we built up a flowsheet for process simulation. These simulations revealed, that this technology can be feasible almost in its present unoptimized form, and it can follow the "zero landfill" theory at the same time, i.e. it is operating without generating further waste streams, but used up almost every produced whey having environmental risk.

Thus we intend to continue this research more deep, to better understand the influencing factors effect, and to further optimize the operations and finally the whole biorefinery technology.

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