Multidisciplinary approach in industrial baker´s yeast production: From manufacture to integrated sustainability

Von der Naturwissenschaftlichen Fakultät der

Gottfried Wilhelm Leibniz Universität Hannover

zur Erlangung des Grades

Doktorin der Naturwissenschaften (Dr. rer. nat.)

genehmigte Dissertation

von

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[2019]

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Tag der Promotion: 18.04.2019

Acknowledgements

Firstly I would like to express my gratitude to my mentor Prof. Dr. Stéphan Barbe for giving me the opportunity to conduct a study in his laboratory. His ideas, motivation, patience and immense knowledge helped me in performing the experiments as well as writing my thesis. Thanks to his advices, the adjustment of living in Germany was rapid, practical as well as personal. I could not have imagined having a better mentor. Special thanks go to Prof. Dr. Thomas Scheper for being my supervisor and supporting my work.

My sincere thanks go to Dr. Marek Mösche who provided me an opportunity to join his team and opened the door to his laboratory in Uniferm GmbH & Co. KG. Very special gratitude goes to Jendrik Hof for his tremendous support, optimism, valuable advices and friendship.

I would like to thank Thomas Millenautzki and Lothar Müller for helping me set up the equipment and providing the assistance. I thank all the students and colleagues who helped me during this work.

To my friends, Maja, Valentina, Ivana A. B., Silvia, Anton, Dijana and Anja, thank you for listening and supporting me through this whole process. To all the new friends I met in Germany, thank you for introducing me to the German culture and making me feel accepted.

My deep and sincere gratitude goes to my dear parents, Anka and Josip, and my sister Petra for their continuous and unconditional love, help and support. Your advices always helped me see the light at the end of the tunnel.

Last but not least, I would like to thank my husband Stjepan. The last three years have been challenging to me, both academically and emotionally. Thank you for taking this journey with me, lifting me up and being proud of me. None of this would be possible without you.

Kurzfassung

Die vorliegende Arbeit zeigt, wie die industrielle Produktion von Backhefe, durch die Kombination multidisziplinärer Entwicklungen, zu einem innovativen und strategischen Konzept in einen Mehrzweck-Bioprozess umgewandelt werden kann. Dieses Konzept stellt die zusätzlichen Schnittstellen dar, an den dieser Produktion gekoppelt werden kann; neben der Herstellung von Hefe, werden die Sektoren der Abfallentsorgung, die Herstellung wertvolle Biomolekülen und die Gewinnung von Energie vorgestellt.

Zu Beginn der Studie wurde die vollständige Massenbilanzierung während der Fed-Batch-Fermentation der Backhefe durchgeführt. Entsprechende Analysen der Melasse und der resultierenden Vinasse zeigen, dass Vinasse für die Gewinnung von Betain, Invertase, Aminosäuren und Proteinen sehr gut geeignet ist. Ein membranbasiertes Verfahren zur effizienten und gleichzeitigen Gewinnung von wertvollen Biomolekülen aus der Fermentationsbrühe wurde entwickelt. Dieser Prozess könnte in einer industriellen Backhefeproduktionsanlage durch die Implementierung einer Ultrafiltrationsstation und eines Adsorptionssystems realisiert werden. Die Simulation der Erzeugung von Niedertemperaturwärme wurde bei einer industriellen Backhefeproduktionsanlage durchgeführt, die aus sieben 150-m^3-Bioreaktoren bestand. Anschließend wurde ein transkritisches Kohlendioxid-Wärmepumpensystem zur Umwandlung dieser Wärme in Fernwärme der 4. Generation erfolgreich ausgelegt. Schließlich wurde die Machbarkeit und Anwendbarkeit dieses Ansatzes bestätigt und ein Konzept zur Langzeitspeicherung von Energie einschließlich modernster PCM-Einheiten (Phase Change Material) diskutiert. Die Apfelsaftproduktion wurde in ein Bioraffineriekonzept umgewandelt, in dem der Abfallstrom in die Produktion von Pellets, Pektin und Apfeltresterextrakt geleitet wurde. Konzentrierter Apfeltresterextrakt wurde in der Produktion der Backhefe getestet und zeigte eine gute Eigenschaft als Alternative zur Melasse.

Die Kombination all dieser Konzepte würde den aeroben Bioprozess der Backhefeproduktion innerhalb einer nachhaltigen industriellen Umgebung ermöglichen. Schließlich wäre dieser Mehrzweck-Bioprozess vorteilhaft und würde der Backhefe-Unternehmen ermöglichen, auf dem Markt wettbewerbsfähig zu bleiben.

Schlagwörter: Backhefe, Betain, Mehrzweck-Bioprozess, Melasse, Würze

Abstract

The present study shows how industrial baker´s yeast manufacture can be turned into multipurpose bioprocess by combining multidisciplinary developments into innovative strategic concept. It demonstrates additional roles given to this manufacture; besides producing yeast, as waste discharger and as supplier of valuable biomolecules and energy.

First the full mass balance during the fed-batch fermentation of baker´s yeast was performed. Analyses of raw material molasses and corresponding vinasse indicated the suitability of vinasse in recovery of betaine, invertase, amino acids and food grade proteins. Straightforward membrane based process for the efficient and simultaneous recovery of valuable biomolecules from fermentation broth of baker´s yeast was developed. This process can be easily implemented in a conventional baker's yeast production plant by integrating an ultrafiltration station and an adsorption system. Furthermore, simulation of the generation of low grade heat from an industrial baker's yeast production plant consisting of seven 150 m^3 bioreactors was conducted. Subsequently, a transcritical carbon dioxide heat pump system for the conversion of this heat into 4^{th} generation district heat was successfully designed. Economic study confirmed the feasibility and the applicability of this approach and a concept for long-term energy storage including state-of-the-art PCM-units (Phase Change Material) was discussed. Finally, apple juice production was reshaped into biorefinery concept, where waste stream was directed into production of pellets, pectin and apple pomace extract. Concentrated apple pomace extract was tested in the baker´s yeast cultivation and demonstrated a good performance to be employed as an alternative to molasses.

Combining all these concepts allows aerobic bioprocess of baker´s yeast production to be employed within a sustainable industrial environment. Moreover, additional purposes would be of great benefit from an economic point of view and enable baker´s yeast producing companies to stay competitive on the market.

Key words: baker´s yeast, betaine, multipurpose bioprocess, molasses, vinasse

List of symbols

Uppercase symbols

Δ**ABS**	Absorbance value above the reagent blank
$\Delta P_{membrane}$	Transmembrane pressure drop [bar]
A_{ref}	Integral value representing the internal standard
A_x	Integral value of the signal representing betaine
I. A.	Invertase activity [U/mL]
M_{ref}	Molar mass of the internal standard [g/mol]
M_x	Molar mass of betaine [g/mol]
N_{ref}	Number of protons in the internal standard
N_X	Number of protons in betaine
V	Total volume [mL]
V_S	Volume of the sample [mL]
$Y_{inv/X}$	Yield coefficient of invertase activity based on yeast biomass [g/g]
$Y_{X/N}$	Yield coefficient of yeast biomass based on nitrogen consumption [g/g]
$Y_{X/sugar}$	Yield coefficient of yeast biomass based on total sugar consumption [g/g]

Lowercase symbols

a	Absorptivity constant [mL/(µmole · cm)]
b	Cuvette light path [cm]
c_B	Concentration of betaine [g/L]
c_{Ref}	Concentration of the internal standard [g/L]
m_{aS}	Mass of the crucible with the burnt sample [g]
m_c	Mass of crucible [g]
m_{dS}	Mass of the crucible with the dried sample [g]
t	Time or reaction [min]
y	Year

List of abbreviations

AD	Adsorption	**MAP**	Monoammonium phosphate
AEX	Anion exchange adsorption	**MF**	Microfiltration
AP1	Apple pomace 1	**MWCO**	Molecular weight cut off
AP2	Apple pomace 2	**n. d.**	Not determined/detectable
APE	Apple pomace extract	**NS2P**	New Sequential, 2-Profile
CEX	Cation exchange adsorption	**P**	Pellets
CF	Centrifugation	**PCM**	Phase change materials
cPFAPE	Concentrated pectin free apple pomace extract	**PFAPE**	Pectin free apple pomace extract
COP	Coefficient of performance	**PR**	Precipitation
D	Drying	**PT**	Solid pectin
D. S.	Dry substance	**RJ**	Raw juice
DM	Dry matter	***S. cerevisiae***	*Saccharomyces cerevisiae*
dPT	Dried pectin	**SMB**	Simulated moving bed
EL	Ethanolic liquor	**sp**	Set point
EV	Evaporation	**TDF**	Total dietary fibre
FB	Fermentation broth	**UF2**	Ultrafiltration 2 kDa
HSSD	High sugar synthetic dough	**UF30**	Ultrafiltration 30 kDa
LSSD	Low sugar synthetic dough	**VF**	Vacuum filtration

1 Introduction

Baker´s yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) refers to live active biomass yeast used in bakery for dough leavening. What makes doughs rise is the conversion of sugars into ethanol and carbon dioxide and it is one of the most important parameter of commercial baker´s yeast called fermentative capacity (Grba, 2010).

Industrial production of baker's yeast, exclusively for the production of bread doughs, dates from the end of the 19th century. It started with production of distiller's yeast on grain-based mash, which was used in breweries and distilleries. At the beginning of the 20th century in Germany as well as in Denmark, fed-batch process (Zulauf) was introduced, which is still in its basic form applied today (Grba, 2010). During the last decades, major advances have been achieved. Fed-batch fermentation is practical method of incremental feeding which enables the yeast growth without simultaneous production of sizable quantities of ethanol. Transfer of oxygen from the air into liquid cultures of microorganisms (aeration) as well as strict pH control has been recognized as very important parameters in the production. By-product of sugar industry molasses replaced substrate consisted of corn, malt and malt sprouts as carbon and energy source for yeast growth. Furthermore, there has been a rapid shift to automatic control of the fermentation which makes the conducting of the fed-batch cultivations not only easier to control but also easier to monitor (Reed, 1991).

Like in all biotechnological processes, there is a constant research in baker´s yeast production to optimize the process technology and its control in order to produce the highest amount of yeast´s biomass as fast as possible at the lowest cost (Gélinas, 2014). Another burning issue of baker´s yeast producing companies is the volatile market of raw materials. Prices of molasses are increasing due to their use in industrial applications of bioethanol production (Balat et al., 2008). Although studies regarding alternative raw materials in baker´s yeast production were conducted in the last few decades, none of the findings seem to be implemented in industry (Acourene et al., 2011; Bhushan et al., 2006; Ferrari et al., 2000; González, 1996; Nancib et al., 1997). Since baker´s yeast is in the spotlight of the attention, by-product vinasse (cell-free broth obtained after the separation) is being undervalued for any further processing; it is mostly used as animal feed supplement (Iranmehr et al., 2011) as well as field fertilizer (Hidalgo, 2009). Considering that yeast´s growth is an exothermic process, cooling of large

bioreactors by underground water is of extreme importance (Grba, 2010). Exiting warm water is the carrier of the low grade heat which can be used in space and water heating as 4th generation district heat by employing the state-of-the-art heat pump into the industrial surroundings of baker´s yeast production.

The aim of this thesis is defining concepts which represent the basis for the multidisciplinary approach in baker´s yeast production. Following objectives have been covered:

1) For the first time, industrial baker´s yeast production was presented as multipurpose bioprocess. Besides producing baker´s yeast, this manufacture discharges and re-uses waste streams from agricultural and food industry as feedstock. Additional value is given to by-product vinasse by recovering valuable key biomolecules (amino acids, betaine and invertase). Furthermore baker´s yeast production recycles low grade heat and acts as energy supplier.

2) Mass balance during the fed-batch fermentation of *S. cerevisiae* was determined. Molasses and corresponding vinasse were analysed and consequently determined what the yeast exactly utilizes from molasses and which constituents remain in vinasse.

3) Novel process for the simultaneous recovery of key biomolecules from baker´s yeast fermentation broth was developed. Vinasse was processed by ultrafiltration steps, which applied the membrane with a membrane weight cut off (MWCO) of 30 kDa as well as the membrane with MWCO of 2 kDa. Further downstream processing involved evaporation and ion exchange adsorption.

4) Scale-up of the laboratory data, obtained from the fed-batch cultivation of *S. cerevisiae*, were employed in the conversion of low grade heat from an industrial baker´s yeast production plant into 4th generation district heat. Dimensioning of an appropriate transcritical carbon dioxide based heat pump was determined as well as the cost evaluation.

5) Production of apple juice was reshaped into the concept of biorefinery, in which pectin, pellets and concentrated pectin free apple pomace extract were also produced. The latter was tested as raw material in the cultivation of baker´s yeast.

Parts of this thesis were originally published as:

Ivančić Šantek M, Lisičar J, Mušak L, Špoljarić Vrana I, Beluhan S, Šantek B. 2018. Lipid production by yeast *Trichorporon oleaginosus* on the enzymatic hydrolysate of alkaline pretreated corn cobs for biodiesel production. *Energy Fuels*. DOI: 10.1021/acs.energyfuels.8b02231

Lisičar J, Millenautzki T, Scheper T, Barbe S. 2018. New trends in industrial baker's yeast fermentation: Recovery of key biomolecules and low-grade heat conversion. *J Biotechnol.* 280S, 17-18. DOI: 10.1016/j.jbiotec.2018.06.052

Lisičar J, Scheper T, Barbe S. 2017. Industrial baker´s yeast fermentation: From manufacture to integrated sustainability. *J Biotechnol.* 256S, 23-24. DOI: 10.1016/j.jbiotec.2017.06.630

Lisičar J, Scheper T, Barbe S. 2017. Turning industrial baker's yeast manufacture into a powerful zero discharge multipurpose bioprocess. *Ind Biotechnol*. 13 (4): 184-191. DOI: 10.1089/ind.2017.0018

Lisicar J, Sedaghati M, Barbe S. 2018. Looking at baker´s yeast fermentation through new glasses: The neglected potential of vinasse for biotechnological applications. Manuscript of 31st Yeast Conference, Leuven (Belgium), 16.-17.04.2018

Lisičar Vukušić J, Kneer A, Mösche M, Barbe S. 2018. Turning industrial aerobic fermentation plants into thermal power stations. *Int J Energ Res.* DOI: 10.1002/er.4299

Lisičar Vukušić J, Millenautzki T, Sedaghati M, Schallenberg M, Müller P, Hof J, Mösche M, Barbe S. 2018. Fractionation of baker's yeast vinasse via ultrafiltration: Assessment of feasibility. *Int J Food Sci Technol*. DOI: 10.1111/ijfs.14080

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2 Moving from single purpose to multipurpose process of baker´s yeast production

2.1 Objectives

The aim of this chapter is to demonstrate how industrial aerobic manufacture of baker's yeast can be transformed into a powerful multipurpose bioprocess with zero discharge. Following concepts have been discussed:

1) smart integration of side product streams from the food industry in the supply chain
2) recovery of high value biomolecules (betaine and invertase) from by-product vinasse
3) implementation of state-of-the-art heat pumps within the process of baker´s yeast production

New purposes which can be derived from industrial baker´s yeast production result in an attractive and sustainable bioprocess and develop a strategy allowing producers to achieve much more than just yeast.

2.2 Theoretical background

At the beginning of the 20th century, the baker´s yeast production has industrialized independently from the ethanol production in which high alcohol yields result in lower yeast biomass (Gélinas, 2014). Yeast was expensive back then so efforts had to be made in order to reduce production costs and to find cheaper raw materials for the growth media. Diluted molasses with the addition of ammonia, vitamins and minerals proved to be the most suitable substrate for this purpose (Gélinas, 2012). Nowadays baker´s yeast producing companies try to harvest the highest amount of biomass as fast as possible at the lowest cost (Gélinas, 2014). The yearly global production is around 2 million tonnes and expected to grow every year approx. 4 % because of the growing trend of processed and fast food (Josephsen et al., 2004). The current focus of producers is on the improvement of the process, its control and of course cost reductions. Manufactures play a critical role in dictating the material and energy

resources in today´s society. Their process and products have not only a huge impact on the environment but also on energy demand, carbon dioxide emissions, climate change and production of materials and chemicals that are consumed by the products across their life cycle (Duflou et al., 2012). It is of crucial importance for the various industries to make smart integrations among themselves and to create and keep the balance between the society, economy and environment (Figure 2.1) (Lisičar et al., 2017).

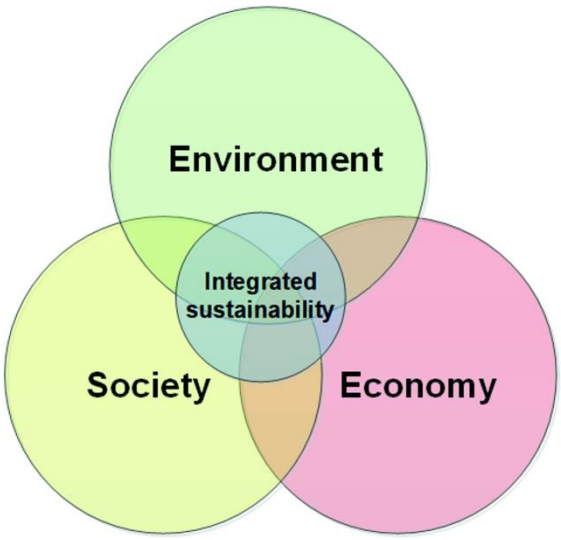

Figure 2.1 Integrated sustainability strongly links industrial processes to society, economy and environment (Lisičar et al., 2017)

2.2.1 Traditional baker´s yeast production

Baker´s yeast is industrially produced according to different technologies: variations in number of generations, duration and level of aeration in each phase, different types of bioreactors and control in the last phase of the cultivation (Grba, 2010). It is an aerobic process based on propagating cells from pure culture to large bioreactors by increasing the volume in each propagation stage in sugar based medium (Lisicar et al, 2018). Molasses is usually diluted with water in the ratio 1:1, clarified by means of separators and steam sterilised. Micro- and macro-elements are added in the form of salts as well as vitamins (Grba, 2010). The initial phase of cultivation of *S. cerevisiae* starts in the laboratory. Sterile flasks with molasses (less than 5 L) are inoculated with pure culture. The growth usually lasts two to four days. The entire yield is then used as the inoculum for the second stage. Usually, this

phase consists of two pure culture cultivations. The capacities of the vessels in this stage are larger (1,140 L to 26,500 L). These batch fermentations last 13 to 24 hours. The content of the bioreactor from the first pure culture is added to the next fermentation vessel, which already contains the nutrients. The main biomass production is achieved in the last fermentation stage (so called final trade cultivation). In this phase four to seven large bioreactors are used (38,000 L to over 250,000 L) and attention is carefully dedicated to the production of high biomass concentration with high fermentative and storage capacity. Bubble column bioreactors are steadily aerated in order to keep the specific amount of dissolved oxygen at the optimal level. Yeasts are facultative anaerobic microorganisms, capable of both aerobic respiration and alcoholic fermentation. Under anaerobic conditions, fermentable sugars are consumed and transformed into ethanol and carbon dioxide resulting in lower biomass yields. In the particular case of *S. cerevisiae*, when the sugar concentration is above a critical concentration (150 mg/L) (Verduyn et al., 1984), the so called Crabtree effect occurs leading to the production of ethanol even if enough dissolved oxygen is present in the growth medium. Therefore, sugar concentration has to be kept under the above mentioned critical concentration during the whole fermentation. In order to achieve that, cultivation is performed as fed-batch fermentation, meaning the incremental and controlled addition of molasses. To satisfy the need of the yeast for macro-elements, molasses is usually supplemented with ammonium salts, phosphoric acid or phosphate salts and magnesium salts. The ammonium solution is usually used as the nitrogen source. The vitamin solution can be added to the molasses before the fermentation or placed in small separate tanks and dosed by rotameters into the bioreactor. Final trade cultivations last 11 to 15 hours and the yield variates from 15,000 to 100,000 kg of compressed yeast per batch, depending on the size of the bioreactor. Once the final amount of the yeast has been reached, the broth is pumped into centrifugal separators. In order to achieve the desirable yeast solids concentration (18 to 21 %), two or three passes are necessary through the continuous dewatering centrifuges. Yeast cream obtained after this phase can be delivered to large-scale bakeries or further dewatered by filtration. Rotary vacuum filters are usually employed for the continuous concentration of yeast cream. The large rotating drum is coated with potato starch containing small amounts of salts. As it rotates it is submerged into the yeast cream and the vacuum enables the filtration through the filter. As the drum rotates, blades at the bottom of the drum remove the yeast. When the filter cake is formed and while the drum is rotating, excess salt is removed by

spraying a small amount of water. Yeast cakes contain 30 to 33 % of solids. The cake is then mixed with water, oil and emulsifiers. Emulsifiers are added for a white appearance and oil helps extrude the yeast. To form blocks of cake, the compressed yeast is extruded through open-throated nozzles. Finally the blocks are cut, wrapped with wax paper and cooled to below 8 °C (Safriet, 1994).

2.2.2 New trends in baker´s yeast production

2.2.2.1 Alternative raw materials

As previously mentioned the most widely used growth medium for the production of the baker´s yeast is molasses. The term molasses is applied to the final effluent obtained in the preparation of sugar by repeated crystallization. Commercial molasses consists of water, sugars (sucrose, glucose, fructose and raffinose), non-sugars (N-components: proteins, peptides and amino acids) and minor components (trace elements, vitamins and growth substances) (Table 2.1) (Olbrich, 1963). Cane and beet molasses are used in the industry as raw materials for the production of baker´s yeast, ethanol and citric acid. The main compound of both molasses is sucrose. The concentrations of other compounds (amino and organic acids, minerals, vitamins) depend on the processing steps of the sugar purification (Nakata et al., 2014).

Table 2.1 Average composition of beet and cane molasses (Olbrich, 1963)

Constituent		Beet molasses [%]		Cane molasses [%]	
Water		16.5		20.0	
Sugars:	Sucrose	51.0		32.0	
	Glucose	-		14.0	62.0
	Fructose	-	53.0	16.0	
or	Invert sugar	1.0		-	
	Raffinose	1.0		-	
Non-sugars: nitrogenous materials, free and bound acids, soluble gummy substances		19.0		10.0	
Silicon(IV) oxide		0.1		0.5	
Potassium oxide		3.9		3.5	
Calcium oxide		0.26		1.5	
Magnesium oxide		0.16		0.1	
Phosphor(V) oxide		0.06		0.2	
Sodium oxide		1.3	~ 11.5		8.0
Iron(III) oxide		0.02		0.2	
Aluminium(III) oxide		0.07			
Soda and carbonate residue (as CO_2)		3.5			
Sulphate residue (as SO_3)		0.55		1.6	
Chlorides		1.6		0.4	
Total		100.0		100.0	

With more than 100 $ per tonne FOB (Free On Board) in 2016, molasses appears to be strongly overpriced when compared to other commodities (steel, coal, oil, sugar) (UM Trading Newsletter, 2015). The high price of this feedstock is mostly due to the high world demand for beet and cane molasses used in ethanol production. This strong coupling between molasses price and ethanol production currently makes price forecasting difficult and the resulting picture of the molasses market became quite complicated (UM Trading Newsletter, 2015). In European countries, beet molasses is the most utilized sugar-containing feedstock (Balat et al., 2008). Although the sugar prices are dropping because of the surplus in the world market, the prices of molasses changed very little and in some crops of Northern Hemisphere have actually risen (UM Trading Newsletter, 2015). Despite this substantial increase in raw material costs, baker's yeast has not raised in price (Tate & Lyle, 2006) and keeping this price at competitive level becomes a serious challenge for baker's yeast producers. Another issue is that the surplus in sugar production leads to increase of molasses generation which will be more exhausted and possess a lower sugar concentration in relation to total solids. This will undoubtedly have a negative effect upon yeast fermentation (Basso et al., 2011).

The prices of common raw materials used in baker´s yeast fermentation are shown in Table 2.2.

Table 2.2 Prices of common raw materials used in the production of baker´s yeast in the year 2000 (Petrides, 2003)

Raw material	Comment	Price [$/kg]
C-Source		
Glucose	Solution 70 % w/v	0.25 – 0.35
Molasses	50 % Fermentable sugars	0.08 – 0.12
N-Source		
Ammonia	Anhydrous, fertilizer grade	0.20 – 0.25
Ammonium sulphate	Technical	0.15 – 0.25
Ammonium nitrate	Fertilizer grade 33.5 % N, bulk	0.15 – 0.20
Urea	46 % N, agricultural grade	0.20 – 0.25
Salts		
Potassium dihydrogen phosphate	Granular	1.65 – 1.85
Potassium sulphate	Granular, purified	2.20 – 2.50
Magnesium sulphate heptahydrate	-	0.25 – 0.35
Zinc sulphate heptahydrate	Agricultural grade, power	0.50 – 0.60
Other		
City water	-	0.0005
Distilled water	-	0.01 – 0.2

Since raw materials used in large-scale fermentation are huge contributors to the cost of low-value baker´s yeast, using agro-industrial residues as a substrate has become an interesting alternative for reducing production costs (Vendruscolo et al., 2008). Cheese whey is the liquid remaining following the precipitation and removal of milk casein during cheese-making. It represents about 85-95 % of the milk volume and retains 55 % of milk nutrients, containing mostly lactose (4.5-5 % w/v), soluble proteins (0.6-0.8 % w/v), lipids (0.4-0.5 % w/v) and mineral salts (8-10 % of dried extract). This by-product represents an environmental problem because of the high volumes produced (more than $145 \cdot 10^6$ t of liquid whey per year) and its high organic matter content (Siso, 1996). The usage of whey as a potential substrate in biotechnological processes has been reported by Moulin et al (1984). Whey could be used for

protein recovery, production of galactose, ethanol and bacterial fermentation. It has also been proved to be adequate for baker´s yeast growth (Moulin et al., 1984). However, *S. cerevisiae* is not able to grow on cheese whey, unless lactose is hydrolysed to glucose and galactose. Various approaches have been suggested: conversion of lactose to glucose and galactose by free and immobilized enzymes or by chemical hydrolysis, conversion of lactose to lactic acid and genetic manipulation of *S. cerevisiae* in order to be able to use lactose directly as a carbon substrate (Champagne et al., 1990). Ferrari et al. have successfully evaluated partial substitution of molasses by cheese whey in the fed-batch fermentation of baker´s yeast. Water used to dilute molasses was replaced with whey. However in this medium the biomass yield was 6-8 % lower than the yield observed for yeast grown only on molasses. Nevertheless, fermentative capacity of baker´s yeast grown on mixture molasses/whey corresponded with the value measured of commercial baker´s yeast. The remaining question is if cost savings in raw materials and wastewater treatment could compensate the extra cost of the enzyme required for lactose hydrolysis (Ferrari et al., 2002). Despite all the efforts to fully exploit this substrate, only about 50 % of world whey production has been turned to good account (Moulin et al., 1984).

Another agricultural residue that has been widely examined is apple pomace. It is a left-over solid residue (25-30 % of total processed fruits) obtained after the extraction of apple juice. Every year thousands of tonnes of apple pomace is generated worldwide (Table 2.3). High moister content (70-75 %) and organic matter content make this by-product convenient for microbial decomposition, which leads to problems regarding its disposal. Apple pomace contains simple sugars, pectin, dietary fibres and natural antioxidants. In former years, dried apple pomace was used as animal feed, fuel to boilers or as soil conditioner. However, since 1980, it was extensively studied as a substrate for microbial growth used in production of organic acids, enzymes, single cell proteins, low alcoholic drinks, ethanol, biogas, pigment and baker´s yeast (Bhushan et al., 2008). Bhushan et al. demonstrated the good performance of apple pomace as a carbon source for the growth of baker´s yeast in the fed-batch mode. They also investigated the dough rising capacity, which was similar to the control commercial baker´s yeast and therefore qualify apple pomace as an alternative to molasses (Bhushan et al., 2006).

Table 2.3 Yearly world apple pomace generation (Bhushan et al., 2008)

Country	Quantity (thousands of tonnes)
Brazil	13.75
Germany	250
India	3-5
Iran	97
Japan	160
New Zealand	20
Spain	20
United States	27

The potential of hydrolysed waste cassava starch for the production of baker´s yeast, with the strong potential for its large-scale use in the industry, has been reported by Ejiofor et al. The baker´s yeast grown on cassava medium had slightly lower biomass concentration than the yeast grown on molasses. However, the dough-leavening activities were identical, agreeing closely with the activity of commercial yeast (Ejiofor et al., 1996).

Acourene et al. (2011) and Nancib et al. (1997) have presented how a major date-producing country can economically use food waste in order to produce high quality baker´s yeast. Not only the date extracts but also the date syrups are shown as a good carbon (containing a large percentage of sugars especially glucose, fructose and sucrose) and nitrogen source for the cultivation of *S. cerevisiae*. Orange peel has proved to be a potential promoter for the growth in baker´s yeast production by Plessas et al. (2006). In batch system improved biomass concentration, maximum growth rate and productivity were observed. The continuous fermentation was also carried out and the system showed high operational stability which is important for the industrialization of the process.

2.2.2.2 Recovery of high value biomolecules

Processing of the cell free residue of the fermentation (also called vinasse) is another issue that attracts a lot of attention. Vinasse, originated from separators, centrifuges and rotary vacuum filters, is characterised by high chemical oxygen demand, dark colour, high concentrations of total nitrogen and non-biodegradable organic pollutants (Rahimpour et al., 2014). It is a low-value and cheap by-product mainly used as feed additive for animals (Iranmehr et al., 2011) or as organic fertilizer (Gemtos et al., 1999). Vinasse consists of the

non-sugar substances that are not being assimilated by *S. cerevisiae* (Rahimpour et al., 2014). Among these substances are amino acids, which are convenient for recovery. Glutamic acid as well as leucine and isoleucine can be recovered by the means of ion exchangers (Olbrich, 1963). Valuable intermediate originating from beet molasses, whose demand on the global market is expected to grow, is betaine. Its usage is widely spread in different fields: as additive to improve metabolism of animals, in sports drinks, cosmetics, detergents, paints and coatings (Grand View Research). Vinasse contains up to 12 % of betaine (Olbrich, 1963). Betaine can be obtained by introducing the vinasse to a chromatographic column containing a salt of a polystyrene sulfonate cation exchange resin or a weakly acid cation exchange resin in H^+-form (Heikkilä et al., 1982; Paananen, 2014). The European patent EP 2,923,749 A1 clearly describes the recovery of betaine from a solution containing betaine and sucrose by nanofiltration (Paananen et al., 2015). Vinasse could be treated in the same way in order to obtain high concentrations of betaine. In this treatment the invertase could also be obtained. Sucrose cannot be directly assimilated by *S. cerevisiae* and has to be hydrolysed in glucose and fructose prior to the biotransfomation. For this reason, large amounts of invertase are excreted during the final trade cultivation. This enzyme is widely used in food industry – bakery, sweets and confectionary products (Kulshrestha et al., 2013). The pilot recovery of invertase via aqueous-two-phase extraction has been extensively reported and investigated in the literature (Kula et al., 1982; Timerman, 2012).

2.2.2.3 Recovery of low grade fermentation heat

Important challenges of the 21st century are securing a reliable, economic and sustainable energy supply as well as the environmental and climate protection. In this regard, reusing waste heat generated in industrial processes can be performed by implementing a heat pump in the industrial unit. Unfortunately their potential is not used as it should or could be, compared to those installed for space heating, due to the lack of knowledge, low awareness of heat consumption in companies and long payback periods (sometimes up to 5 years). Nevertheless, the examples of the existing installations can be found in food and beverage industry, chemical industry, automobile factory and metal processing (Laue, 2014).

During industrial baker's yeast production, huge amounts of heat are released. 18.4 kJ of heat are generated for every gram of yeast´s cell solids produced (Reed et al., 1991). Therefore the

cooling system of the bioreactors is of extreme importance for the efficient cultivation. In the late 1980s, the Sweden located baker´s yeast producing company Jästbolaget AB introduced a system of heat converters and heat exchangers. By 1992 a large heat converter was installed for recovering of all the heat generated in the fermentation process. The energy is transferred to the municipal district-heating network providing heat to about 1,500 homes (Jästbolaget webpage; Sakavets et al., 2015).

2.3 Four-dimensional baker´s yeast production

2.3.1 1st dimension: baker´s yeast production

A conventional baker´s yeast manufacturing plant with a yearly production of 100,000 tonnes of compressed yeast (solid matter content is 25 %) is used as a reference process. Figure 2.2 illustrates the work flow of one of the several bioreactors used during the final trade cultivation. Usually four to seven bioreactors are used in this stage but for the sake of simplicity only one is presented. 100,000 tonnes of molasses, 22,000 tonnes of nitrogen source (ammonia 10 % w/w) and 8,000,000 m^3 of cooling water (van der Pas, 2013) are required for the production of 25,000 tonnes of yeast per year (dry matter content). Furthermore, approx. 230,000 tonnes of vinasse have to be evaporated during the corresponding downstream processing. Cooling water, which is usually pumped from the wells and then released into the rivers, streams or canals, has an exit temperature of approx. 20-24 °C. The emphasis of this process is clearly on yeast biomass production. After the evaporation, concentrated vinasse is transferred to trucks and transported to be used on fields as fertilizer or at farms as feed supplement for animals. In this traditional one-dimensional manufacturing process, one main product (baker's yeast) is obtained from one main substrate (molasses). Beside the sugar producing plants, large bakeries and animal farms, there are no tight linkages with further industrial fields and society within this work flow (Lisičar et al., 2017).

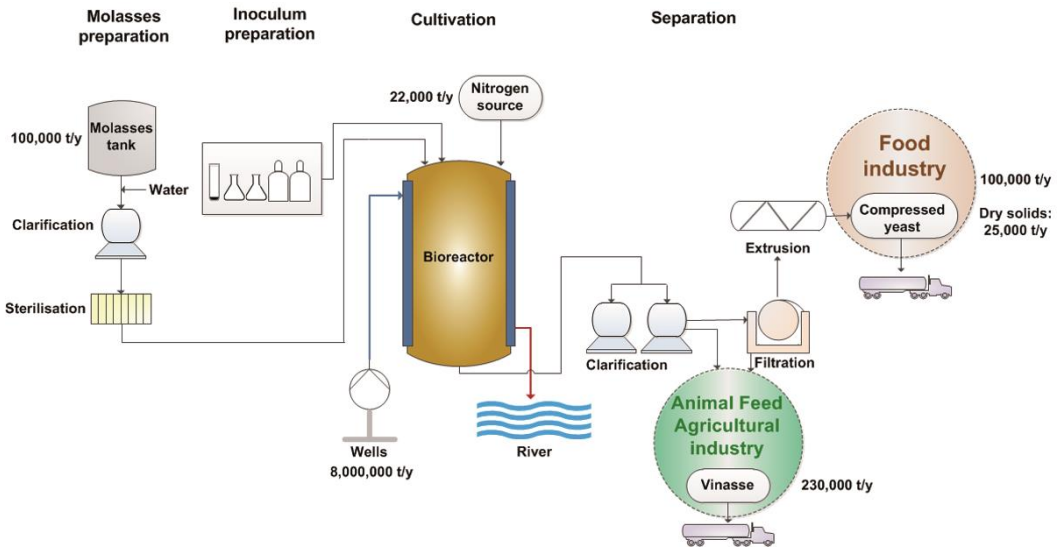

Figure 2.2 Work flow of a conventional baker´s yeast manufacturing plant during final trade cultivation as a one-dimensional bioprocess (Lisičar et al., 2017)

2.3.2 2nd dimension: waste treatment company

As previously mentioned, everyday huge amounts of fruit wastes are produced by growing agro-industrial activities. They are generally an appreciable source of carbohydrates as well as proteins, vitamins, minerals and natural antioxidants. Dried apple pomace consists of up to 50 % of fermentable sugars (Bhushan et al., 2008) and therefore is convenient for the growth of baker´s yeast. Another feasible substrate for baker´s yeast production is cheese whey. Considering *S. cerevisiae* is unable to assimilate lactose, it must be hydrolysed to glucose and galactose by β-galactosidase. However, from an economic point of view, this phase has to be considered as a critical step (Stineman, 1980).

According to this concept, baker's yeast producers would additionally act as waste treatment company (Figure 2.3). Acid whey is processed by solids separators in order to remove suspend, cheese-type particles. The whey is further processed by heating and passed through a series of ultrafiltration membranes. Next step is the lactose hydrolysis in which the bond between glucose and galactose is split. The economically justified enzyme should be applied. The treated whey is then prepared to be added to molasses in the fermentation of baker´s yeast. Apple pomace is first processed in mixer-settler by solid-liquid extraction. To obtain sugar syrup the solid-liquid separation through filtration or hydraulic press is applied. These

alternative substrates are not used to fully substitute molasses but to dilute it (water is being saved) and provide an additional source of carbon and nitrogen. Following the idea of integrated sustainability, this strategy offers a way to discharge and re-use waste from agricultural and food industry (Lisičar et al., 2017).

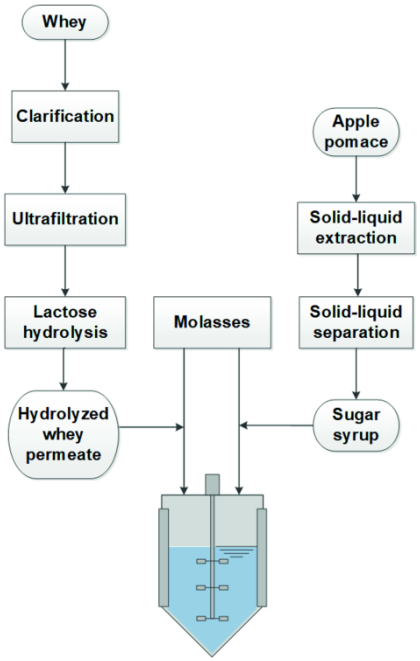

Figure 2.3 Treatment of the acid whey and apple pomace used in the production of baker´s yeast (Lisičar et al., 2017)

2.3.3 3rd dimension: supplier of key biomolecules

Baker´s yeast production can act as a supplier of key biomolecules (Figure 2.4). In order to avoid unnecessary transportation costs, membrane and chromatographic systems used for the treatment of vinasse should be placed near the baker's yeast plant or integrated in it. Vinasse is filtrated through microfiltration membranes (cross-flow filtration) in order to remove residual solids. Subsequently, invertase is being captured from the corresponding permeate via protein chromatography (e.g. membrane chromatography or expended bed adsorption). Betaine as well as amino acids (glutamic acid, leucine and isoleucine) is purified via ion exclusion chromatography by using water as an eluent and concentrated by multi-stage evaporation. Such an evaporation system is usually present in conventional baker's yeast manufactures for the dewatering of vinasse.

In this concept a wastewater treatment is integrated, which is usually considered as a separate part of the industrial activity. Increasing water supply and costs for processing the wastewater is the problem many industries are facing. One of the steps in this approach is to regenerate the wastewater and recycle it as process water. In this phase membrane technology plays an important role (Van der Bruggen et al., 2006). The development of pilot scale nanofiltration system for yeast industry wastewater treatment has been reported by Rahimpour et al (2014). The water should be first passed throughout nanofliltration membranes and then treated by reverse osmosis. After the treatment the obtained processing water can be used in the preparation of the molasses.

The gained values of the amino acids, betaine and invertase have been estimated (explained later in thesis). If the yearly production of the considered baker´s yeast company is 100,000 tonnes, the recovered amount of amino acids is 190 tonnes per year and the recovered amount of betaine equals 4,500 tonnes per year. The amount of invertase obtained is 2,600 tonnes of enzyme preparation with an activity of 1,200 IU/mL. Still out of 230,000 tonnes of molasses, which can be obtained by producing 100,000 tonnes of baker´s yeast, large amounts of key molecules are recovered (Lisičar et al., 2017).

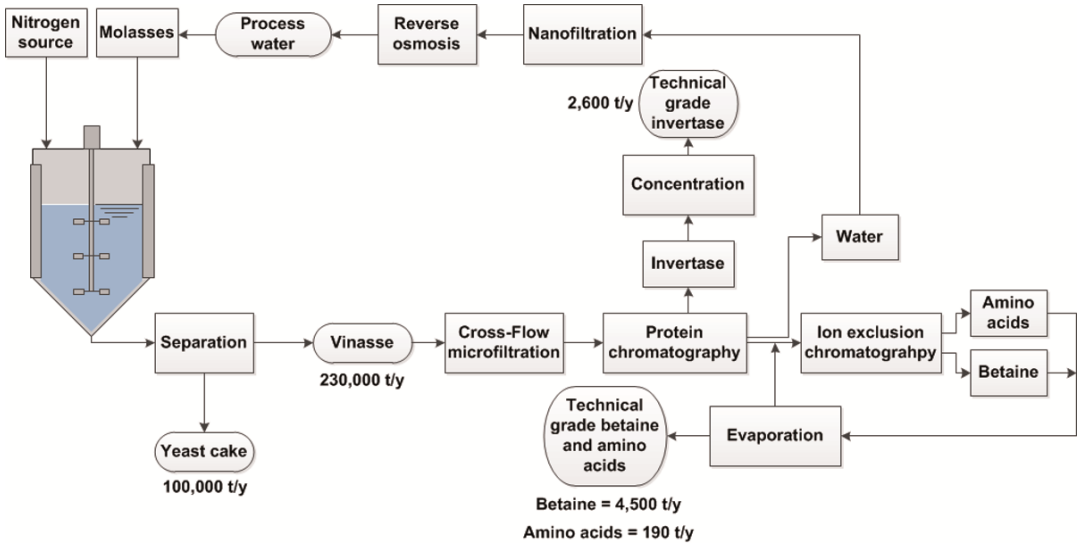

Figure 2.4 Recovery of key biomolecules and water recycling in baker´s yeast production (Lisičar et al., 2017)

2.3.4 4th dimension: energy supplier

The concept developed at Jästbolaget AB (Sweden) was slightly modified and adapted for the reference process from chapter 2.3.1. The released fermentation heat can be estimated by following two approaches and the mean value was used for further calculations:

1) Considering a yearly production of 100,000 tonnes of compressed yeast with 25 % of dry matter, it comes to 25,000 tonnes of yeast on dry matter or 0.7927 kg/s. According to Reed et al. 18.4 kJ are evolved per gram of cell solids produced and the released fermentation heat equals 14.6 MW.
2) The yearly demand for cooling water totals 8,000,000 m^3 (van der Pas, 2013). The water originating from the wells enters the bioreactors cooling system at 8 °C and exists with the temperature of 21 °C. Specific heat capacity for water is 4.187 kJ/(kg · K) and the fermentation heat released equals 14.2 MW.

The mean value of released fermentation heat equals 14.4 MW which is enough to cover the needs of approx. 25,000 people (living in Germany) for space heating and hot water. In the proposed concept (Figure 2.5), cooling water is pumped from the wells and kept in a tank at constant level. It enters the cooling system of the bioreactors with a temperature of 8 °C and exits with a temperature of 21 °C. It is then directed to two heat pumps. The heat of one part of the exiting cooling water (2.2 MW) is converted into process heat (3.14 MW) in a first heat pump requiring an electrical demand of 0.96 MW. The remaining heat from the exiting cooling water (12.2 MW) is converted in a second heat pump generating 17.9 MW of district heat. The corresponding electrical demand is 5.7 MW. By this strategy, low grade fermentation heat is re-used, water management is improved and the resulting district heat is distributed to local community offering a new and positive interaction with the society. District heat is playing a major role in Germany where the implementation of 4th generation district heat is planned for 2020. Its objective is to further promote climate protection and sustainable energy production by minimizing heat loss during energy transportation, recycling low grade heat from industrial processes and applying new standards in building isolation making low temperature heating possible.

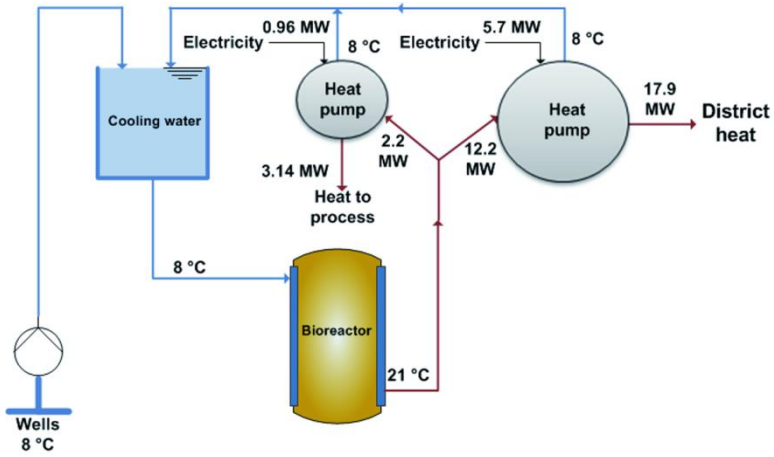

Figure 2.5 Heat pump system integrated in the baker´s yeast production (Lisičar et al., 2017)

2.4 Conclusion

Following the concept of integrated sustainability, Figure 2.6 depicts the industrial baker´s yeast production as a multipurpose process. The efforts of baker´s yeast producing companies need to be set on the search for cheaper sources in order to stay competitive. The reduction of the usage of molasses due to the partial replacement with apple pomace and whey makes a clear connection with food and agricultural industry. By this step, agro-industrial residues from the food industry are re-used and transformed in higher value products. Vinasse obtained after centrifugation and filtration is processed throughout chromatography and key biomolecules are recovered which can be traded to pharmaceutical, chemical and food industry. The processed water is recycled and re-used for the preparation of molasses. Cooling water is not released into the river but directed to a heat pump system where the huge amount of low grade fermentation heat is converted to higher grade heat. This in turn can be either reinjected into the processes or transferred to the surrounding cities as district heat. The water from the heat pump is recycled for cooling and the shortages are replaced with water from the wells. With this concept, the producing company is able to keep its status in the future as a leading baker´s yeast producer and additionally acts as waste treatment company, a producer of key biomolecules and an energy supplier. In order to convince baker's yeast producers, necessary investments and related risks have to be estimated. The implementation of these

multidisciplinary advances will also require changes in mentality, company philosophy and marketing strategies.

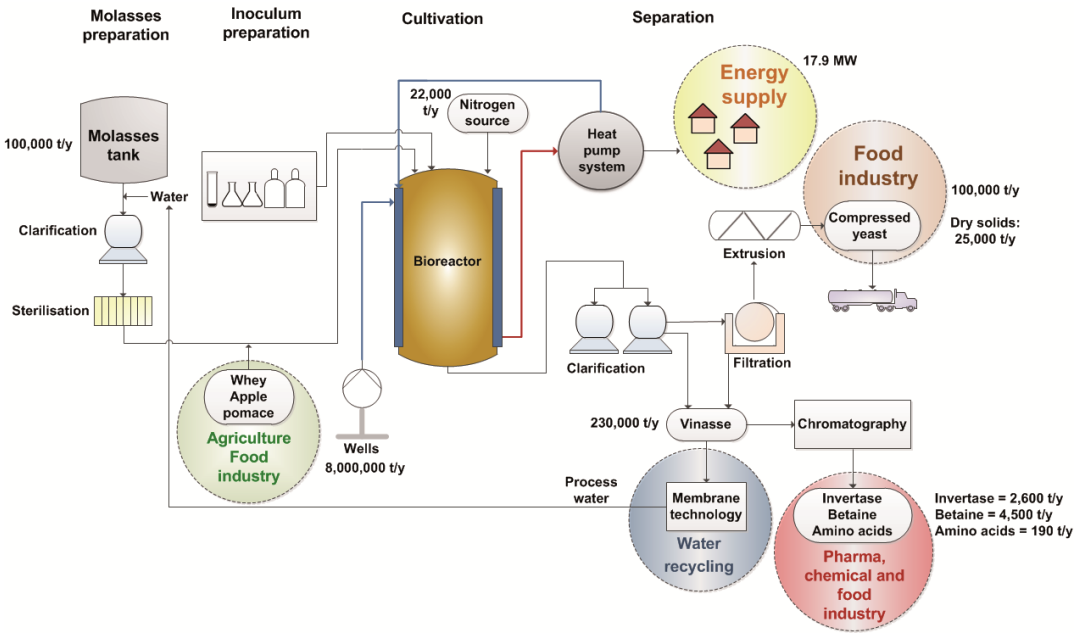

Figure 2.6 Four-dimensional baker´s yeast manufacturing plant as a zero discharge multipurpose process (Lisičar et al., 2017)

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3 Full mass balance analysis during baker´s yeast fermentation

3.1 Objectives

As previously mentioned, vinasse could be employed in the production of biomolecules which would transfer a cheap by-product of baker´s yeast production into valuable substrate. Due to the lack of reliable data regarding full mass balance analysis during industrial baker's yeast fermentation and the composition of the resulting vinasse, following steps were performed:

1) mass balance of raw materials and products during the fed-batch fermentation of *S. cerevisiae* was determined

2) detailed compositions of molasses and vinasse were analysed and compared to establish the degree of utilization of molasses nutrients during the fermentation

3) potential recovery of key biomolecules was discussed

3.2 Theoretical background

The industrial production of baker´s yeast is an aerobic process based on propagating cells from pure culture to large bioreactors by increasing the volume in each propagation stage in sugar based medium. To overcome the well-known Crabtree effect, which results in lower biomass yield and ethanol production (Vieira et al., 2013), the dosage of sugar, usually molasses, has to follow a specified feed rate profile during the fed-batch fermentation in order to maintain its concentration below the critical value (50-100 mg/L) (Di Serio et al., 2001). This way, the specific growth rate is controlled by the feed rate profile of sugar (van Hoek et al., 1998). Ammonia is the usual nitrogen source for this fermentation (ter Schure et al., 2000) and also has to be added according to a specified rate profile. In the later phase of the cultivation, the dosage of substrates is intentionally lowered or even stopped for one or both substrates. In this stage, substrate limitation occurs and the cells switch from the exponential to the stationary phase leading to the accumulation of reserves in form of glycogen and trehalose. This is a crucial phase during industrial baker's yeast fermentation which allows

cells to survive the upcoming stress (osmotic and mechanical stress during conditioning and packaging) and to build up their storage stability (Lillie et al., 1980). Traditionally, feed rate profiles for molasses and ammonia are built on the basis of company historic data and are specific to a determined strain and process conditions (pH-value changes, temperature profile) (Vieira et al., 2013). Despite of the long history of industrial baker's yeast fermentation, a full mass balance analysis has not been reported yet for this process. Such an analysis could be very beneficial for scientists and yeast producers aiming to improve their process or valorise the resulting vinasse.

In this study, vinasse is referred as the cell-free broth obtained after cell separation at the end of the fed-batch fermentation. Cell separation is usually achieved by centrifugation and continuous filtration via rotary vacuum filters. Approximately 2.3 tons of liquid and unconcentrated vinasse is recovered per ton of baker's yeast. After concentration and partial precipitation, vinasse is often used in Europe as an additive or feeding supplement for ruminants and non-ruminants (Hidalgo, 2009), due to its influence on animal performance leading to an increase in daily weight gain (Iranmehr et al., 2001). Since the beginning of the 20^{th} century, vinasse has also been used as field fertilizer (Hidalgo, 2009), due to significant content of macronutrients (nitrogen, potassium, calcium sulfate, magnesium) and micronutrients (iron, manganese, zinc, copper) (de Raad, 2011). All over the world, its application in agriculture reported positive feedback to soil properties (Rajagopal et al., 2014).

However, vinasse could play more important role as raw material for the production and recovery of key biomolecules such as betaine or technical enzymes (Lisičar et al., 2017). The composition of vinasse originating from ethanol production has been widely studied in order to assess its potential for animal feeding and to eliminate the environmental pollution caused by this product (Scull et al., 2012; Rodrigues et al., 2017). Yalçin et al. (2010) have investigated nutritive values of modified dried vinasse from baker´s yeast production intend to be used as animal feed. Surprisingly, very little attention has been dedicated to the investigation of vinasse resulting from industrial baker's yeast fermentation and information regarding its composition could not be found in the literature.

3.3 Materials and methods

3.3.1 Chemicals and equipment

Table 3.1 List of chemicals used in the fed-batch fermentation and analysis of molasses and corresponding vinasse

Chemical	Manufacturer
2-Hydroxy-3,5-dinitrobenzoic acid	Merck, Darmstadt (Germany)
9-Fluorenylmethyl chloroformate	Merck, Darmstadt (Germany)
Acetonitrile	Merck, Darmstadt (Germany)
Amino acid standard (additionally added glutamin, tryptophan, asparagin)	Sigma-Aldrich, St. Louis, Missouri (USA)
Ammonia 10 %	Bernd Kraft, Duisburg (Germany)
Antifoam	Uniferm, Monheim am Rhein (Germany)
Borate buffer pH 10	Merck, Darmstadt (Germany)
Borate buffer pH 8	Merck, Darmstadt (Germany)
Bromphenol blue	Carl Roth, Karlsruhe (Germany)
Buffer solution pH 4	Merck, Darmstadt (Germany)
Buffer solution pH 7	Merck, Darmstadt (Germany)
Coolant Type HKF 15.1	Conzelmann, Kammeltal (Germany)
Copper(II) sulphate pentahydrate	Merck, Darmstadt (Germany)
D-Biotin	VWR, Pennsylvania (USA)
Deuterium oxide	Deutero, Kastellaun (Germany)
Dithioerythritol	Merck, Darmstadt (Germany)
Dowex X8 50W 50-100	Alfa Aesar, Haverhill, Massachusetts (USA)
Ethanol 99 %	Merck, Darmstadt (Germany)
Hydrochloric acid	Merck, Darmstadt (Germany)
Hydrochloric acid 37 %	Carl Roth, Karlsruhe (Germany)

Iron(III) chloride hexahydrate	Merck, Darmstadt (Germany)
Mercaptopropionic acid	Merck, Darmstadt (Germany)
Methanol	Merck, Darmstadt (Germany)
Methyliodid	Merck, Darmstadt (Germany)
Monoammonium phosphate	Sigma-Aldrich, St. Louis, Missouri (USA)
Monosodium phosphate pH 7.8	Merck, Darmstadt (Germany)
o-Phthaldialdehyde	Merck, Darmstadt (Germany)
Pantothenic acid	Alfa Aesar, Haverhill, Massachusetts (USA)
Phosphoric acid	Merck, Darmstadt (Germany)
Pyridoxine hydrochloride	Alfa Aesar, Haverhill, Massachusetts (USA)
Sucrose	Merck, Darmstadt (Germany)
Sodium azide	Merck, Darmstadt (Germany)
Sodium hydrogen carbonate	VWR, Pennsylvania (USA)
Sodium hydroxide	Merck, Darmstadt (Germany)
Sodium potassium tartrate	VWR, Pennsylvania (USA)
Sulphuric acid 25 %	Geyer Th, Lohmar (Germany)
Tert-butylamine hydrochloride	Sigma-Aldrich, St. Louis, Missouri (USA)
Thiamine nitrate	Alfa Aesar, Haverhill, Massachusetts (USA)
Trichloroacetic acid	Merck, Darmstadt (Germany)
Tris/Glycin	Carl Roth, Karlsruhe (Germany)
Tris/HCl	Carl Roth, Karlsruhe (Germany)

Table 3.2 List of test-kits used in analysis of molasses and corresponding vinasse

Test-Kit	Manufacturer
Ammonium cuvette test 2.0-47.0 mg/L NH_4-N LCK	Hack, Düsseldorf (Germany)
BCA protein assay kit	Merck, Darmstadt (Germany)
Enzytec D-Glucose / D-Fructose / Sucrose	R-Biopharm AG, Darmstadt (Germany)

Table 3.3 List of equipment used in the fed-batch fermentation and analysis of molasses and corresponding vinasse

Equipment	Manufacturer
3 Canal gasifier	Sykam, Fürstenfeldbruck (Germany)
7.5 % Mini-PROTEAN TGX gel	Bio-Rad, Munich (Germany)
Autoclave Tuttnauer 5075 ELV	Biomedis Laborservice, Gieben (Germany)
Balance AT200	Mettler Toledo, Columbus, Ohio (USA)
Biostat Qplus bioreactor (1 L)	Sartorius Stedim Biotech, Göttingen (Germany)
Camera ChemiDoc™ XRS+ system	Bio-Rad, Munich (Germany)
Column heating device T-1	Techlab, Blacksburg, Virginia (USA)
Conductivity meter LF330	WTW, Weilheim (Germany)
Frigomix FX-100	Sartorius Stedim Biotech, Göttingen (Germany)
Heraeus Pico 21 centrifuge	Thermo Fischer Scientific, Waltham, Massachusetts (USA)
HPLC pump Agilent 1200	Agilent, Santa Clara, California (USA)
Injector Triathlon	Spark Holland BV, Emmen (Netherlands)
Mini-Protean tetra system	Bio-Rad, Munich (Germany)
Minisart PES syringe filter 0.2 µm	Sartorius Stedim Biotech, Göttingen (Germany)
Muffle furnace S27 Controller L3	Nabertherm, Lilienthal (Germany)
NMR-spectrometer Ascend 400	Bruker, Billerica, Massachusetts (USA)

pH Meter LAB 850	Schott Instruments, Mainz (Germany)
RF-10AXL fluorescence detector	Shimadzu, Kyoto (Japan)
Shaker KS 250	IKA, Staufen (Germany)
Spectrophotometer DR3900	Hach Lange, Düsseldorf (Germany)
Spectrophotometer DR5000	Hach Lange, Düsseldorf (Germany)
Vacuum furnace	Shel Lab, Cornelius, Oregon (USA)
Zorbax Eclipse Plus C18, 3.5 µm,4.6 mm x 150 mm	Agilent, Santa Clara, California (USA)

3.3.2 Strain

Large-scale yeast inoculum *S. cerevisiae* was kindly provided by Uniferm GmbH & Co. KG, Monheim am Rhein, Germany.

3.3.3 Medium and cultivation

Fed-batch fermentation was conducted in the laboratory in Uniferm GmbH & Co. KG, Monheim am Rhein. A 1 L bioreactor was filled with 0.5 L water and 2.7 g ammonium dihydrogen phosphate and autoclaved at 121 °C for 20 minutes. 466.05 g of a molasses mixture (10 % cane and 90 % beet molasses) and 183.95 g of tap water (together with 25 % sulfuric acid required to adjust pH-value to 5.4) was supplemented with a trace element solution and 300 µL of antifoam and autoclaved at 121 °C for 20 minutes. Directly after the sterilization, the molasses mixture was placed on a shaker at 150 rpm, aerated with air (2 slpm) for 1 hour and then supplemented with vitamin stock. Medium´s weight lost due to water evaporation during the sterilization and aeration was adjusted with sterilized water.

The fermentation carried out in this study was performed according to the method developed at Uniferm GmbH & Co. KG. In this regard, dosage profiles, pH profile, temperature profile and aeration profile are designed to simulate large-scale fermentation, and enable maximum yield of yeast with high fermentative capacity and long storage stability. The bioreactor filled with 500 g of water and 2.7 g of ammonium dihydrogen phosphate was inoculated with 18.47 g of yeast (concentration 0.731 g/g). The medium was then gradually added to the bioreactor

and the fed-batch fermentation was started. After 20 hours and 40 minutes of fermentation, the bioreactor was discharged and the yeast cake was separated from the vinasse by means of vacuum filtration. Molasses mixture (after the treatment) and vinasse, as by-product, have been further analysed.

3.3.4 Determination of dry matter

Vacuum furnace was used for the determination of dry matter. 1 mL of sample was pipetted, weighted and heated for 24 hours at the temperature of 105 °C (Bougrier et al., 2005). Dried sample was once again weighted and the dry matter content (DM) was calculated according to the following formula:

$$\mathrm{DM} = \frac{m_{dS} - m_C}{V_S}\ [\mathrm{g/L}] \quad (1)$$

m_{dS} = mass of the crucible with the dried sample [g]

m_C = mass of crucible [g]

V_S = volume of the sample [mL]

3.3.5 Determination of ash content

For the determination of ash content a muffle furnace was used. 1 mL of the sample was pipetted, weighted and heated for 2 hours at the temperature of 550 °C. Ash content was determined according to the following formula:

$$\text{Ash content} = \frac{m_{aS} - m_C}{V_S}\ [\mathrm{g/L}] \quad (2)$$

m_{aS} = mass of the crucible with the burnt sample [g]

m_C = mass of crucible [g]

V_S = volume of the sample [mL]

3.3.6 Determination of betaine concentration

Betaine concentration was determined by the method proposed by Chastellian (1976) with minor modifications. The samples were acidified with 37 % hydrochloride acid to pH 3. A

column was filled with 55 mL of a cation-exchange resin (Dowex X8 50W 50-100), washed with 75 mL of 2.5 M hydrochloride acid and subsequently neutralized with 75 mL of water prior to measurements. 50 mL of the sample (molasses mixture and vinasse) was injected in the column and eluted with 50 mL of distilled water (fraction 1). The column is then injected with 75 mL of 2.5 M HCl and washed with 75 mL of distilled water (fraction 2). Finally column is acidified with 50 mL of 2.5 M HCl and 100 mL of distilled water (fraction 3). Fractions have been collected and analysed via NMR spectroscopy. For this purpose, 300 µL of the corresponding fraction was filled in a NMR-tube and 300 µL of internal standard (tert-butylamine hydrochloride, 33.33 mg/mL) as well as 100 µL of deuterium oxide was added. The betaine concentration was calculated according to the following formula:

$$c_B = c_{Ref.} * \frac{N_{Ref.}}{N_x} * \frac{M_x}{M_{Ref.}} * \frac{A_x}{A_{Ref.}} \text{ [g/L]} \quad (3)$$

c_B = concentration of betaine [g/L]

c_{Ref} = concentration of the internal standard [g/L]

N_{ref} = number of protons in the internal standard

N_X = number of protons in betaine

M_x = molar mass of betaine [g/mol]

M_{ref} = molar mass of the internal standard [g/mol]

A_x = Integral value of the signal representing betaine

A_{ref} = Integral value representing the internal standard

3.3.7 Determination of sugar concentration

The concentrations of sucrose, glucose and fructose have been determined with commercial kits.

3.3.8 Determination of protein concentration

The protein concentrations were determined with the commercial kit. For the SDS-page electrophoresis the samples were precipitated with 60 % trichloroacetic acid and then treated

with 10x Tris/Glycin (Yamamichi et al., 2011) and Laemmli buffer according to Laemmli with minor differences (Laemmli, 1970). The 5x Laemmli buffer contained 1.5 M Tris/HCl, 0.01 % Bromphenol Blue and none of 2-mercaptoethanol. The gel with proteins was recorded.

3.3.9 Determination of amino acid content

The samples were treated with methanol in ratio 1:4 and frozen overnight in order to precipitate the proteins. Proteins were removed via centrifugation at 14,000 rpm for 15 minutes and filtered. The samples were diluted with borate buffer (0.4 M) to reach pH-value 10. For the determination of cystin/cysteine concentration 100 µL of the sample was mixed with 250 µL of borate buffer (pH 8), 50 µL of 20 mM dithioerythritol, 50 µL of 400 mM methyliodid and 100 µL of 4 M natrium hydroxide. After 10 minutes the reaction was stopped with 100 µL of 4 M hydrochloric acid and 350 µL of borate buffer (pH 10) was added.

Two mobile phases were prepared: A (40 mM monosodium phosphate pH 7.8 and 5 mM sodium azide) and B (acetonitrile, methanol and water in ration 45:45:10). Two derivatization reagents were prepared; OPA-reagent: 10 mg o-phthaldialdehyde, 6.5 mg mercaptopropionic acid in 500 µL methanol and 500 µl borat puffer (0.4 M, pH 10) and FMOC-reagent (9 mg/mL 9-fluorenylmethyl chloroformate in acetonitrile). Injection diluent was prepared with 100 mL of eluent A and 0.4 mL of concentrated phosphoric acid. The injected samples consisted of the following: 15 µL sample, 20 µL OPA-reagent, 20 µL FMOC-reagent and 50 µL of injection diluent. The HPLC apparatus consisted of a pump, a gasifier, an injector, a column heating device, an analytical column a detector and computer program (Clarity 5.0; DataApex, Prague, The Czech Republic).

3.3.10 Determination of invertase activity

The invertase activity was determined by the method of Timerman (2012). One unit U of enzyme activity is defined as the amount of enzyme required to either hydrolyse 1 µmole of sucrose per minute or produce 1 µmole of invert sugar per minute. This method relies on the spectrophotometric determination of monosaccharides in the presence of an alakaline 3,5 dinitrosalicylate solution (DNS) (0.20 M NaOH, 23 mM 3, 5-dinitrosalicylate and 0.53 M sodium potassium tartrate). 1 mL of sucrose solution (100 mM) was mixed with 0.1 mL of the sample in a glass-tube. After 5 minutes, 2 mL of DNS was added, the tubes were placed in

boiling water for 10 minutes and afterwards diluted with 3 mL of deionized water. The absorbance of the samples was measured at 540 nm (Spectrophotometer DR5000, Hach Lange; Düsseldorf, Germany). Finally, the invertase activity was determined according to Beer-Lambert law:

$$\text{I. A.} = \frac{\Delta\text{ABS} \cdot \text{V}}{\text{a} \cdot \text{b} \cdot \text{t} \cdot \text{V}_\text{S}}\ [\text{U/mL}] \qquad (5)$$

I. A. = invertase activity [U/mL]

ΔABS = absorbance value above the reagent blank

V = total volume [6.1 mL]

a = absorptivity constant [2.0 mL/(μmol · cm)]

b = cuvette light path [1 cm]

t = time or reaction [5 min]

V_S = volume of the sample [mL]

3.3.11 Determination of raw protein content in yeast

Quantitative determination of nitrogen was performed according to the method of Kjeldahl (1883). The protein content in yeast is calculated by multiplying with factor 6.25.

3.3.12 Determination of residual ammonium ions in vinasse

The concentration of residual ammonium ions in vinasse was determined with commercial kit.

3.4 Results and discussion

Figure 3.1 depicts the general mass balance of the fermentation process. During the feeding phase, 349.8 g of molasses mixture and 48.7 g of 10 % ammonium solution were pumped into the bioreactor. 9.9 g of 25 % sulphuric acid was required to correct the pH-value during the fermentation. The cultivation yielded 241.7 g of yeast, with a dry matter content of 27.23 % (approx. 7.4 g dry cell weight per kg of broth) and a protein content of 49.03 %. The achieved

high cell density and protein content are very similar to the values obtained by operating an industrial process (Reed et al., 1991). After vacuum filtration, 645.8 g of vinasse was obtained.

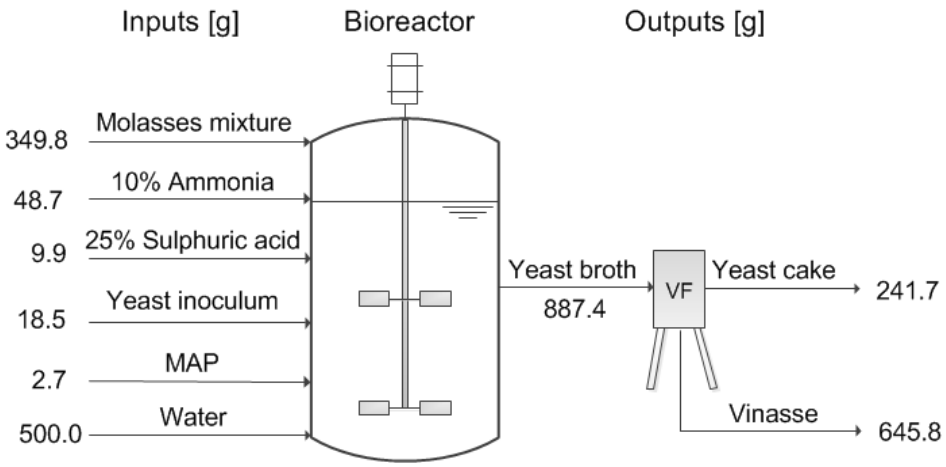

Figure 3.1 General mass balance analysis of the fed-batch fermentation performed in this study

3.4.1 Utilization of sugars and invertase production

Absolute masses and contents of sucrose, glucose and fructose measured in molasses mixture and vinasse are summarized in Table 3.4. As expected, sucrose was found to be the prevailing sugar in the molasses mixture, followed by fructose and glucose. The sugar composition measured for this medium is in a good agreement with the literature (Olbrich, 1963; Teclu et al., 2009).

Table 3.4 Absolute masses of sugar [g] and sugar contents [g/L] measured in molasses mixture and vinasse

Sugars	Molasses		Vinasse	
	[g]	[g/L]	[g]	[g/L]
Glucose	6.88	23.49	0	0
Fructose	9.88	33.73	0	0
Sucrose	85.49	291.76	0	0
Total	**102.25**	**348.98**	**0**	**0**

The yield coefficient of yeast biomass based on total sugar consumption ($Y_{X/sugar}$) was 0.584 g/g, which fits well with data reported in the literature (Reed et al., 1991; George et al., 1998). Sucrose is a disaccharide which has to be hydrolysed in order to be metabolized by *S. cerevisiae*. For this purpose, external invertase (21.3 U/mL) was released in the cultivation medium by *S. cerevisiae* during the fermentation. In this regard, a yield of activity based on yeast biomass $Y_{inv./X}$ = 210.9 U/g was determined. Invertase is a key biomolecule which currently belongs to the most important technical enzymes and should be regarded as an attractive by-product of this process. SDS-PAGE analysis confirmed the presence of external invertase, with a molecular weight of 270 kDa (Kulshrestha et al., 2013) (Figure 3.2). Furthermore, only peptides and proteins with low molecular weights were found in the molasses mixture while the protein fraction of vinasse seems to consist of larger proteins which can be easily separated from other molecules by size exclusion (e.g. ultrafiltration).

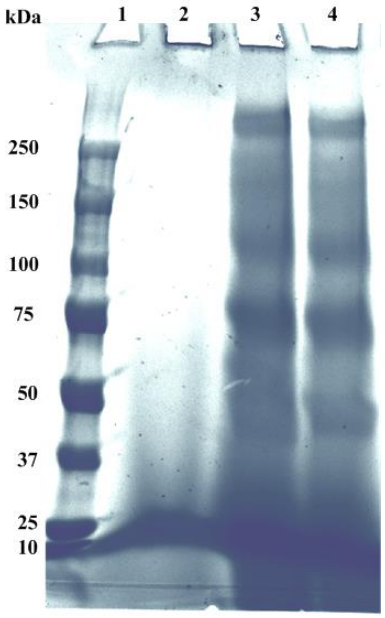

Figure 3.2 SDS-PAGE analysis [Lane 1; molecular weight markers (250 – 10 kDa), lane 2; molasses mixture, lane 3; vinasse with 2-mercaptoethanol and lane 4; vinasse; (20 µL)]

3.4.2 Utilization of ammonia and protein production

As already mentioned 48.7 g of 10 % ammonia were pumped into the bioreactor during the fed-batch fermentation and only 0.5 g of unassimilated nitrogen measured in form of

ammonium ions was found in the vinasse. This finding confirms the substantial utilization of ammonia by *S. cerevisiae* and reveals that absolute nitrogen limitation did not occur during the late step of the process. Ammonia is then converted into glutamate and glutamine in order to be used as nitrogen source (ter Schure et al., 2000). The yield coefficient of yeast biomass based on nitrogen consumption ($Y_{X/N}$) was 16.04 g/g respectively. Baker´s yeast utilizes the nitrogen source in anabolic processes for the synthesis of proteins, enzymes and nucleic acids (Walker, 2014). Approx. 29.6 g of new synthesized protein was found in yeast biomass. According to Table 3.6, the mass of protein in molasses mixture and vinasse amounted 27.1 g and 19.2 g respectively. This in turn suggests that molasses proteins and peptides were catabolized during the fermentation. The presence of proteins with higher molecular weight as well as the measured invertase activity indicates the release of new synthesized proteins in the fermentation medium due to controlled secretion or cell lysis.

3.4.3 Utilization of amino acids

It is known that amino acids are metabolized by *S. cerevisiae* (Olbrich, 1963). The distribution of amino acids measured in the molasses mixture and the vinasse are summarized in Table 3.5. According to the literature (Šárka et al, 2013), amino acid with the highest content present in molasses is aspartic acid. Other amino acids having a majority share are asparagine and glutamic acid. A much lower absolute mass of amino acids was measured in vinasse (0.59 g in vinasse vs. 2.56 g in molasses) which confirms the utilization of amino acids during the last hours of the fermentation when substrate limitation occurred. With exception of aspartic acid and glutamic acid, all amino acids present in the molasses mixture were almost fully metabolized. The considerable amount of glutamic acid might result from the combined utilization and production during ammonia conversion. Glutamine and histidine were not detected in the molasses mixture. However, they were measured in small amounts in the vinasse, meaning they were synthesized in the course of the fermentation. Glutamine could have been produced during the growth on ammonia, as mentioned above. In this pathway glutamate and α-ketoglutarate are being produced as well as histidine.

Table 3.5 The distribution of amino acids in molasses mixture and vinasse presented as absolute mass [g] and concentration [g/L]

Amino acid	Molasses		Vinasse	
	[g]	[g/L]	[g]	[g/L]
Alanine	0.2577	0.8794	0.0096	0.0161
Arginine	0.0441	0.1505	0.0071	0.0118
Asparagine	0.2231	0.7614	0.0167	0.0280
Aspartic acid	0.4189	1.4298	0.1896	0.3177
Cysteine	0	0	0	0
Glutamine	0	0	0.0012	0.0020
Glutamic acid	0.3117	1.0639	0.2569	0.4303
Glycine	0.0624	0.2129	0.0143	0.0240
Histidine	0	0	0.0013	0.0022
Isoleucine	0.2001	0.6828	0.0087	0.0146
Leucine	0.1767	0.6032	0.0078	0.0131
Lysine	0.0146	0.0498	0.0017	0.0028
Methionine	0.0219	0.0749	0.0023	0.0038
Phenylalanine	0.0396	0.1352	0.0034	0.0056
Proline	0.1443	0.4925	0.0138	0.0231
Serine	0.1220	0.4165	0.0059	0.0098
Threonine	0.0604	0.2063	0.0061	0.0102
Tryptophan	0.0455	0.1554	0.0062	0.0104
Tyrosine	0.2552	0.8710	0.0277	0.0464
Valine	0.1574	0.5373	0.0077	0.0130
Total	**2.5557**	**8.7226**	**0.5880**	**0.9849**

3.4.4 Mass balance of betaine

Betaine is a well-known amino acid derivative originating from beet molasses. The betaine content in molasses mixture was 53.91 g/L and represented 8 % of dry matter weight which is in a good agreement with literature data (Heikkila et al., 1992). Approx. 70 % of the betaine present in the molasses mixture was found in the vinasse (Figure 3.3). According to literature, betaine is not being metabolized to any significant extent by *S. cerevisiae* (Thalasso et al., 1999). Most likely is that a part of the betaine kept entrapped in pores of the yeast cake after vacuum filtration. Nevertheless, an appreciable amount of betaine remained in the vinasse (11.04 g at 18.5 g/L) which also has a considerable economic potential. Natural betaine is an important biomolecule in animal feeding which is expected to gain relevance over its synthetic form. Supplementation with natural betaine improves carcass lean deposition particularly under production stress and reduced the impact of coccidia challenge on intestinal lesion scores and positively affected nutrient digestibility and feed efficiency in broilers (Amerah et al., 2015).

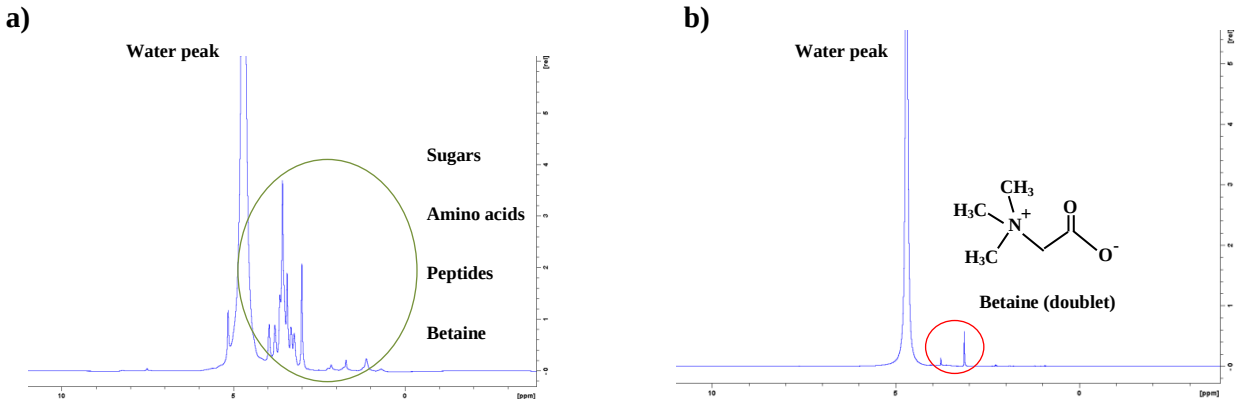

Figure 3.3 NMR-images of molasses (a) and corresponding vinasse (b)

3.4.5 Overview of molasses and vinasse´s composition

The full compositions of molasses mixture and vinasse are shown in Table 3.6. Figures 3.4-3.5 depict the results of the chemical analyses of the molasses mixture and the vinasse. Just a glance over these diagrams clearly shows that the vinasse is much less complex medium than the molasses mixture. During the fermentation, baker´s yeast "purified" betaine from the

molasses mixture by assimilating almost all available sources of carbon. The composition of the vinasse gained from ethanol production has higher content on organic matter and contains sugar. Furthermore, it is characterized by a higher amino acid content and a considerable ethanol concentration (Rodrigues et al., 2017; Scull et al., 2012). The amount of invertase and betaine has not been reported yet for this kind of vinasse. However, it is important to state that the determined vinasse composition found in this work resulted from a specific molasses mixture (10 % sugar cane molasses and 90 % beet molasses).

Table 3.6 Composition of molasses mixture and vinasse presented as absolute mass [g] and concentration [g/L]

	Molasses		**Vinasse**	
	[g]	**[g/L]**	**[g]**	**[g/L]**
Amount	349.75	-	645.79	-
Dry matter	196.62	671.90	49.09	82.23
Ash content	27.94	95.36	16.40	27.47
Betaine	15.80	53.91	11.04	18.50
Sucrose	85.49	291.76	0	0
Glucose	6.88	23.49	0	0
Fructose	9.88	33.73	0	0
Total protein	27.08	92.41	19.17	32.11
Amino acids	2.56	8.72	0.59	0.98

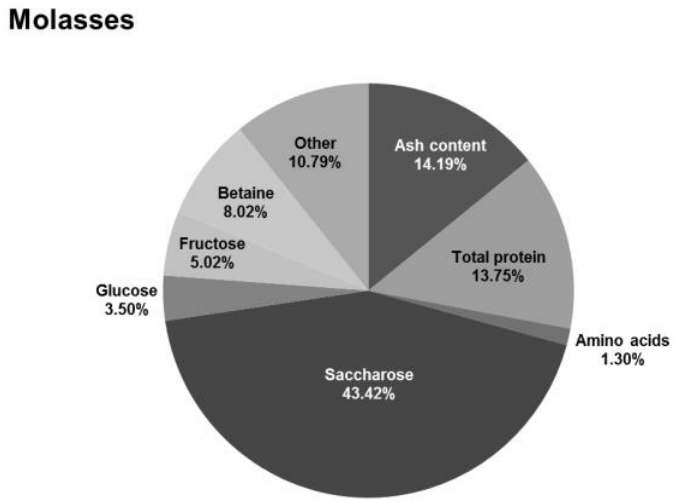

Figure 3.4 Composition of molasses mixture based on dry matter shown in percentage

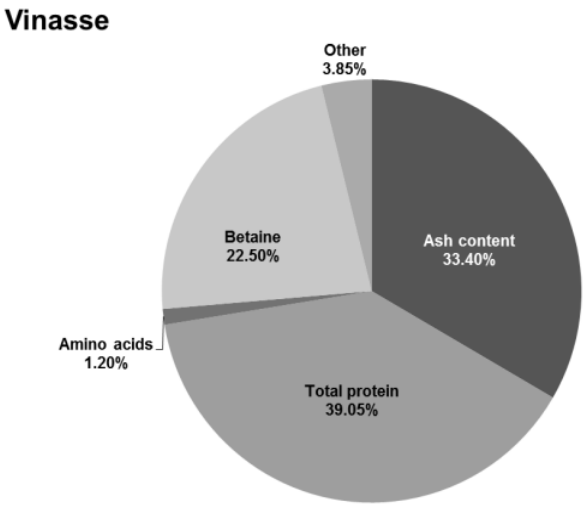

Figure 3.5 Composition of vinasse based on dry matter shown in percentage

3.5 Conclusion

Considering that the fed-batch fermentation of baker´s yeast performed in this study is a well-established process developed over the years by scientists and experts in Uniferm GmbH & Co. KG, the results obtained allow the upscaling to a large-scale process in the industrial environment. A yearly production of 40,000 tons of compressed baker´s yeast would generate 92,000 tons of vinasse which would need to be processed a bit differently than in the usual baker´s yeast plant. External invertase, as a large glycoprotein with a molecular weight of approx. 270 kDa, can be separated and concentrated by ultrafiltration (e.g. 100 kDa MWCO). The remaining protein fraction can be removed from small molecules (betaine, aspartic acid, glutamic acid and ashes) by an additional ultrafiltration step (e.g. 1 kDa MWCO). At neutral pH value, betaine exhibits zero net charge and does not bind on any ion exchanger. Under these conditions, aspartic acid and glutamic acid are negatively charged so that betaine purification (de-ashing and removal of aspartic acid and glutamic acid) can be achieved by ion exchange. According to the findings, it would lead to the bioproduction of approx. $1.8 \cdot 10^{12}$ invertase units. The current market price for invertase involved in the production of invert sugar is approx. 2.5 $ for 10^6 invertase units (activity 20 U/mg) (Stowers et al., 2008). Combining this price with the large vinasse volumes resulting from this process reveals a high economic potential for this by-product. In this industrial production, the recovered amount of food grade proteins amounts 2,730.4 t/year. These proteins, which are obtained through valorisation of baker´s yeast vinasse, are high added-value products and as such are expected to have a higher average price of 7 – 8 kg/€ compared to traditional proteins (Probst et al., 2015). Although baker´s yeast consumes most of the amino acids available in molasses, still the appreciable amount could be recovered from vinasse (Table 3.7). The largest share of the amino acid market is consisted of feed amino acids used in the nutrition of swine, poultry and ruminants (Leuchtenberger et al., 2005). The recovered amount of theses amino acids from vinasse is 5.5 t/year. Furthermore, 64.1 t/year of amino acids used in human nutrition as food enhancers could also be recovered. Other amino acids (recovered amount of 14.2 t/year) are employed as raw materials for synthesis of chiral active ingredients, which find application in pharmaceutical, cosmetics and agriculture industries (Leuchtenberger et al., 2005). The yearly volume of vinasse released by a baker's yeast production plant, would allow producing up to 1,700 tons of natural betaine per year. With a current market price of approx. 1,000 $/ton (Xu

et al., 2014) recovering natural betaine from industrial baker's yeast fermentation could have a very high impact on the profitability of this process.

Table 3.7 Yearly production of amino acids for feed and food sector

Amino acid		Quantity [t]
Feed amino acids	Glycine	2.043
	Lysine	0.239
	Methionine	0.321
	Threonine	0.868
	Tryptophan	0.883
	Valine	1.101
Food amino acids	Aspartic acid	27.014
	Glutamic acid	36.595
	Phenylalanine	0.478

3.6 References

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4 Fractionation of baker´s yeast vinasse via ultrafiltration

4.1 Objectives

By-product of baker´s yeast production vinasse (cell-free broth) occurs in separators, centrifuges and rotary vacuum filters. Overall, industrial fermentation usually results in approx. 30 % of compressed yeast and 70 % of vinasse. Vinasse, with approx. 6 % of dry matter, is further concentrated via evaporation leading to vinasse concentrate containing approx. 70 % of dry matter. It is further directed into a centrifugation station, where two products are obtained: vinasse salt is the solid part and used as field fertilizer, while the liquid part is used for animal feeding (Figure 4.1).

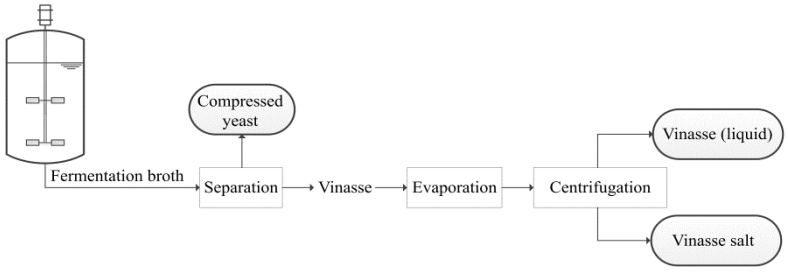

Figure 4.1 Typical downstream processing of baker´s yeast vinasse in industry (Lisičar Vukušić et al., 2018)

However, modifying this process could upgrade vinasse from a low-value stream to more valuable by-product, which can be employed for the recovery of high-value biomolecules such as technical enzymes (e.g. invertase), organic osmolytes (e.g. betaine) and food grade proteins. This chapter aims to assess the feasibility of the fractionation and recovery of invertase, food grade proteins and betaine from baker's yeast vinasse via cross-flow ultrafiltration. In this regard, following steps have been performed:

1) separation of invertase via ultrafiltration with MWCO of 30 kDa
2) separation of betaine rich food grade protein fraction via ultrafiltration step with MWCO of 2 kDa

3) obtaining a salt mixture (fertilizer) and a betaine concentrate by evaporation and centrifugation

4) purifying the betaine by ion exchange adsorption

4.2 Theoretical Background

The potential of baker's yeast vinasse for the recovery of valuable molecules (enzymes, betaine and amino acids) has been recognized (Villa et al., 1980; Hamstra et al., 1997, Paanamen et al., 2014). Natural betaine, a trimethyl derivative of glycine, attracts more and more attention as a feed additive, especially due to the restrictions of using antibiotics in animal feeding. Betaine is an important osmolyte involved in the control of osmotic pressure inside the cells. This results in increased proliferation of the intestinal structure which could have a positive impact on animal health and nutrient digestibility. Also as methyl donor, it provides three methyl groups which can be used in transmethylation reactions for the synthesis of numerous substances (carnitine and creatine). It improves the efficiency of coccidiostats which indicates improved animal performance (Eklund et al. 2005; Kettunen et al., 2001). Humans obtain betaine from food (e. g. wheat bran, spinach, beets and shrimps). As osmolyte it protects the cells from high concentrations of electrolytes and urea in kidneys and improves liver function. Furthermore, betaine-rich diet may lower the risk of cardiovascular diseases (Craig, 2004). Cholewa et al. (2013) demonstrated how betaine supplementation improves body composition, muscle size, work capacity as well as the indication of lowering the homocysteine thiolactone level, which has been associated with vascular disease, insulin resistance, diabetic retinopathy, seizures and Alzheimer's disease. Natural betaine originates from sugar beet molasses. When sugar beet is processed for the recovery of sucrose, betaine is being concentrated in the molasses. Beet molasses usually contains 3-8 % of betaine calculated on dry solids (D. S.) basis (Heikkila et al., 1992). Natural betaine extracted from sugar beet molasses and vinasses (fermented molasses) using a patented chromatographic separation process has been introduced by Dupont® (Danisco Animal Nutrition, 2013). The corresponding NS2P process (New Sequential, 2-Profile) involves a combined batch process and SMB (Simulated Moving Bed) and a three-fraction chromatography system, which allows producing new betaine revenue stream. Produced betaine has a purity of 67-78 % in D. S. with a recovery of 90-98 % (Burris et al., 2009). The

current price of this product is approx. 6-7 €/kg (Jose Manuel de la Fuente, DuPont Industrial Biosciences, personal communication, 07th March 2018).

Proteins are one of the main constitutes of foods. Besides its nutritional value, food proteins are usual ingredients in food formulation and play an important role in the expression of sensory properties of foods (Damodaran and Paraf, 1997). They are also employed as food-gelling and thickening agents and stabilizers for foams and emulsions (Tolstoguzov, 1991). Commercially used animal and plant proteins include whey protein isolate, casein, ovalbumin, soy and bovin serum albumin (Lam and Nickerson, 2013).

S. cerevisiae is the main strain used for the production and purification of invertase (Kulshrestha et al., 2013). It produces two types of invertase: non-glycosylated intracellular form and glycosylated secreted form (Sainz-Polo et al., 2012). Secretion of extracellular invertase, also called external invertase, is subjected to glucose repression even at low glucose concentrations (0.1 %), while intracellular invertase, whose function is unknown, is produced constitutively at low concentrations (Briggs et al., 2004). A considerable proportion of external invertase has been found to be entrapped in the cell wall of the yeast, where it catalyses the hydrolysis of sucrose into glucose and fructose, which the yeast cell can import (Esmon et al., 1987). For this reason, external invertase is industrially obtained from yeast extract after cell disruption. However, during the baker´s yeast production a portion of the external invertase is released in the fermentation broth, where it accumulates (Villa and Phaff, 1980). The resulting mixture of glucose and fructose is called inverted sugar syrup which is sweeter than sucrose and offers technical advantages. Invertase is one of the most widely used enzymes in food engineering, especially in the preparation of jams and candies (Shankar et al., 2013). Other application of this enzyme can be found in drug and pharmaceutical industries, as well as in cosmetics as plasticizing agents (Kulshrestha et al., 2013). Market price of invertase is approx. 2.5 $ for 10^6 invertase units (activity 20 U/mg) (Stowers et al., 2008).

Ultrafiltration process has become an essential technique for concentrating and separating in the food industry processing due to its advantages over conventional separation treatments: gentle product treatment, high selectivity and low energy consumption (compared to condensers and evaporators) (Mohammad et al, 2012). Application of cross-flow

ultrafiltration membranes can be found in the dairy industry (processing of milk and whey) and beverage industries (beer, fruit juices and wine) (Lipnizki, 2010).

4.2.1 Novel approach for the simultaneous recovery of betaine and invertase

The presented approach is designed to be easily integrated in a baker´s yeast producing company as part of the downstream processing (Figure 4.2). It would require minor infrastructural changes of the plant and would not affect the baker's yeast production. The first stage of the downstream processing remains unchanged, the fermentation broth is directed to separators, centrifuges and rotary vacuum filters, whence vinasse is collected. A first cross-flow ultrafiltration is intended to retain invertase. Since the molecular weight of this enzyme is 270 kDa, using a membrane with a MWCO of 30 kDa, invertase will be concentrated in the corresponding retentate. A subsequent cross-flow ultrafiltration step employs a membrane with lower MWCO, in this case 2 kDa. This step concentrates food grade proteins, valued in human food and animal feed. The further treatment involves the concentration of the permeate by using the existing evaporation – centrifugation station. Two product-streams flow out of this process: salt mixture and concentrated liquid fraction. The latter is finally treated via ion exchange adsorption in a so called tandem system. It includes two adsorption beds which are intended to be alternately operated; while one bed is adsorbing, the other one is being regenerated. As a result of the ion exchange adsorption step natural betaine (similar to Betafin®) is recovered. This fourth product is known to have a high economic potential and may be regarded as an enhancement for the concretization of this new technology. CF-P is a concentrate of inorganic salts which can be used as fertilizer (Lisičar Vukušić et al., 2018).

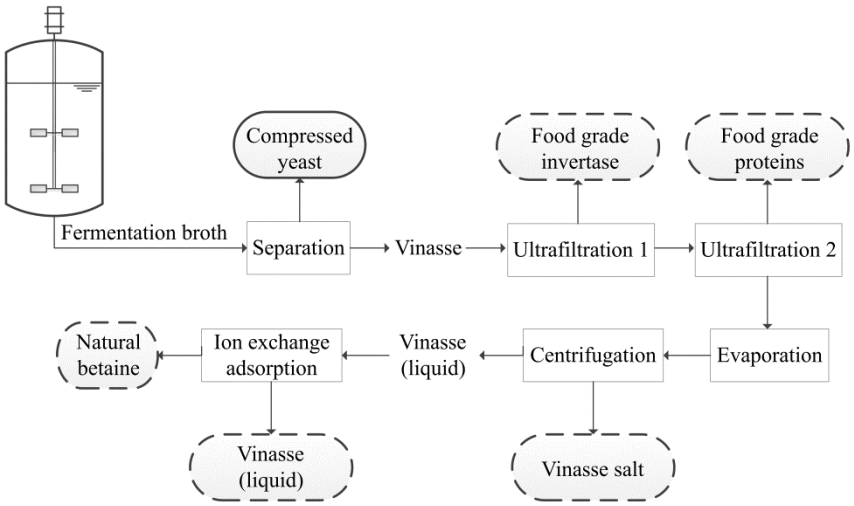

Figure 4.2 Novel straightforward downstream processing of baker´s yeast vinasse (Lisičar Vukušić et al., 2018)

4.3 Material and methods

4.3.1 Chemicals and equipment

Table 4.1 List of chemicals used in fed-batch fermentation and downstream processing

Chemical	Manufacturer
Ammonium solution 10 %	Carl Roth, Karlsruhe (Germany)
Ammonium sulphate	Carl Roth, Karlsruhe (Germany)
Biotin	VWR International, Radnor, Pennsylvania, (USA)
Calcium-D(+)-pantothenate	Carl Roth, Karlsruhe (Germany)
Casamino acids	AMRESCO, Inc. VWR International, Radnor, Pennsylvania, (USA)
Certipur® Buffer pH 4	Merck, Darmstadt (Germany)
Certipur® Buffer pH 7	Merck, Darmstadt (Germany)
Citric acid	Alfa Aesar, Haverhill, Massachusetts (USA)

Copper(II) sulphate pentahydrate	Merck, Darmstadt (Germany)
Dowex Monosphere MR-450 UPW	Sigma-Aldrich, St. Louis, Missouri (USA)
Ethanol 99.8 %	Merck, Darmstadt (Germany)
Fructose	AMRESCO, Inc. VWR International, Radnor, Pennsylvania, (USA)
Glucose	Alfa Aesar, Haverhill, Massachusetts (USA)
Hydrochloric acid 37 %	Carl Roth, Karlsruhe (Germany)
Iron(III) chloride hexahydrate	Carl Roth, Karlsruhe (Germany)
Magnesium sulphate	VWR International, Radnor, Pennsylvania, (USA)
Maltose	SERVA Electrophoresis GmbH, Heidelberg (Germany)
Monoammonium phosphate	VWR International, Radnor, Pennsylvania, (USA)
Nicotinic acid	VWR International, Radnor, Pennsylvania, (USA)
Oxygen	Westfalen, Münster (Germany)
Potassium dihydrogen phosphate	Merck, Darmstadt (Germany)
Pyridoxine hydrochloride	VWR International, Radnor, Pennsylvania, (USA)
Sodium chloride	Merck, Darmstadt (Germany)
Sodium hydroxide 2.5 M	Carl Roth, Karlsruhe (Germany)
Sorbitol	Merck, Darmstadt (Germany)
Sucrose	Südzucker, Mannheim (Germany)
Sulphuric acid 25 %	Merck, Darmstadt (Germany)
Tert-butylamine hydrochloride	Sigma-Aldrich, St. Louis, Missouri (USA)
Thiamine hydrochloride	Carl Roth, Karlsruhe (Germany)
Tri-sodium citrate dihydrate	Merck, Darmstadt (Germany)

Table 4.2 List of equipment used in fed-batch fermentation and downstream processing

Equipment	Manufacturer
15 L bioreactor Techfors-S	Infors, Bottmingen (Switzerland)
Alcocontrol	Cetotec, Bad Honnef (Germany)
Flojet-D49X003B pump	RY Brook, New York (United States)
Hydrosart® Sartocon® Slice 200 Microfiltration Cassette (0.45 µm)	Sartorius, Göttingen (Germany)
Hydrosart® Sartocon® Slice 200 Microfiltration Cassette (2 kDa)	Sartorius, Göttingen (Germany)
Hydrosart® Sartocon® Slice 200 Microfiltration Cassette (30 kDa)	Sartorius, Göttingen (Germany)
Kern CPB 30 kg 5N (balance)	Kern, Balingen (Germany)
Minizentrifuge IKA® mini G	IKA Werke GmbH & Co. KG, Staufen im Breisgau (Germany)
Muffle furnace S27 Controller L3	Nabertherm, Lilienthal (Germany)
Peripex W2 peristaltic pump IP65	Bio components (Switzerland)
pH meter PCE-PHD 1	PCE Deutschland GmbH, Meschede (Germany)
Refractometer 0-32 %	Greiner Glasinstrumente GmbH, Lemgo (Germany)
Rotary evaporator RV 10 digital	VWR by IKA, Radnor, Pennsylvania (SAD)
Rotina 420/420R centrifuge	Hettich, Tuttlingen (Germany)
Sorvall RC-5B Plus Superspeed Centrifuge RC-5B Plus	Thermo Fisher Scientific, Waltham, Massachusetts (SAD)
Systec DE-150 autoclave	Systec GmbH, Linden (Deutschland)
TB6 balance	PCE Deutschland GmbH, Meschede (Germany)
Temperature switch model TDS-30	Wika, Klingenberg (Germany)
Vacuum drying oven	Shel Lab; Oregon, USA

4.3.2 Strain

Large-scale yeast inoculum *S. cerevisiae* was kindly provided by Uniferm GmbH & Co. KG (Monheim am Rhein, Germany).

4.3.3 Fed-batch cultivation

4.3.3.1 Pilot scale fermentation set-up at the Faculty of applied natural sciences, TH Köln

Fed-batch fermentation technique developed at the laboratory of Uniferm GmbH & Co. KG (Monheim am Rhein, Germany) was modified and adapted to 15 L bioreactor located at the Faculty of applied natural sciences, TH Köln (Leverkusen, Germany). The adaptation and the optimization were performed over the period of nine months. All the fermentations conducted in this thesis were performed the same way. The set-up is shown in Figure 4.3.

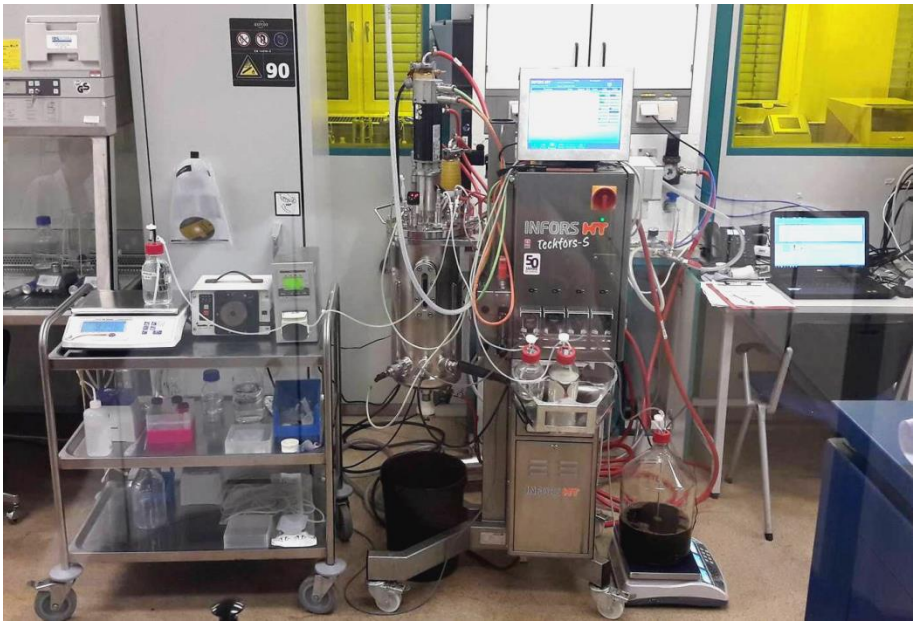

Figure 4.3 Pilot scale set up for conducting fed-batch fermentations

Preparation for the fed-batch fermentation started with calibrating the pH probe, first in the buffer pH 7 and then pH 4. During the calibration (usually lasts 45 minutes for each buffer) 5 L of tap water with 30 g of MAP together with two funnels, large empty molasses bottle (10 L) and tubes for molasses medium and inoculation were sterilised at 121 °C for 20 minutes.

The water for the fed-batch fermentation is not being sterilised together with the bioreactor (considering it is in situ sterilisable bioreactor) because of the variable water loss of up to 0.5 kg during the sterilisation. Ethanol probe was calibrated using three solutions containing following ethanol concentrations (w/w): 0.02, 0.05 and 0.1 %. After the calibration of pH probe, O_2 and ethanol probes were connected to the bioreactor as well as two temperature probes (one belonging to the bioreactor system and the other one used for ethanol measurements) and all the Luer-Lock connection fittings at the top of the bioreactor were closed. Bioreactor was sterilised for 20 minutes at 121 °C containing few litres of water. Afterwards water in the bioreactor is poured out and sterile 5 L was poured in by sterile funnel. O_2 was polarised overnight. The next day sterile mixture of beet and cane molasses, with the Brix value approx. 45, was poured into the large bottle placed on the balance and connected to the bioreactor. Additionally vitamin and mineral solutions were added into molasses (exact composition cannot be specified due to confidentiality reasons). Molasses serves as carbon source, while 10 % ammonium solution is employed as nitrogen source. Considering that acid and base pumps of the bioreactor system are only used for pH control, an external pump was used for dosing the ammonium solution. O_2 probe was calibrated at 100 % saturation by aerating the medium with air. The fed-batch fermentation begun by starting a software programme IRIS, in which recipe for controlling the dosage of substrates, pH value and temperature was applied. Inoculation of 180 g of yeast was performed by means of a tube and a pump. When the O_2 concentration dropped at 0 %, the second point of O_2 calibration was performed. The fed-batch cultivation was performed within a temperature range from 30 °C to 36 °C and the pH-range from 4 to 6.2 (kept at specific value by 25 % sulphuric acid). Substrates molasses and ammonia were added incrementally according to the specific profile enabling maximum yeast growth with minimum ethanol production. The dissolved-oxygen concentration was kept above 10 % of air saturation by following sequence:

Sequence

```
#0, Begasung
IF(pO2.v<pO2.sp-0.1){Stirrer.sp=Stirrer.sp+50}
IF(pO2.v>pO2.sp+2 AND Air_Flow.sp<10){Stirrer.sp=Stirrer.sp-50}
IF(Stirrer.sp>1150){Stirrer.sp=1150}
IF(Stirrer.sp<800){Stirrer.sp=800}

IF(pO2.v<pO2.sp-0.1 AND Stirrer.sp>1145){Air_Flow.sp=Air_Flow.sp+0.5}
IF(pO2.v>pO2.sp+2 AND Gas_Mix.sp<1){Air_Flow.sp=Air_Flow.sp-0.2}
IF(Air_Flow.sp>10){Air_Flow.sp=10}
IF(Air_Flow.sp<5){Air_Flow.sp=5}

IF(pO2.v<pO2.sp-0.1 AND Stirrer.sp>1149 AND Air_Flow.sp>9.9 ){Gas_Mix.sp=Gas_Mix.sp+1}
IF(pO2.v>pO2.sp+2 AND Air_Flow.sp<10.5){Gas_Mix.sp=Gas_Mix.sp-1}
IF(Gas_Mix.sp>50){Gas_Mix.sp=50}
IF(Gas_Mix.sp<0){Gas_Mix.sp=0}
```

Figure 4.4 Sequence of aeration control used in fed-batch fermentations (sp = set point)

Lack of dissolved oxygen in the medium (below 10 %) was first regulated by increasing the impeller speed. When the maximum of 1150 rpm was reached, more air was added into the medium (parameter Air_Flow). When the maximum air flow of 10 NL/min was reached, pure oxygen was added into the medium (parameter Gas_Mix) (Figure 4.4). The parameters controlled are listed in Table 4.3. Fed-batch fermentation lasted approx. 20 hours.

Table 4.3 Controlled parameters during the fed-batch fermentation of *S. cerevisiase*

Parameter	Unit	Explanation	
Temperature	°C	-	
pH	-	-	
Feed	%	molasses addition	% are given as measurement of pump´s rotation speed
Feed2	%	10 % ammonia addition	
Antifoam	%	% are given as measurement of pump´s rotation speed	
EtOH	%	ethanol measurement	
pO_2	%	-	
Stirrer	rpm	-	
Air_Flow	NL/min	-	
Gas_Mix	%	This parameter controls the opening and closing the valve which regulates the dosage of oxygen.	

Baker´s yeast produced in pilot-scale at the Faculty of applied natural sciences was investigated by the company Uniferm GmbH & Co. KG in order to confirm the quality of the produced yeast (measured parameters: dry matter, protein content and fermentative capacity). It proved to be very similar to the company's baker´s yeast, which was a confirmation of scaling-up and process validation of fed-batch fermentation.

4.3.3.2 Fed-batch fermentation

Fed-batch fermentation was performed as described in chapter 4.3.3.1. Dosage profiles (molasses mixture and ammonia solution), pH profile, temperature profile and aeration profile are similar to an industrial process and enable maximum yield of yeast with high fermentative capacity and long storage stability. During the fermentation 3,876 g of molasses medium and 503 g of 10 % ammonium solution were pumped into the bioreactor. Samples were taken after 4, 6, 16, 18 and 20 hours, centrifuged for 10 minutes at 6,000 rpm, washed with 0.9 % sodium chloride (for dry yeast biomass determination) or demineralized water (for fermentative capacity determination) and centrifuged one more time. The concentration of dry yeast biomass was determined via vacuum furnace at 105 °C for 24 hours. Fermentative capacity was calculated from the increase of ethanol concentration (Ethanol UV Method, R-Biopharm; Darmstadt, Germany) in two different synthetic doughs (LSSD and HSSD) according to the method proposed by Bell et al., (2001).

4.3.4 Cross-flow microfiltration and ultrafiltration

All cross-flow ultrafiltration cassettes used in this work had the same dimensions and an effective filtration area of 0.02 m². Each filtration was performed individually in a batch mode by applying a transmembrane pressure of 0.9 bar ($\Delta P_{membrane}$). In a batch configuration, the feed/retentate is recirculated through the filtration module (Figure 4.5) with a recycling flow rate of 0.7 – 0.8 L/min. For this purpose, a self-priming diaphragm pump (FLOJET-D49X003B) was used in this work. The transmembrane pressure was set by the throttle valve V-1. The recycling flow rate was not measured during the ultrafiltration trials in order to keep the dynamic of the filtration undisturbed. Prior to filtration trials and after membrane cleaning, the water flux of each cassette was measured with deionized water by applying a transmembrane pressure drop of 1.5 bar. Membrane cleaning was achieved by rinsing the corresponding cassette for 15 min with deionized water in a single pass mode (transmembrane pressure drop was set to 1.5 bar). In this regard, 1,220 g of vinasse was treated by a first ultrafiltration (UF30) using the ultrafiltration cassette with a nominal cut off of 30 kDa (Table 4.4). The retentate (UF30-R) was immediately analysed. The permeate (UF30-P) was further processed by means of a second ultrafiltration (UF2). For this step, the ultrafiltration cassette

with a cut off of 2 kDa was employed. The retentate (UF2-R) was again analysed and the permeate (UF2-P) was then subject to evaporation.

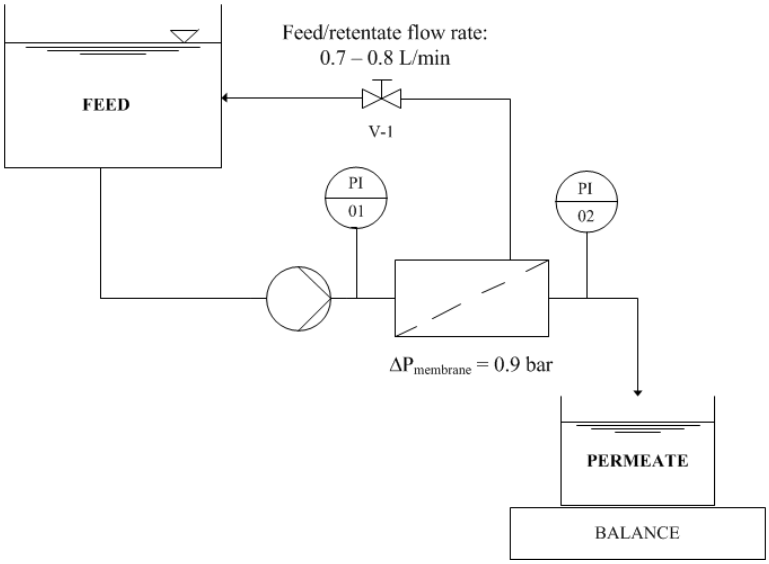

Figure 4.5 Batch configuration of ultrafiltration mode used in this research (Lisičar Vukušić et al., 2018)

Table 4.4 Mass balance of fractions obtained via microfiltration and ultrafiltrations

Step	Fraction		Mass [g]
UF30	Feed	Vinasse	1,220.0
	Retentate	UF30-R	190.0
	Permeate	UF30-P	1,010.0
UF2	Feed	UF30-P	950.0
	Retentate	UF2-R	200.0
	Permeate	UF2-P	690.0
EV	Feed	UF2-P	669.4
	Water	EV-W	606.0
	Concentrate	EV-C	62.6
CF	Feed	EV-C	62.6
	Pellet	CF-P	11.0
	Supernatant	CF-S	49.0
AD	Feed	CF-S	30.0
	Product	AD-P	20.0

4.3.5 Evaporation – centrifugation

The evaporation of UF2-P was conducted in rotary evaporator. 669.4 g of UF2-P was subject to evaporation for 54 minutes at a temperature of 78 °C, an absolute pressure of 130 mbar and rotation speed of 100 rpm. Finally 62.6 g of concentrate (fraction EV-C) were gained and further centrifuged at 4,000 rpm for 10 minutes resulting in 49 g of supernatant (CF-S) and 11 g of sediment (pellets) (CF-P).

4.3.6 Adsorption

25 g of Dowex Monosphere MR-450 UPW were added to 30 g of concentrated supernatant (CF-S) (pH value was preliminary adjusted to 7 with 2.5 M potassium hydroxide) in Falcon™ Tube and manually stirred for 1 minute. The suspension was allowed to phase separate for 1 hour and then centrifuged for 10 min at 4,000. The supernatant (AD-P) was finally poured out of the Falcon™ Tube and collected in a sample vessel.

4.3.7 Analyses of membrane processed concentrates

Following analyses were performed the same way as in the chapter 3.3:

- determination of ash content
- determination of protein concentration
- determination of amino acid content
- determination of invertase activity

4.3.7.1 Determination of betaine concentration

The betaine concentration was determined by the method of Chastellain and Hirsbrunner (1976) with minor modifications. 0.3 mL of raw sample was mixed with 0.3 mL of internal standard tert-butylamine hydrochloride (10 g/L) and 0.1 mL of deuterium oxide. The samples were analysed via ^{1}H-NMR spectroscopy. The betaine concentration was calculated according to the formula (3).

4.3.7.2 Determination of dry matter

Determination of dry matter was determined in the same way as in chapter 3.3.4, only 2 mL of the sample was pipetted, weighted and dried.

4.4 Results and discussion

4.4.1 Fed-batch cultivation and vinasse production

9.011 kg of fermentation broth was harvested after 20 hours. The concentration of dry yeast biomass reached 73.5 g/L, which is significantly higher than found in literature (Vieira et al., 2013; Zamani et al., 2008) and also higher than the values reported during industrial baker´s yeast fermentation (George et al., 1998). Fermentative capacity (Figure 4.6) was determined one day after cell harvest (yeast samples were kept cooled at 4 °C) and results indicate a good fermentative capacity in LSSD medium (2.431 $mmol_{Ethanol}/(g_{Yeast} \cdot h)$) and a lower but still satisfying fermentative capacity was measured in HSSD medium (2.181 $mmol_{Ethanol}/(g_{Yeast} \cdot h)$) which is known to be caused by overexpression of invertase and intrinsic osmotolerance triggered by high sugar environment (Bell et al., 2001). A considerable invertase activity of 383.3 U/mL was found in the fermentation broth and a much lower value was measured in vinasse (39.5 U/mL). This finding may be explained by the entrapment of the heavily glycosylated external invertase in the cell wall. As expected, an appreciable amount of betaine of 0.0181 g/g was measured in vinasse.

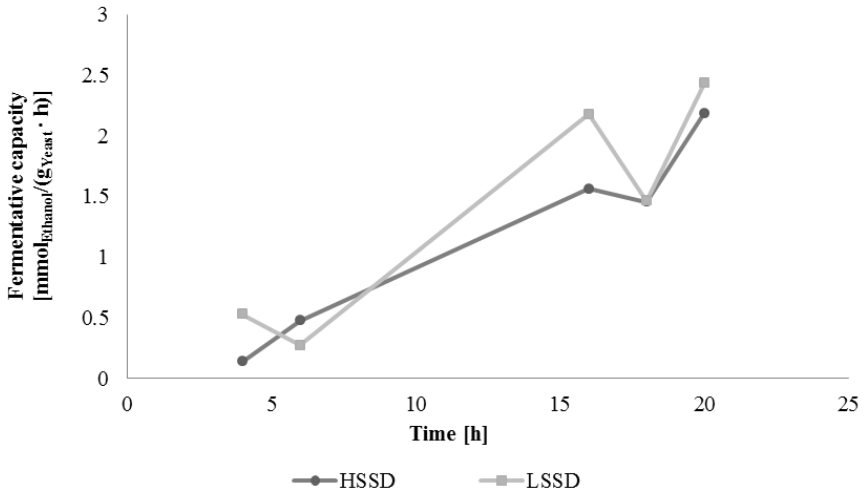

Figure 4.6 Fermentative capacity measured in two synthetic doughs

4.4.2 Purification via cross-flow ultrafiltration

The permeate flux from the ultrafiltration of vinasse (Figure 4.7) was nearly constant and corresponds to 75-90 % of the water flux measured with this module (2.22 kg/(bar · m^2 · min)). The continuous decline of permeate flux (UF30-P) may result from the progressive formation of a polarisation layer during the filtration. After membrane cleaning with deionized water, 98.2 % of the water flux was recovered indicating that no irreversible membrane fouling occurred during the filtration. An activity of 117.12 U/mL was measured in the corresponding retentate (UF30-R) and a low activity (0.0996 U/mL) was measured in the corresponding permeate (UF30-P) due to the retention and concentration of the invertase (270 kDa) by the 30 kDa membrane. The specific invertase activity increased from 2.23 to 7.24 U/mg$_{Protein}$. Overall, ultrafiltration seems to be an appropriate technique for the recovery and concentration of invertase from baker's yeast vinasse but the achieved specific activity is too low for an industrial-strength application and this fraction has to be further purified.

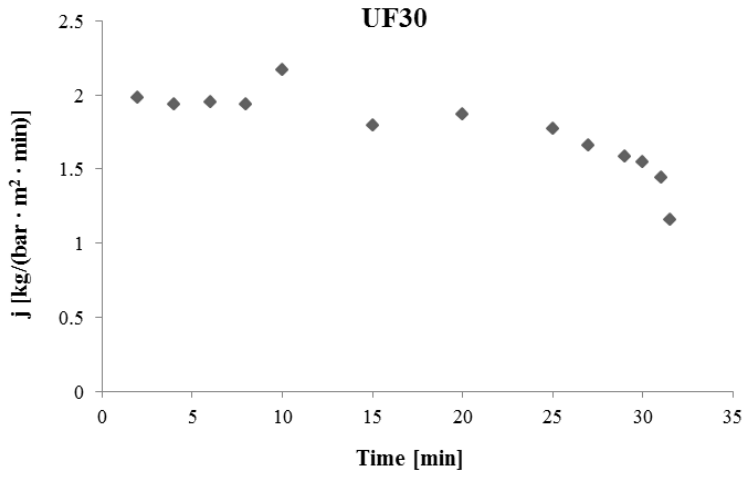

Figure 4.7 Permeability of the membrane used in the first ultrafiltration step (UF30) (Lisičar Vukušić et al., 2018)

During the second ultrafiltration step, which was operated with a 2 kDa module, the permeate flux was 10-25 % lower than the corresponding water flux (0.16 kg/(bar · m^2 · min)) (Figure 4.8). The progressive formation of a polarization layer was also observed during this filtration first 20 min of the filtration leading to a steep decrease of the permeate flux. 97.1 % of the water flux was recovered after membrane cleaning and allows us to conclude that no irreversible membrane fouling occurred during this second filtration step. This resulting retentate (UF2-R) has a very interesting composition. Based on D.S., it consists of 16.3 % of betaine, 44.7 % proteins and 16.4 % of ash. This betaine rich protein fraction has a high potential as functional food ingredient. Since it is originated from plant raw materials (sugar beet) and no genetically organisms are involved in the production, producer should not expect to face critical acceptance issues. Protein concentrates usually exhibit high ash contents due to the coordination of minerals on the surface of proteins (Brasileiro et al., 2012; Quiquampoix et al., 2007). This phenomenon also explains the lower ash contents found in permeates compared to their respective retentates. The concentration and purification of this fraction could be achieved by evaporation and isoelectric precipitation.

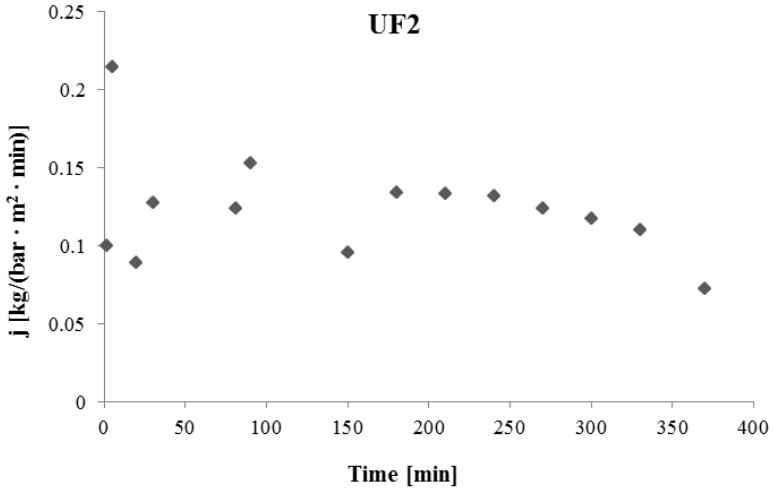

Figure 4.8 Permeability of the membrane used in the second ultrafiltration step (UF2) (Lisičar Vukušić et al., 2018)

4.4.3 Purification and concentration of betaine

Evaporation with subsequent centrifugation is an usual sequence of unit operations in baker's yeast production plants which is used for vinasse concentration before its usage as feed or fertilizer. The same concept was used in this study; UF2-P was treated via evaporation in order to induce the precipitation of minerals and deashing. The resulting concentrate was treated by centrifugation leading to two fractions (salt mixture CF-P and a liquid fraction CF-S). As expected, high ash content was found in CF-P (0.714 g/g). In CF-S, betaine concentration of 188 mg/g was measured, which is more than 10 times higher than measured in fractions before evaporation (Table 4.5). This fraction contained 9.2 g of betaine with a purity of 45.8 % in D.S.

Table 4.5 Compositions of the fraction obtained by cross-flow filtration

Fraction	Dry matter [mg/g]	Ash content [mg/g]	Protein content [mg/g]	Betaine		
				Concentration [mg/g]	Purity [% in D.S.]	Recovery [%]
Vinasse	80.6 ± 0.1	21.78 ± 0.03	17.5 ± 0.4	18.1 ± 0.9	22.6	100
UF30-R	76.5 ± 4.4	20.6 ± 0.6	16.1 ± 0.7	14.9 ± 0.7	19.4	12.8
UF30-P	83.0 ± 0.4	14.6 ± 0.9	15.4 ± 1.1	15.8 ± 0.8	19.0	72.3
UF2-R	75.6 ± 3.7	12.5 ± 0.6	34.0± 0.9	12.4 ± 0.6	16.3	11.2
UF2-P	85.8 ± 3.3	16.9 ± 0.6	7.3 ± 0.5	14.7 ± 0.7	17.1	45.9
CF-S	409.9 ± 4.4	94.5 ± 1.3	n.d.	188.0 ± 8.9	45.8	41.7
CF-P	760.2 ± 33.7	713.9 ± 53.7	n.d.	-	-	-
AD-P	220.0 ± 1.2	39.7 ± 1.8	n.d.	165.9 ± 1.0	75.5	24.6*

*** =** considering the full processing of the supernatant obtained after centrifugation
n. d. = not detectable

Finally, CF-S was neutralized and mixed with a mixed bed ion exchange resin. At neutral pH value, betaine is a zwitterion; it cannot be adsorbed by the resin and was recovered from the fraction collected after the adsorption step (AD-P). Overall, 5.43 g of betaine with a purity of 75.5 % in D.S. can be recovered from 1.22 kg of vinasse. Amino acid content (Table 4.6) measured in UF2-P and AD-P demonstrates the efficient purification achieved with the final adsorption step. The overall recovery rate for betaine is 24.6 %. It is lower than the reported value obtained with the NS2P process and mostly relies on the simultaneous recovery of four different product fractions (invertase, food grade proteins, vinasse salt mixture, liquid vinasse and betaine) as well as its very simple implementation in an existing baker's yeast production plant. Nevertheless, it is still acceptable regarding the large volumes to be daily treated (approx. 300 t/day for a baker's yeast production plant).

Table 4.6 Distribution of amino acids in permeate of the second ultrafiltration step (UF2-P) and in the fraction after adsorption (AD-P)

Amino acid	UF2-P [mg/g]	AD-P [mg/g]
Alanine	0.090	0.077
Arginine	0.288	0.036
Asparagine	0.083	0.074
Aspartic acid	0.362	0.304
Cysteine	0	0
Glutamine	0.017	0.020
Glutamic acid	0.478	0.387
Glycine	0.017	0.019
Histidine	0.061	0.027
Isoleucine	0.039	0.025
Leucine	0.026	0.017
Lysine	0.011	0.004
Methionine	0.003	0.003
Phenylalanine	0.012	0.010
Proline	0.059	0.017
Serine	0.028	0.022
Threonine	0.003	0.010
Tryptophan	0.175	0.058
Tyrosine	0.220	0.120
Valine	0.051	0.032
Total	**2.023**	**1.263**

4.4.4 Upscaling and implementation of the novel downstream processing in a baker´s yeast producing plant

A large-scale mass balance was calculated by considering a yearly production of baker´s yeast of 50,000 t (Figure 4.9), the resulting amount of vinasse that needs to be processed is 110,000 t/year or 209.3 kg/min. Vinasse would be obtained by using the existing separators. Since enzymatic activity is generally not desired in food grade proteins, a 30 kDa UF-system is necessary to separate invertase from the remaining proteins. The retentate (UF30-R) has to be further purified (e.g. chromatography) in order to produce an invertase rich fraction with a specific activity > 20 U/mg. 176.7 kg/min of permeate UF30 enters a 2 kDa UF-system. This step provides a betaine rich protein fraction (UF2-R) which could be concentrated by evaporation (using the existing evaporation station) and further purified by precipitation. UF2-P is processed in the existing evaporation – centrifugation station leading to a stream of salt mixture (CF-P) and a concentrate stream (CF-S 10.21 kg/min). Due to the abundant inorganic content, CF-P could be further used as field fertilizer. The final step of the downstream processing is a deashing step via ion exchange that leads to the production of natural betaine with a purity of 75.5 % (based on D.S.). The corresponding adsorption station embodies a tandem ion exchange adsorption system as described by Barbe et al. (2006). It consists of parallel connected anion and cation exchange adsorption beds working in alternating modes adsorption and regeneration (one is in adsorbing mode while the other is being regenerated).

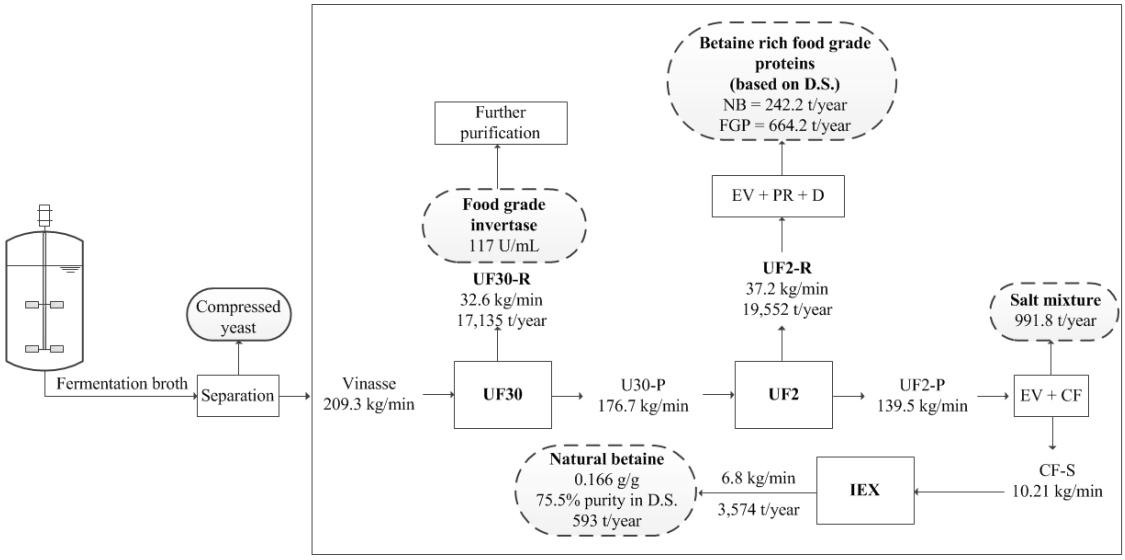

Figure 4.9 Upscaling of the pilot process and mass balance expectations for the simultaneous recovery of betaine and food grade proteins (Lisičar Vukušić et al., 2018)

4.5 Conclusion

The fractionation of baker´s yeast vinasse to recover a betaine rich food protein fraction, betaine and fertilizer is technically feasible. The strong entrapment of the external invertase in baker's yeast cell wall was confirmed. Consequently, a very low invertase activity was found in vinasse (39.5 U/mL) and the corresponding specific activity could not be sufficiently increased via ultrafiltration. The benefit of betaine in human and animal nutrition has been extensively reported in the last decade. These findings open very interesting opportunities for the mentioned betaine rich protein fraction as functional food ingredient and nothing comparable could be found on the market. Finally, a betaine concentrate was obtained with a concentration of 166 mg/g, a purity of 75.5 % and a recovery yield of 24.6 %. Considering a baker's yeast plant with a yearly production of 50,000 tonnes of compressed baker´s yeast on dry matter, the recovery of 1,486 t/year of betaine rich food proteins and 593 t/year Betafin® similar natural betaine could be achieved by implementing an ultrafiltration plant and an adsorption system.

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5 Conversion of waste heat from the industrial baker´s yeast production into 4th generation district heat

5.1 Objectives

The industrial aerobic bioprocess of baker´s yeast production requires large amounts of water to cool down large bubble column bioreactors. As result an appreciable quantity of low grade heat is generated. Because of the low temperature level of the water (approx. 25 °C) exiting the bioreactors cooling system, very little attention has been dedicated to heat recovery and conversion from this stream. However if it is redirected into state-of-the-art heat pump system, 4th generation of district heat which employs low-temperature water (30-70 °C) as heat carrier can be generated. In this chapter the conversion of low grade heat, from an industrial baker´s yeast production plant, into 4th generation district heat with an appropriate heat pump system is studied. The key point of this work focused on large industrial aerobic bioprocess as well as the production scheduling and real time fluctuations which occur in every manufacture. Therefore, the following steps have been performed:

1) fed-batch fermentation of baker´s yeast at pilot scale bioreactor
2) upscaling to an industrial baker´s yeast production plant consisted of seven 150 m^3 bioreactors
3) simulation of the conversion of low grade heat into 4th generation district heat with an appropriate heat pump system
4) heat pump dimensioning as well as cost evaluation of the corresponding equipment
5) alternative concepts for the energy storage

The implemented district heat generation is based on the system developed at Jästbolaget AB, in which the cooling cycle of the bioreactor system is coupled with the heating cycle of the heat pump to generate 4th generation district heat. However, it could be also implemented in other large industrial production processes (e. g. citric acid) (Lisičar Vukušić et al., 2018).

5.2 Theoretical background

High energy prices, limiting fossil fuels supplies and increasing concern regarding environmental consequences of fossil fuels have driven interest in biofuels. However alternative fuels should not only have the environmental benefits over the traditional fuels but should also be economically competitive with it (Hill et al., 2006). One of the most prominent renewable energy resources is biodiesel, which is produced from renewable biomass by transesterification of triacylglycerols. In its production various renewable lipids have been used, including vegetable oils, animal fats and wasting oils. However, the economic restriction lies in the cost of the oil raw materials (Meng et al., 2009). That is why the interest in the production of microbial lipids has been raised. Oleaginous yeast *Trichosporon oleaginosus* has the ability to accumulate up to 60 % of dry cell weight with the concentration on lipids of about 70 g/L. In order to lower the costs of the biodiesel production, raw materials need to be replaced with low-cost substrates. Regarding this, the cultivation of the mentioned yeast was conducted to produce lipids on the enzymatic corn cobs hydrolysates by two bioprocess configurations: separate hydrolysis and lipid production and simultaneous saccharification and lipid production. The latter process proved to have a great potential of the production of microbial lipids from renewable lignocellulosic biomass on the industrial scale, which can be used as a feedstock for biodiesel production (Ivančić Šantek et al., 2018). In this process as well as in the production of the ethanol, microorganism and its metabolites are used for the production of biofuels, contributing to the sustainable development of the renewable energy. In this chapter a different approach is presented, in which an aerobic process is presented as the tool for generating energy.

Aerobic baker's yeast growth is a strong exothermic process which leads to the release of an appreciable quantity of low grade heat. Under fully aerobic conditions, 18.4 kJ of heat is being produced per gram of solids of baker´s yeast (Reed, 1991). Therefore large amounts of water are required to cool down the corresponding bioreactors. Because of the low temperature level of the water (approx. 25 °C) exiting the bioreactors cooling system, it is usually released in rivers, streams and canals (Lisičar et al, 2017). However, redirecting this stream into heat pump system would generate large amounts of water with a higher temperature level (e.g. 70 °C) (Lisicar et al, 2018a).

Heat pumps employ a refrigeration cycle to transfer heat, in which a refrigerant (known as the "working fluid") is compressed (as a liquid) then expanded (as a vapour) to absorb and remove heat (Hepbasli et al., 2008). The interest in heat pumps as means of energy recovery is steadily growing due to the increase in fuel costs as well as the concerns regarding global warming. It is the only known process that recirculates waste heat back into a production process, providing energy efficient and environmentally friendly heating and cooling for process industries as well as in domestic and commercial buildings (Chua et al., 2010). Industrial heat pumps were first installed in late 1970s and early 1980s. They often used the chlorofluorocarbon (CFC) refrigerant R114, because of its advantageous properties (high critical temperature and a low pressure level). An important disadvantage of this refrigerant is its harmfulness to the earth's ozone layer. With the ratification of the Montreal protocol enacted in 1987 CFC and hydrochlorofluorocarbon (HCFC) refrigerants have been banned in Germany since 1996. The requirements for an adequate refrigerant are low global warming potential (GWP) as well as harmless and non-flammable properties (Wolf et al., 2012). Carbon dioxide is one of the few natural refrigerants, which is neither toxic nor flammable. It is widely available, inexpensive and does not affect the global environment as many other refrigerants. GWP of HCFC is over 1000, while CO_2 has GWP of 1. However, net global warming impact when used as a technical gas is zero, since the gas is the waste product from industrial production (Nekså, 2002). Properties that make this refrigerant unique are low critical temperature and high working pressure required to use CO_2 under typical heat pump conditions (Austin et al., 2011). Critical point, at which gas can no longer be distinguished from liquid, for CO_2 is at 31.1 °C and 73.7 bar. In a conventional pump, the refrigerant operates below its critical point, hence the name subcritical cycle (Bensafi et al., 2007). At low pressure, heat absorption occurs by evaporation and heat rejection takes place by condensation after compression. In a transcritical cycle, an evaporator also serves the heat absorption function, but heat rejection occurs via gas cooling in the supercritical domain (Nekså, 2002; Austin et al., 2011).

Low temperature water (70-80 °C) from heat pump systems allows generating 4^{th} generation district heat, which is planned for the period of 2020-2050. According to Lund et al., the future conditions for heat demand and supply will change. Heat demands are expected to be lower within new built buildings. Lower heat demands in buildings involve major

opportunities for low temperature district heating systems to take an important role in future energy system, in which smart integration between district heating, electricity sector and transport sector is realized (Lund et al., 2014). Expected temperatures of this generation are 50-55 °C for supply and 30 °C at the highest for the return. The goal is to provide the consumers with 50 °C district heating (Kamal, 2017). Some of the challenges this generation is facing, in order to fulfil its role in future sustainable energy systems, are ability to supply low-temperature district heating for space and water heating to existing buildings as well as to energy efficient buildings and to distribute heat in networks with low grid losses (Figure 5.1). 4th generation district heat needs to be an integrated part of smart energy systems, in which smart electricity, thermal and gas grids are combined and coordinated in order to achieve an optimal solution for each and every individual segment as well as for the overall energy system (Lund et al., 2014).

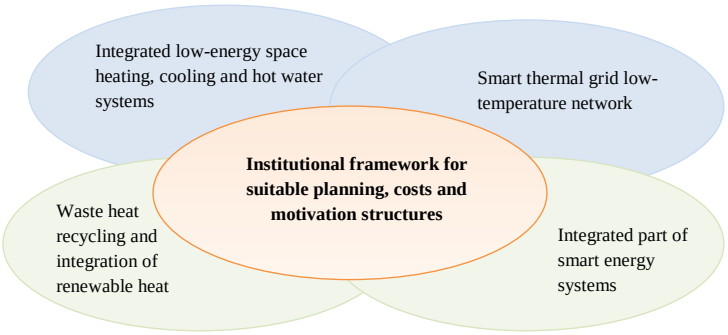

Figure 5.1 Illustration of the concept of 4th generation district heating including smart thermal grids (Lund et al., 2014)

The only known example of converting low grade heat from industrial bioprocess into 4th generation district heat can be found in Swedish baker´s yeast producing company Jästbolaget, where the system of heat converters and heat exchangers was already introduced in the late 1980s. By 1992, a large heat converter was installed for recovering of all the heat generated in the fermentation process. The heat at a specific temperature of 70 °C (personal communication with Petter Fetz, Jästbolaget AB, April 2018) is transferred to the municipal district-heating network, providing 30 GWh of energy to about 1,500 homes (Lisičar et al., 2017). However, very little reliable data regarding conversion of waste heat from an industrial bioprocess into district heat can be found in academic literature (Franzén, 2014).

5.3 Materials and methods

5.3.1 Fed-batch cultivation

Baker's yeast growth was investigated during a fed-batch fermentation (similar to an industrial process) performed in a pilot scale bioreactor. The resulting data combined with data from the literature (Bjarre, 2016; Franzén, 2014) have been subsequently used for the simulation and the up-scaling process.

Chemicals and equipment used were the same as in the chapter 4.3.1. Fed-batch fermentation was performed as described in the chapter 4.3.3.1. Samples were taken and centrifuged for 10 minutes at 6,000 rpm and washed twice with 0.9 % sodium chloride. The concentration of dry yeast biomass was determined via vacuum furnace at 105 °C for 24 hours.

5.3.2 Process simulation

The simulation was conducted by means of CoolPack Software package developed at Technical University of Denmark. It is freeware and a user-friendly interface consisting of a collection of programs used for analysing, designing, dimensioning and optimizing refrigeration systems (Jakobsen et al., 2001; Jakobson et al., 1999), which can be downloaded from http://www.en.ipu.dk/Indhold/refrigeration-and-energy-technology/coolpack.aspx. This package has been successfully used for the design of subcritical and transcritical CO_2 cycles (refrigeration and heat pumps) and their investigation (Almeida et al., 2011; Campbell et al., 2007; Alves-Filho, 2015).

5.4 Results and discussion

5.4.1 Laboratory scale fed-batch fermentation of baker´s yeast

Fed-batch fermentation lasted approx. one day (the exact time cannot be specified due to confidentiality reasons). Produced baker´s yeast (845 g on DM (Figure 5.2)) harvested at the end of the fed-batch fermentation was significantly higher than found in literature (Vieira et al., 2013; Zamani et al., 2008). At the end of the fermentation, the dosage of the substrates

was stopped in order to enable yeast to accumulate intracellularly energy-storing carbohydrates trehalose and glycogen (Lillie et al., 1980). This led to a slower yeast growth at the end of the fermentation.

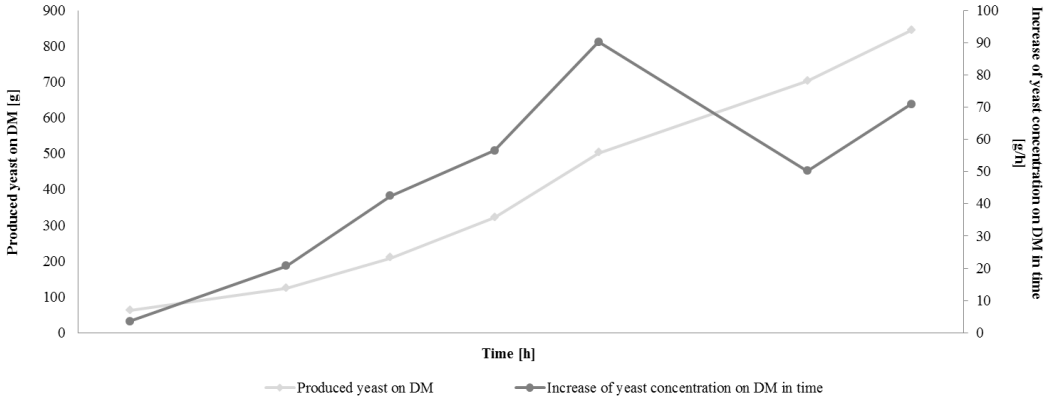

Figure 5.2 Increase of yeast concentration on dry matter in 15 L bioreactor during the fed-batch fermentation of baker's yeast (Lisičar Vukušić et al., 2018)

The production of one gram cell solids evolves 18.4 kJ of heat (Reed et al., 1991). The above mentioned experimental data regarding the evolution of yeast concentration during the fermentation was used to calculate the heat released. The corresponding results are shown in Figure 5.3.

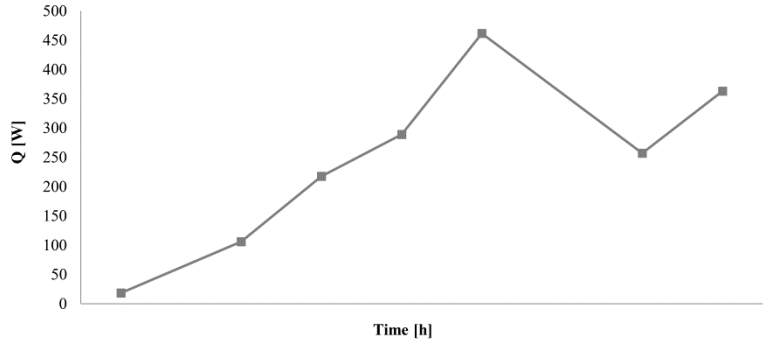

Figure 5.3 Heat release in 15 L bioreactor during the fed-batch fermentation of baker's yeast (Lisičar Vukušić et al., 2018)

As expected, heat generation during the process is coupled to yeast growth. Heat release reached its maximum in the 14th hour (461 W), when yeast is highly active and is multiplying the fastest.

5.4.2 Simulation of large-scale industrial baker´s yeast production

The baker´s yeast plant considered in this case study is presented in Figure 5.4. Molasses, as a carbon source, is diluted, clarified and steam sterilised. Micro- and macro-elements are added to the molasses in the form of salts as well as vitamins. Molasses and ammonia are pumped into bioreactors according to a specific dosage profile. Final fed-batch fermentations are performed in 150 m^3 bubble column bioreactors (15.4 m high and 3.52 m diameter), which are being constantly cooled down by using cold water from the wells. The fermentation broth is then processed by means of separators and rotary vacuum filters, resulting in compressed baker´s yeast (30 % of dry matter) and vinasse. For the sake of simplicity, cultivations of yeast in the laboratory, propagation in larger vessels and inoculation process as well as the aeration and cooling process are not depicted.

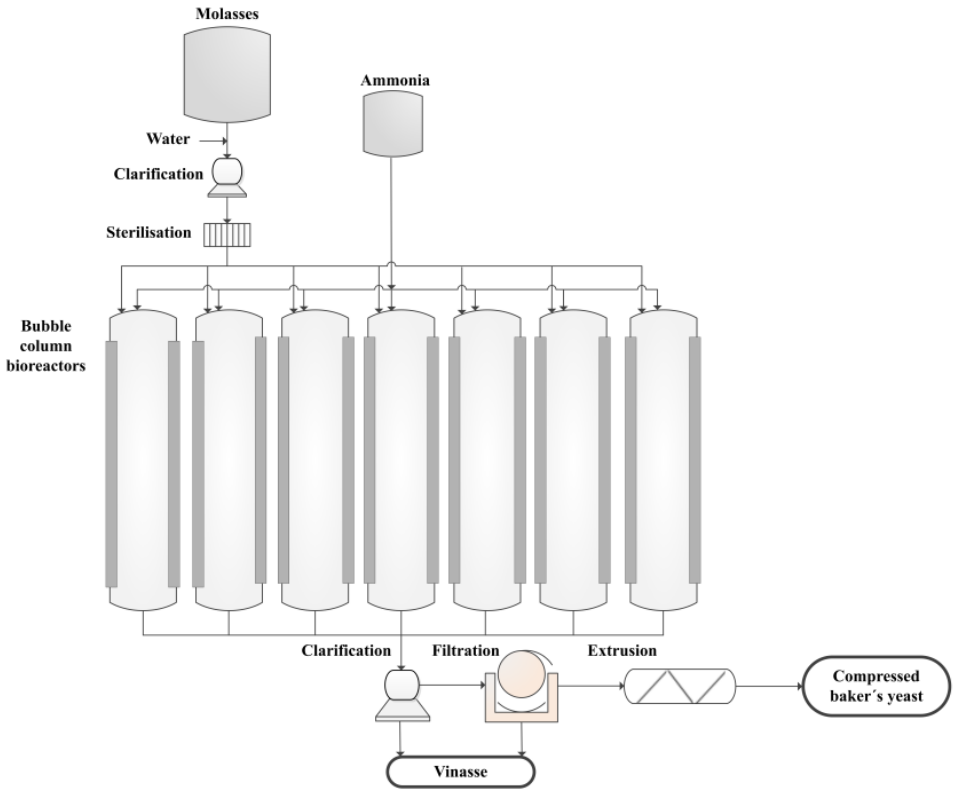

Figure 5.4 Scheme of baker´s yeast plant used for the simulation (Lisičar Vukušić et al., 2018)

In an industrial setting, usually five bioreactors are occupied simultaneously, while the other two are in the phase of sterilisation or downstream processing (Figure 5.5). The heat released in one 150 m³ during the fermentation is shown in Figure 5.6. Five hours are needed for downstream processes and the preparation for the next fed-batch fermentation. 350 fermentations are performed in one bioreactor per year. According to the experimental data, this plant would produce 20,850 tonnes of compressed baker´s yeast (in dry matter) in all seven bioreactors per year.

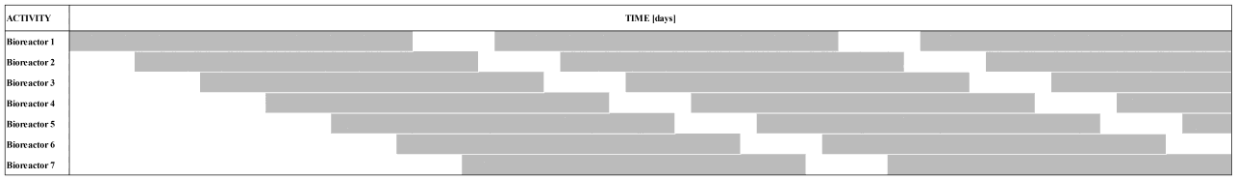

Figure 5.5 Gantt chart for the production scheduling in baker´s yeast plant (Lisičar Vukušić et al., 2018)

In 150 m^3 bioreactor the released heat goes up to 4.4 MW (Figure 5.6).

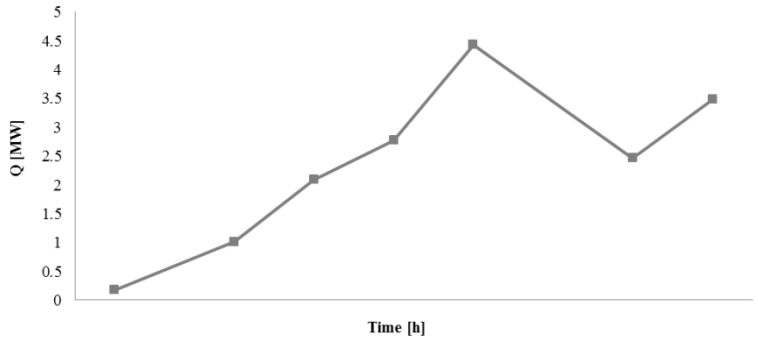

Figure 5.6 Heat released during the fed-batch fermentation in one 150 m^3 bioreactor (upscaling from laboratory data) (Lisičar Vukušić et al., 2018)

The total heat release has been stimulated in seven 150 m^3 industrial bioreactors (Figure 5.7). Our calculation predicts a fluctuating value between 8 and 14 MW with an average value of 11 MW. This actually represents the energy produced by 2 to 3 wind turbines and is enough to supply approx. 19,090 people with hot water and room heating (Lisicar et al, 2017). The mean value of 11 MW has been taken into consideration for the further analysis and simulation.

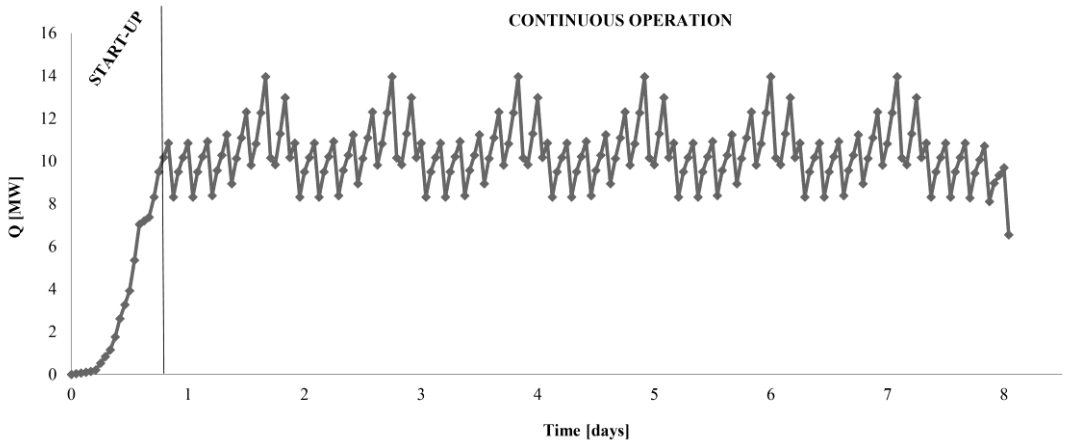

Figure 5.7 Total energy release over time in seven 150 m^3 industrial bioreactors (Lisičar Vukušić et al., 2018)

5.4.3 Design of a transcritical CO_2 heat pump system

The corresponding heat pump system mainly consists of an evaporator, a compressor, a gas cooler and an expansion device (Figure 5.8). Heat source for the heat pump is the returning water from the process with a temperature of 25 °C. It exits the cooling system of the seven bioreactors of the baker´s yeast production plant. It serves as carrier of the heat obtained in fed-batch fermentations (in our case an average value of 11,000 kW). The CoolPack software package (Technical University of Denmark) was used for the design of the heat pump system. Core part of this system is the compressor which has to be electrically powered with 5,743.5 kW. The heat pump uses a refrigerant cycle to extract heat from the cooling water and transfer it to a second water cycle with a higher temperature level which can be used as 4^{th} generation district heat for space and water heating in households. It supplies 16,184 kW by means of water with a temperature of 70 °C. The cold water, with the temperature of 14 °C exiting the heat pump, is then directed to the cooling system of the bioreactors and the cycle is repeated. Efficiency of the system (coefficient of performance – COP) is given as ratio between the heating capacity and the power input and in this case study is 2.9.

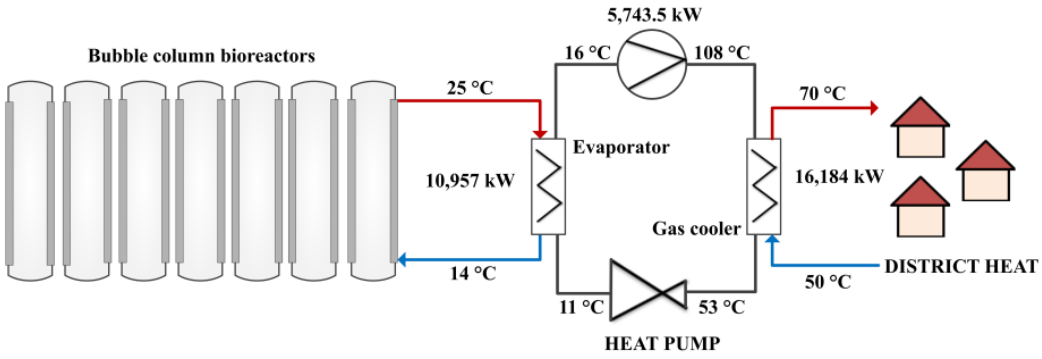

Figure 5.8 Schematic description of transcritical heat pump with natural refrigerant CO_2 (Lisičar Vukušić et al., 2018)

The corresponding transcritical refrigeration cycle is depicted in Figure 5.9. CO_2 evaporation takes place at 11 °C (46.15 bar) and the corresponding vapour is overheated to 16 °C. After compression, the supercritical CO_2 reaches a temperature of 108 °C (142.2 bar). It is then subjected to isobaric cooling down to a temperature of 53 °C and reinjected into the evaporator after expansion. The most relevant design parameters for the considered heat pump system are summarized in Table 5.1.

Table 5.1 Design parameters for simulating the cooling systems of the heat pump

Parameters	
CO_2-mass flow	103.76 kg/s
Isotropic efficiency	0.85
Compressor heat loss	10 % (574.35 kW)
Suction line: flow velocity	10 m/s
Suction line diameter	322.1 mm
Discharge line: flow velocity	12 m/s
Discharge line diameter	195.9 mm
Liquid line: flow velocity	5 m/s
Liquid line diameter	201.8 mm

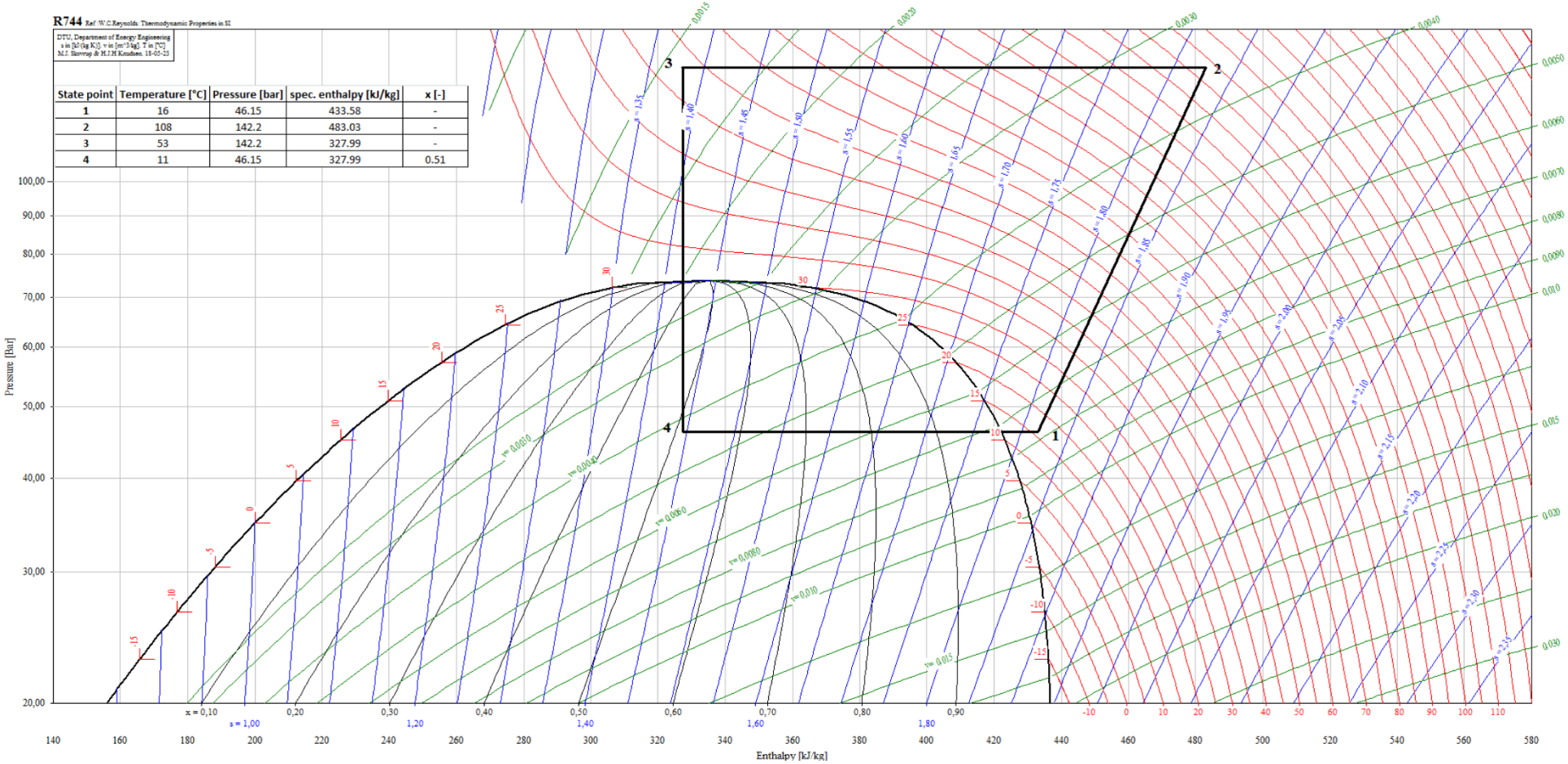

Figure 5.9 Refrigeration cycle of the designed CO_2 transcritical heat pump obtained after simulation by using the CoolPack Software package (Technical University of Denmark) (Lisičar Vukušić et al., 2018)

5.4.4 Cost estimation

Cost estimation is a crucial step for the investigation of the feasibility of the presented approach. According to current hardware prices, the capital costs for the proposed transcritical heat pump would be approx. 4.47 million €. Additional costs for planning installation, equipment and materials (fittings and pipes), would be approx. 813.040 €. This heat pump requires extra electricity consumption of approx. 35,314.2 MWh/year, which leads to the additional cost of 2.83 million € (considering the price of 0.08 €/kWh) (Statista, 2018). The produced amount of energy by this heat pump system is 141,771.84 MWh/year. Current price for 1 MWh of energy is 65.2 € (SWU, 2018), which leads to a potential yearly turnover of approx. 9.24 million €. When considering the investment that need to be made by the company and the gain of distributing the district heat, the payback period would be around 10 months.

5.4.5 Long-term energy storage during baker´s yeast production

Day–night or summer–winter time shift show different energy demands. For this purpose, thermal storage systems have been widely discussed (Agyenim et al., 2010; Alva et al., 2018; Sarbu et al., 2018). The main problem is the long-term energy storage which cannot be easily solved because of the huge storage capacity requirements. Short-term storage concepts are mainly based on phase-change materials to increase the energy-density (paraffin, salt hydrates), whereas paraffin has a much lower melting energy than salt hydrates. Some salt hydrates are not very stable concerning the number of melting-solidification cycles and they can also be corrosive. The energy density of phase change materials can be between 3 up to 100 times higher compared to water. One of the major challenges of short-term energy storage is the low heat conductivity of phase change materials (PCM). In order to increase the effective heat conductivity, open pored cellular metallic structures like metal foam can be used whereas the pores are filled with PCM. Consequently the effective heat conductivity can be increased from 0.23 W/(m · K) up to 7 W/(m · K). For fast charging and discharging operations the PCM-foam composite is absolutely necessary (Kneer et al., 2011). To be able to store huge amounts of energy, PCMs need huge installations and the systems have to be efficiently isolated. In the case of baker´s yeast plant, two PCM elements should be placed in a tandem connection (Figure 5.10). One unit is completely loaded with heat (using the

complete heat capacity) and the effluent is led back to the heat pump system. In the next step the first unit generates heat for the district heat network, while the second unit is loaded and the whole procedure is repeated (Lisicar et al., 2018b).

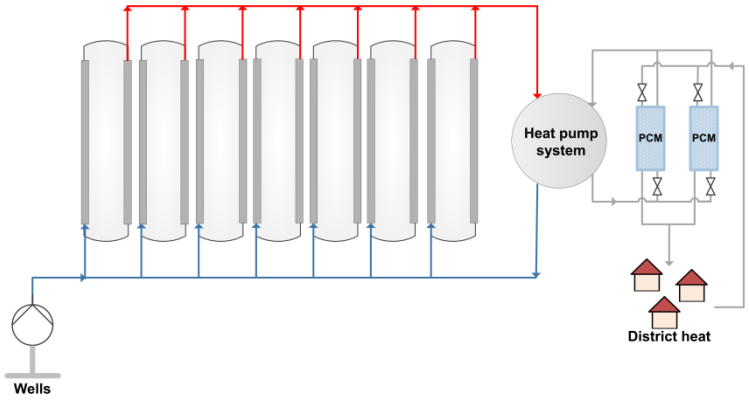

Figure 5.10 PCM and heat pump system integrated in baker´s yeast production plant (Lisičar Vukušić et al., 2018)

Interesting approach for long-term energy storage is the development of geological porous storage concepts like petrothermal systems. If the thermal conditions and the porosity allow pumping water into the permeable zones, it is possible to store energy from an industrial process in the underground during summer time and rejected during winter time. The capacity of such systems are much larger compared to phase change materials or adsorption systems. Another possibility of long term energy storage is the usage of underground pressurized gas tanks and if available high gas caverns (from salt production). This process however needs a transformation of the energy to a higher temperature level in order to produce electricity. This is only possible by using heat pumps and an evaporation and condensation process. Furthermore, the vapour must be at such conditions that the expansion within a turbine is possible. Installed huge gas tanks can be used to store compressed gas for later electricity production. Caverns may have a diameter of around 70 m and a height of 200 m (cavern Staßfurt (Warren, 2006)). The storage pressure is about 180 bar, therefore it can be used for long-term storage issues. However the exploration of cavern is very expensive so the usage of existing cavern is much more economical challenging.

5.5 Conclusion

The conversion of low grade heat from industrial baker's yeast production into 4th generation district heat is technically and economically feasible. The investigated process is able to generate up to 140 GWh and a potential turnover up to 9.2 million € in Germany. It would supply the needs of space heating and hot water of approximately 28,000 German citizens. Investment including the heat pump device and installation would cost a company roughly 5.3 million €. It is important to mention that additional energy input of 5.74 MW is required for operating the heat pump. All being considered, payback period is 10 months which suits very well with the investment practice in the industry (usual payback period is 1 to 2 years). Possible concepts of long-term energy storage have been presented. In this regard, PCM-metal foam composite seems to be an appropriate option. However, further research addressing size and capacity of these latent heat storage modules needs to be conducted.

5.6 References

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6 Reshaping apple juice production into biorefinery concept

6.1 Objectives

In the last decade, the utilization of waste by-product apple pomace has been extensively researched (due to its difficult disposal) and currently finds beneficial usage in various industries; as substrate for microbial growth or recovery of pectin, xyloglucan and polyphenols. The aim of this chapter is to demonstrate a zero discharge biorefinery process, in which waste from apple juice production is converted into three valuable products enabling connections between different industries. Following objectives have been accomplished:

1) production of apple juice at pilot scale (similar to industrial manufacture)
2) further processing of apple pomace into pectin, pellets and concentrated apple pomace extract
3) extensive mass and heat balance were conducted
4) fed-batch cultivation of baker´s yeast with apple pomace extract as alternative to molasses

6.2 Theoretical background

Apple is one of the most favoured and consumed fruits by mankind widely grown in temperate regions of the world (Shalini et al., 2010; Perussello et al., 2017). Its yearly production in 2016 was 89.33 million tonnes (FAO, 2018). Most of the fruit is used fresh and around 13 % in apple juice manufacture (U.S. Apple Production). In 2017 739 thousand tonnes of apple juice was produced in Germany (Figure 6.1).

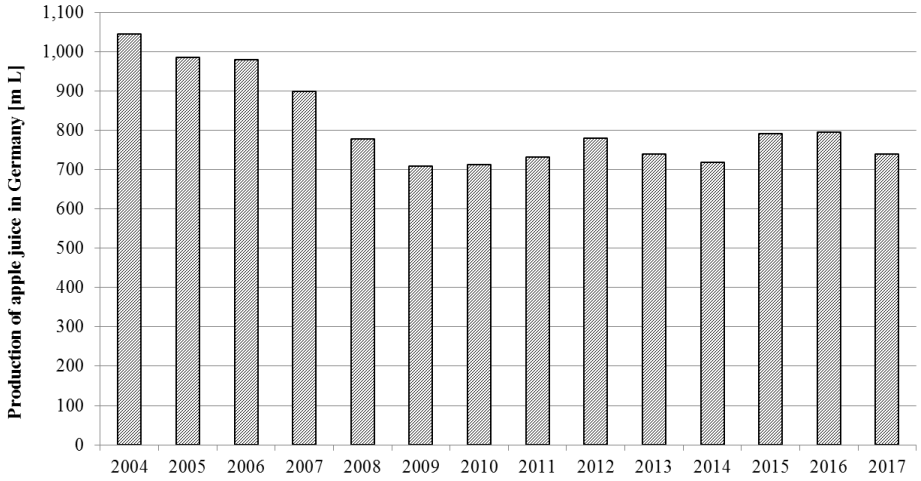

Figure 6.1 Production of apple juice in Germany in the years 2004 to 2017 (in millions of L) (Statistisches Bundesamt, 2018)

In large-scale apple juice production, the raw juice represents 75 % of the processed apple mass and the remaining 25 % is the by-product, apple pomace (Shalini et al., 2010). The processing of apples begins with washing and sorting (removal of damaged and diseased fruit). Apples are then grinded by a disintegrator, hammer mill or grating mill. In order to achieve higher yields of juice (break down cell walls), lower the viscosity and reduce pulp slipperiness, commercial macerating enzymes are usually added. The extraction is accomplished through pressing chopped apple continuously (by belt or screw press) or in batches (by hydraulic or bladder press). The produced apple juice is extremely cloudy and contains particles that can be removed by screening. In this case a cylindrical "cider" screen can be used which revolves on a system of rollers. The screen is kept clean by revolving actions causing the pomace to gather into small balls and finally into a continuous roll which falls off the end of the slightly sloping screen. For the "natural" look associated with fresh apple juice, the ground apple pulp is treated with ascorbic acid before pressing to minimize browning. The juice is screened or settled, but not otherwise filtered. Ascorbic acid is added directly to the mill, to be mixed with the pulp after the apples are crushed and pulp exposed to air. If the final product is meant to be cloudy, then the enzyme step is not applied. Otherwise, after juice extraction, the raw apple juice is treated with enzymes in order to remove suspended solids, which would consequently clog filters, slow the production and may cause the juice to form a haze. These enzymes hydrolyse pectin, hemicellulose and other polymers

and colloids that increase viscosity of the juice. Enzyme treatment can either be performed as hot method (at 54 °C for one to two hours) or cold method (at room temperature, 20 °C for six to eight hours). Further processing involves heat clarification, fining, filtration and pasteurisation. Basic idea of heat clarification is that heated particles within the apple juice coagulate and are easily removed through filtration. In the fining process, bentonite clay particles absorb tannins and protein-tannin complexes. By filtration large particles, certain proteins and microorganisms are being removed from the apple juice. The final and the most important step for preserving the juice is pasteurisation, which involves heating to a given temperature long enough to destroy all organisms that can develop. Flash pasteurization is rapid heating to near the boiling point (above 88 °C) for 25 to 30 seconds, in which steam or hot water passes the juice between plates or through narrow tubes that are heated (Bates et al., 2001).

Considering the large volumes of apple juice being produced, by-product apple pomace is generated worldwide in thousands of tonnes (Table 2.3). Apple pomace is generally composed of skin and flesh (95 %), seeds (2 % to 4 %), and stems (1 %). Its composition has been widely explored (Table 6.1) and differ depending on the variety, origin and processing technology prior to its generation (Perussello et al., 2017).

Table 6.1 Examples of physical-chemical composition of apple pomace (expressed in % of dry weight basis) (TDF = total dietary fibre, n. d. = not determined)

Composition [%]	Joshi (2006)	Kołodziejczyk (2007)	Bhushan (2008)	Sato (2010)	Reis (2012)	O´Shea (2015)
Moisture	3.97 – 5.4	3.0	3.9 – 10.8	11.43	7.9	9.0
Ash	1.6	n. d.	0.5 – 6.1	1.8	1.1	1.6
Fat	3.49 – 3.9	1.5	1.2 – 3.90	1.53	2.3	2.27
Protein	4.45 – 5.67	n. d.	2.94 – 5.67	2.74	3.3	2.37
Carbohydrate	48.0 – 62.0	n. d.	48.0 – 62.0	n. d.	n. d.	84.76
Starch	n. d.	n. d.	n. d.	n. d.	7.8	5.6
Total sugar	n. d.	n. d.	n. d.	n. d.	n. d.	54.2
Glucose	22.7	6.7	19.5 – 19.7	12.57	n. d.	n. d.
Fructose	23.6	19.9	40.3	17.93	n. d.	n. d.
Saccharose	1.8	11.8	3.8 – 5.8	7.04	n. d.	n. d.
TDF	4.7 – 48.72	55.2	4.7 – 51.1	43.63	42.1	30.15
Pectin	3.5 – 14.32	n. d.	3.5 – 14.32	15.27	n. d.	7.84

The disposal of apple pomace into the environment, due to its high bio-chemical oxygen demand, represents a pollution problem (Lisičar et al., 2017). Direct dumping is also difficult, because of the high costs of transportation and the generation of foul smell. In the past, dried apple pomace was used as animal feed, fuel for boilers or added to soil as a conditioner. In the last 30 years its potential has been extensively studied as a substrate for microbial growth and used in the production of value products. Since fresh apple pomace is quite liable to spoil, it must be preserved in order to be stored and used over a long period of time. Preservation can be achieved by drying. Dried apple pomace has demonstrated its worth on animal health and can be utilized for animal feed (Bae et al., 1994; Gutzwiller et al., 2007). It has also been characterized by high pellet-ability (Maslovarić et al., 2015).

One of the most practical approaches in the utilization of apple pomace is for pectin production (Bhushan et al., 2008). Pectin is a structural linear polysaccharide contained in the primary cell walls of terrestrial plants (Srivastava et al., 2011). Commercial pectin is characterised by high content of galacturonic acid, which has become part of the definition for

pectin to be used as food additive or for pharmaceutical purposes. Usual requirements are a minimum of 65 % of galacturonic acid on the ash and moisture-free substances (May, 1990). Excellent water binding and gel forming properties even at low concentrations is the main reason to be employed as thickener and stabiliser in the food industry as gelling agent in jams, confectionary and bakery fillings, as well as stabilizer in yoghurts and milk drinks. It is also used in the cosmetics, personal care (paints, toothpaste and shampoos) and pharmaceutical industry (gel caps), including new utilization as nutraceutical ingredient. Since the early 2000s, pectin has been recognized to have several beneficial health and nutritional effects as a dietary fibre and prebiotic. Conventional pectin production is generally expensive, requiring extraction factories having a close, large-scale source of raw material (dried citrus peel or apple pomace). In 2015 the average price exceeded 15 $/kg and the market (exceeding 60,000 tons) was close to reach $ 1 billion (Ciriminna et al., 2016).

6.3 Materials and methods

6.3.1 Chemicals and equipment

Chemicals and equipment used were the same as in chapter 4.3.1. Additional equipment is listed in Table 6.2.

Table 6.2 Equipment used in the production of apple juice, pectin, pellets and pectin free apple pomace extract

Equipment	Manufacturer
Depth filters Seitz K 100	Pall Filterysystems GmbH, Bad Kreuznach (Germany)
EcoWorxx Pelletmaker PM22E	EcoWorxx GmbH, Raddestorf (Germany)
Preserving boiler Eltac EKA 179	Eltac, Viersen (Germany)
Filter paper MN 540 we, ø 150 mm	Macherey-Nagel GmbH & Co. KG, Düren (Germany)
Hydropresse 20 Liter	Speidel, Ofterdingen (Germany)
IKA Calorimeter C200	IKA GmbH & Co., Staufen (Germany)
Pellet abrasion tester Bioenergy Tumbler 1000	Bioenergy Anlagenplanung GmbH, Vienna (Austria)
Rectification apparatus DN50	Normag Labor- und Prozesstechnik GmbH, Ilmenau (Germany)
Scraped surface evaporator	Normag Labor- und Prozesstechnik GmbH, Ilmenau (Germany)
Shredder Shark Fruit 1,6 kW	Vares Mnichovice a.s., Mnichovice (Czech Republic)
Sheet filter 20x20 FZ 20	Zambelli, Vicenza (Italy)
Sorvall RC-5B Plus Superspeed Centrifuge	Thermo Fisher Scientific, Waltham, Massachusetts (USA)
Vacuum drying oven	Heraeus Instruments, Hanau (Germany)
Vares Hydraulic Profi 18L/2t	Vares Mnichovice a.s., Mnichovice (Czech Republic)
Vario-Pumpsystem VP-ANTR. TEIL	Ismatec, Wertheim (Germany)

6.3.2 Production of apple juice

46.8 kg of Braeburn apples (New Zewaland Apple LTD, Whakatu, New Zealand) were washed and grinded by Shark Fruit 1.6 kW. Crushed apples were pressed at the pressure of 3

bar resulting in 30.3 kg of raw juice (RJ) and 15.5 kg of apple pomace 1 (AP1). Raw apple juice was filtrated and pasteurized at 73 °C for ten minutes and filled in Bag-in-Box containers (Figure 6.2).

6.3.3 Extraction of pectin and sugar

Prior to the extraction, the pH value of demineralized water was adjusted to 1 by 37 % hydrochloric acid. The extraction was performed by mixing 22.25 kg of water with 15.5 kg of AP1 (ratio 1.44) and boiled at 90 °C for one hour while constantly being stirred. The mixture was cooled down to 40 °C and then pressed (Vares Hydraulic Profi 18L/2t, Vares Mnichovice a.s.; Mnichovice, Czech Republic). The solid fraction – so called apple pomace 2 (AP2) – was used to produce pellets (P) and the liquid fraction – so called apple pomace extract (APE) was collected. 10.5 kg of APE was mixed with 4.3 kg of 93.8 % (w/w) ethanol and cooled down to 4 °C overnight. The following day, it was pressed by hydraulic press. The gained solid pectin (PT) was dried at 40 °C for seven days and the ethanolic liquor (EL) was subjected to distillation in order to produce a pectin free apple pomace extract (PFAPE).

6.3.4 Distillation and production of PFAPE

Batch distillation was performed by a rectification apparatus DN50. The column, filled with Raschig-rings, had a diameter of 50 mm and a filling height of 1200 mm, whereby 7 theoretical plates are realized. The capacity of the boiling flask is 10 L and is operated with electric heating device (maximum heat output 1.6 kW). For the separation of EL, a heating power of 0.72 kW was set. At the beginning of the batch distillation, the unit was retracted at reflux ratio 0.2 with a starting head temperature of 76.5 °C. After 165 minutes, 6.47 kg of apple pomace extract was obtained and 2.37 kg ethanol with purity of 88.34 % (w/w) were recovered. PFAPE was concentrated to obtain 0.58 kg concentrated pectin free apple pomace extract (cPFAPE) by evaporation in a scraped surface evaporator for six hours and 30 minutes at a temperature of 80 °C, pressure of 130 mbar and rotation speed of 150 rpm.

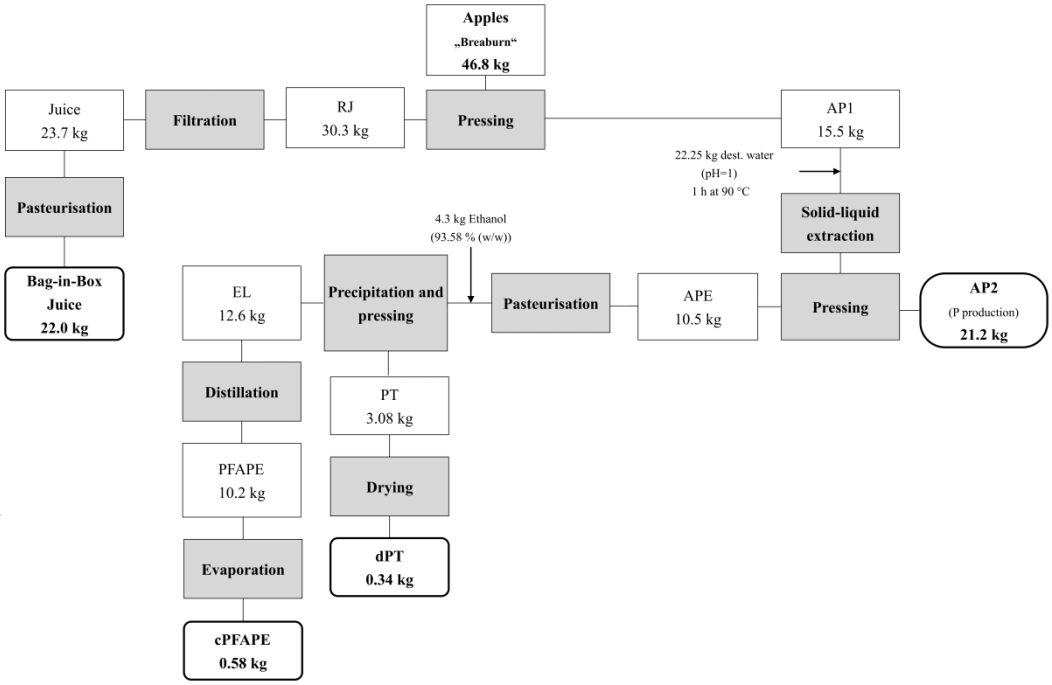

Figure 6.2 Production of apple juice, pectin and pectin free apple pomace extract

6.3.5 Production and analysis of pellets

21.2 kg of AP2 were dried at 60 °C for 24 hours and then used as raw material for pellet production. Moisture content of P was determined at 105 °C until constant weight. Durability was determined according to the standard ISO 17831-1:2015(en). Net calorific value was determined according to ISO 18125:2017(en).

6.3.6 Determination of dry matter

The solid content was determined by using drying oven. Three to five grams of sample was heated for 24 hours at the temperature of 105 °C.

6.3.7 Determination of ash content

A muffle furnace was used for the determination of ash content. The dried samples were heated for two hours at the temperature of 550 °C.

6.3.8 Determination of sugar concentrations

The concentrations of sucrose, glucose, and fructose have been determined enzymatic with the commercial kit as well as by refractometer.

6.3.9 Fed-batch fermentation of baker´s yeast

Large-scale yeast inoculum *S. cerevisiae* was kindly provided by Uniferm GmbH & Co. KG; Monheim am Rhein, Germany.

Two fermentations were conducted; one with the molasses and the second one with the mixture of molasses and cPFAPE as carbon source. All the other steps and course of the fermentation were the same for both cultivations as described in chapter 4.3.3. The molasses medium, a sterile mixture of sugar beet and cane molasses, was kindly provided by Uniferm GmbH & Co. KG, Monheim am Rhein, Germany. The medium for the cultivation contained 93 % of the molasses and 7 % of cPFAPE. Prior to mixing, cPFAPE was first centrifuged and neutralized with a 2.5 M sodium hydroxide solution. Brix value of 73 was lowered to 44 with the addition of demineralized water. The noticeable solid particles were removed via filtration and the medium was sterilised. 4 kg of substrate (molasses or molasses-cPFAPE mixture) were supplemented with a vitamin stock solution. The samples were centrifuged for 10 minutes at 6,000 rpm, washed twice with 0.9 % NaCl for the determination of dry yeast biomass and fermentative capacity. The concentration of dry yeast biomass was determined via vacuum furnace at 105 °C for 24 hours. Fermentative capacity, the increase of the ethanol concentration was determined in two different synthetic doughs (LSSD and HSSD) (Bell et al., 2001).

6.3.10 Process simulation

The simulation of continuous distillation was conducted by means of software ChemSep (ChemSep Lite v7.3) (Table 6.3). Figures were made by softwares e!Sankey 4 and Microsoft Visio 2010.

Table 6.3 Input parameters for continuous distillation in ChemSep Lite v7.3

Operation	
Type of Simulation	Equilibrium column
Configuration	
Operation	Simple Distillation
Condenser	Total (Liquid product)
Reboiler	Partial (Liquid product)
Number of stages	15
Feed stages	12
Properties	
Thermodynamics	
K-value	DECHEMA
Equation of state	Ideal gas law
Activity coefficient	UNIFAC
Vapour pressure	Antoine
Enthalpy	Excess
Enthalpy/Exergy	
Reference state	Vapour 298,15 K
Heat of formation	Excluded
Surroundings T	298,15 K
Heat Capacity IG	T correlation
Heat Capacity L	Mol fraction average
Specifications	
Reflux ratio	1.50
Mass fraction water [/]	
Feed	0.727521
Top	0.0930107
Bottom	0.990247

6.4 Results and discussion

6.4.1 Mass and heat balance in the production of apple juice, pectin, pellets and pectin free apple pomace extract

Mass balance of apple processing into apple juice and further into pectin, pellets and pectin free apple pomace extract are presented in Figure 6.3. It is important to mention that unlike in industrial production, pectin-degrading enzyme was not used during the production of apple juice in this research.

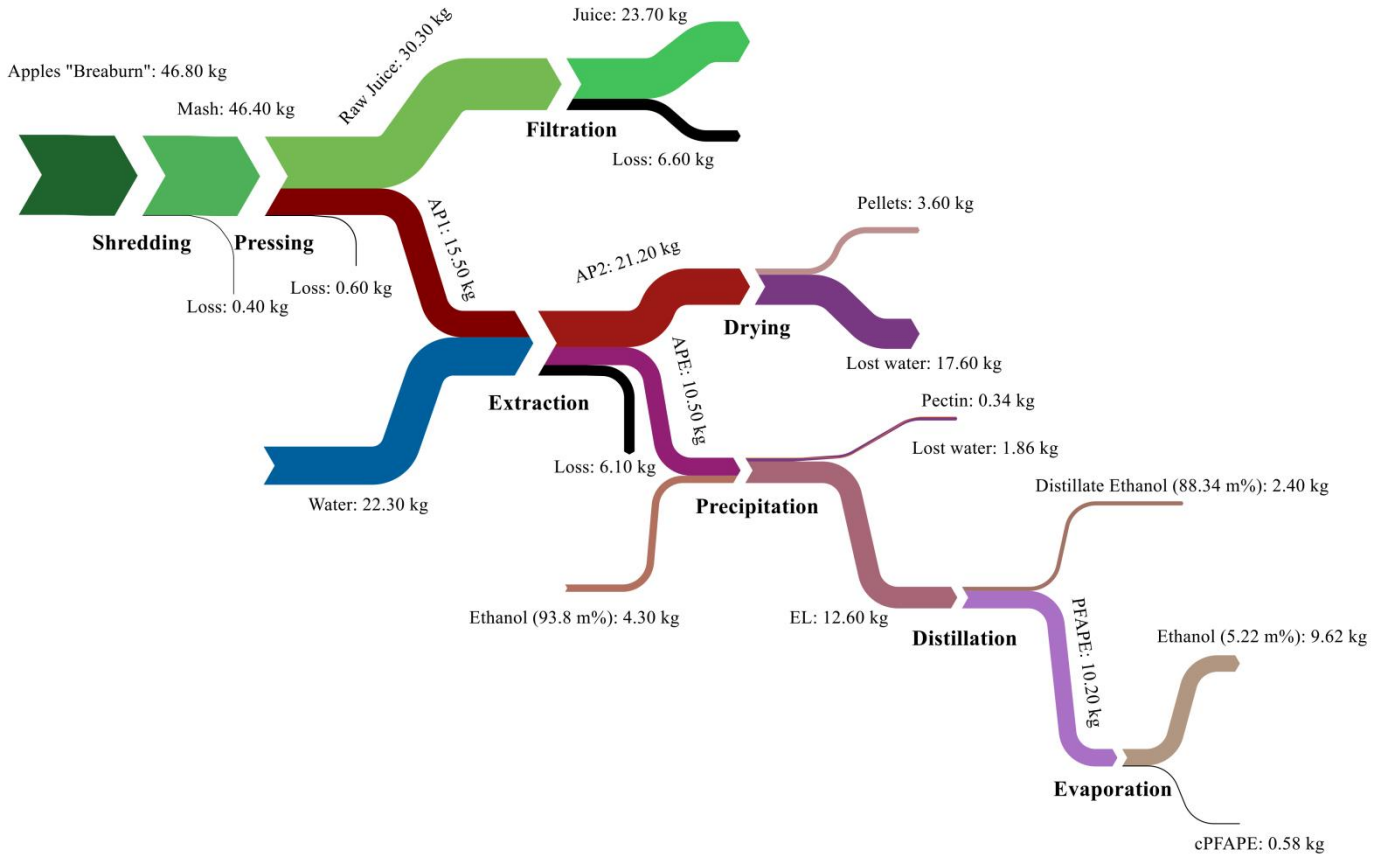

Figure 6.3 Mass balance of apple processing into apple juice, pectin, pellets and pectin free apple pomace extract

After shredding and pressing, 30.3 kg of raw apple juice was obtained, meaning 65 % of apples was utilized for juice production, which corresponds the literature (Bhushan et al., 2008). The losses that remained in the equipment during the production as well as for sampling were taken into consideration. Bag-in-box apple juice contained total sugar concentration of 102.7 g/L, which is in good agreement with the literature (van der Sluis et al., 2002). The extraction of AP1 is a critical step from an economic point of view. Namely, the more water added, the more energy is required during the drying process, more ethanol is need for the precipitation and more energy is required for the distillation and concentration processes. In this research, the extraction was conducted with a water/AP1 ratio 1.44 which resulted in 21.2 kg of AP2 and 10.5 kg of APE. Dried AP2 was employed as substrate for pelleting. Apple pellets, with the moisture content of 17.1 %, were further analysed. Pellet durability index amounted 96.9 %, which is slightly lower than found in literature (Maslovarić et al., 2015). However, measured net calorific value of 20.312 MJ/kg is significantly higher than found in literature (Wojdalski et al., 2016) and very similar to the value obtained with wood pellets (Telmo et al., 2011). Precipitation of extract with ethanol resulted in 3.08 kg of soluble pectin, i.e. in 0.34 kg of dried pectin. To be exact, AP contained 11.89 % of pectin on dry matter (which corresponds to literature in Table 2). Batch distillation proved to be a successful step in recovering ethanol and obtaining PFAPE (Figure 6.4). The head temperature was kept at ethanol´s boiling point (78.3 °C). Starting bottom temperature was 82 °C and by having less and less ethanol in the fraction and more water content, the temperature tended to rise. The reflux ratio could be kept below 1 for 108 minutes. Then it required to be higher and was kept at 7 for 37 minutes. The distillation was stopped as the head temperature suddenly increased because of the presence of water in the distillate. By distillation 2.4 kg of 88.34 % (w/w) ethanol was recovered and 10.2 kg PFAPE obtained as bottom product.

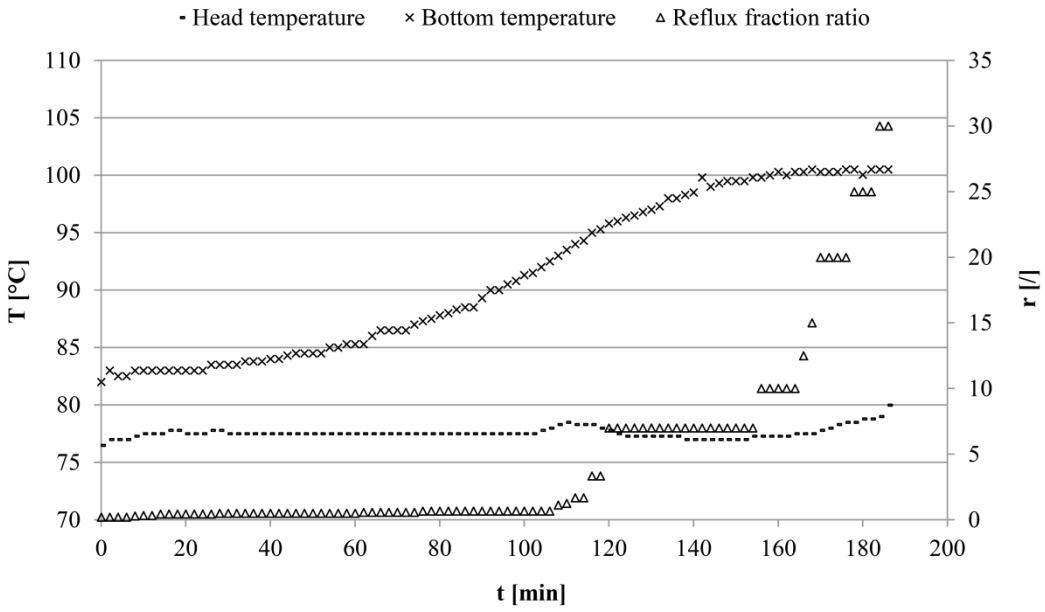

Figure 6.4 Relation of temperatures and reflux ratio during the batch distillation

APE still contained ethanol (5.22 % (w/w)) and water which were removed by evaporation. Obtained final product cPFAPE amounted 0.58 kg with Brix value of 73.

Energy demands for drying of AP2, drying of pectin, distillation and evaporation were calculated (Figure 6.5). As mentioned the extraction is a critical step. Water was mixed with AP1 in ratio 1.44. It resulted in 21.2 kg of AP2, which required energy of 2.892 MJ/kg_{AP1} for the drying process to evaporate 17.6 kg of water, considering an evaporation enthalpy of 2,546.5 kJ/kg (VDI, 2013). This step consumes the most energy. To gain dPT the energy demand was 0.306 MJ/kg_{AP1}. Batch distillation process consumed 1.833 MJ/kg_{AP1}, based on the evaporation enthalpy of ethanol 839 kJ/kg (VDI, 2013). 1.621 MJ/kg_{AP1} was required in the evaporation process to gain cPFAPE. Calculated total energy obtained from the pellets combustion is as high as 3.938 MJ/kg_{AP1}. However, the usual energy efficiency of boilers is approximately 90 % (Deutsches Pelletinstitut, 2015) meaning the pellets are generating 3.544 MJ/kg_{AP1} energy. Combusting the pellets does not cover all energy demands required for the drying of AP2, distillation, evaporation and drying of pectin; an additional input of 3.108 MJ/kg_{AP1} is required.

Figure 6.5 Heat balance in the production of pectin, pellets and pectin free apple pomace extract in MJ/kg$_{AP1}$

6.4.2 Fed-batch cultivation

cPFAPE was tested in fed-batch fermentation of baker´s yeast in order to explore its potential as an alternative to overpriced molasses (Lisičar et al., 2017). The results are presented in Table 6.4. Concentration of dry yeast biomass obtained on the medium consisted of molasses and cPFAPE is slightly lower than the yield obtained on the medium containing only molasses. However, baker´s yeast end concentration is still higher than found in literature (Bhushan et al., 2006). It has been reported, and was also proved in this research, that baker´s yeast shows lower fermentative capacity in HSSD-medium, due to the overexpression of invertase and intrinsic osmotolerance triggered by high sugar environment (Bell et al., 2001). Slightly lower production of ethanol in HSSD is achieved by the yeast grown on the medium containing cPFAPE. However, the higher fermentative capacity is achieved in LSSD medium by the yeast produced on cPFAPE. These results indicate that cPFAPE can be used as an alternative substrate for the production of baker´s yeast. cPFAPE ´s Brix value was 73, which is significantly higher than the value in diluted molasses used in baker´s yeast production but nearly the same as the Brix value of pure molasses (Olbrich, 2006). Considering the high prices of molasses, if only a portion of molasses is replaced with cPFAPE (for example 10

%), it would significantly reduce the costs of producing baker´s yeast. If baker´s yeast manufacture requires 100,000 tonnes of molasses/year, with the substitution of 10 %, the cost reduction would amount $ 1 million. Additionally cPFAPE is being converted from the waste by-product into valuable source of nutrients which contributes to integrated sustainability in the industrial environment, where the waste from one industrial process is used as feedstock for another (Wallance, 2005).

Table 6.4 Maximum biomass yield (on dry matter in g) and fermentative capacity in two synthetic doughs (in $mmol_{Ethanol}/(g \cdot h)$) of *S. cerevisiae* produced on molasses and molasses and cPFAPE

Medium	Dry yeast biomass [g]	Fermentative capacity $[mmol_{Ethanol}/(g \cdot h)]$	
		HSSD	LSSD
Molasses	630	1.806	4.014
Molasses and cPFAPE	620	1.764	4.181

6.4.3 Industrial concept of biorefinery

Apple juice was successfully produced at pilot scale. Furthermore, by-product apple pomace was processed so that three valuable products were obtained. However, in order to process large amounts of apple pomace, modifications have to be implemented. As previously mentioned, the most critical step in this work flow is the extraction process, considering that water needs to be removed in latter phases which require large amounts of energy. Batch distillation is not profitable on industrial scale. That is why the simulation of continuous distillation was performed and three scenarios were developed (Table 6.5). Different water/AP1 ratios for extraction process were investigated (1.08 and 0.72) as well as the same one used in the experimental part of batch distillation (1.44), in order to reduce the energy input into the overall process. It is assumed that the water content in AP2 was constant for water/AP1 ratios > 0.4.

Table 6.5 Theoretical scenarios of energy requirements and demand by applying different water/AP1 ratio for extraction process

Ratio water/AP1 for extraction process	**1.44**	**1.08**	**0.72**
Energy requirements		**[MJ/kg$_{AP1}$]**	
Drying of apple pomace 2	2.892	2.892	2.892
Continuous distillation	0.718	0.538	0.359
Evaporation	1.621	1.216	0.811
Drying of pectin	0.306	0.306	0.306
Total	**5.536**	**4.951**	**4.366**
Energy generated from pellets	3.938	3.938	3.938
Energy demand	1.598	1.013	0.428

The energy demand is reduced for every step, except for AP2 and pectin drying and combustion of pellets, which remained the same. When applying water/AP1 ratio of 1.44 in extraction process, the energy demand input for overall process, in which continuous distillation is applied, is almost 60 % reduced than in process in which batch distillation is used (reduction of 1.598 MJ/kg$_{AP1}$). Simulation showed that if 7.068 kg of water is mixed with 15.5 kg of AP1 (ratio 0.455) in the extraction process, the energy demand for overall process would be entirely covered by the combustion of pellets (Figure 6.6).

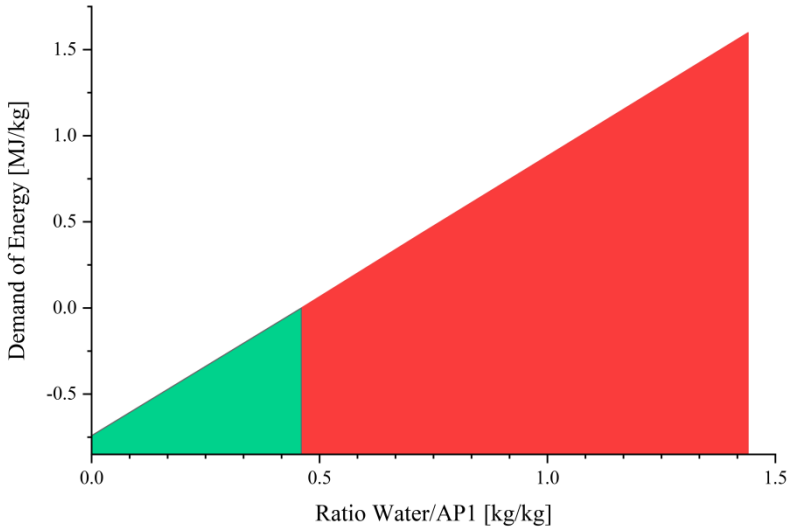

Figure 6.6 Energy input (in MJ/kg$_{AP1}$) required for the process in correlation with ratio water/AP1

The developed biorefinery concept is shown in Figure 6.7. Besides the equipment for the industrial production of apple juice, the distillation apparatus and equipment for producing pectin are integrated in the plant. By producing and combusting pellets, the energy demand can be covered in this chain, with the emphasis of carefully designing the extraction process. cPFAPE partly substitutes raw material molasses in the production of baker´s yeast. This concept does not only include the valorisation of the entire waste steam but also generates energy.

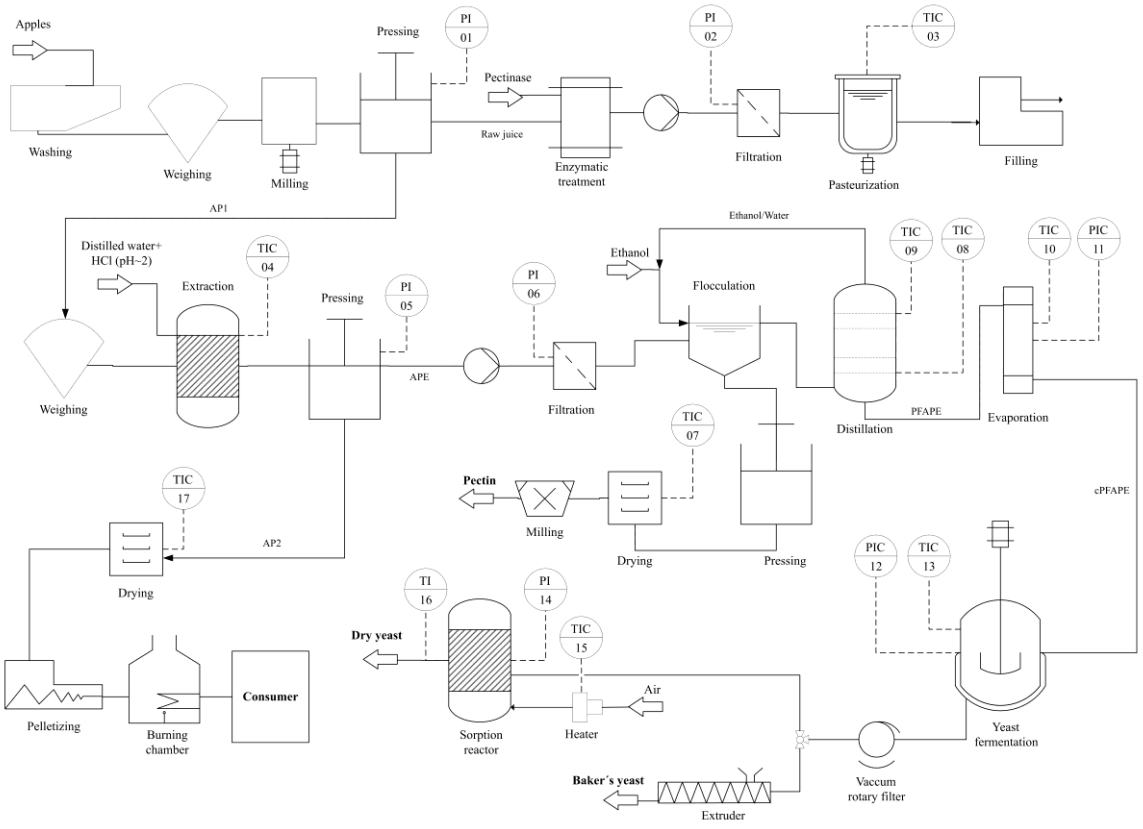

Figure 6.7 Apple juice production turned into biorefinery platform

Considering the results obtained in this study and amount of apple juice produced in Germany in 2017 (Figure 6.1), the corresponding amount of apple pomace produced is approx. 398,000 tonnes, i.e. 73,400 tonnes on dry matter. The amount of pectin that could be recovered equals 8,730 tonnes, which corresponds to almost 15 % of global production accomplished in year 2015 and has worth of more than $ 130 million. Amount of pellets that could be produced equals 92,400 tonnes, which by combusting generate energy of over 1.4 million GJ. The amount of concentrated pectin free pomace extract produced would be over 15,000 tonnes.

6.5 Conclusion

By-product of apple juice production can be fully valorised, resulting in pectin, pellets and pectin free apple pomace extract. The process of apple juice production was similar to industrial, only commercial enzyme of any kind and ascorbic acid has not been used. Critical step of extraction has been discussed as well as the possible scenarios of industrial continuous distillation, considering batch process was applied in recovering ethanol. Total of 3.6 kg of pellets was produced. The net calorific value of 20.3 MJ/kg is practically the same as the one reported for wood pellets. Apple pomace proved to contain 11.9 % of pectin. Concentrated pectin free apple pomace extract showed potential to be employed as a carbon source for the growth of baker´s yeast. Slightly lower biomass yield is obtained in the medium containing cPFAPE and molasses than in the medium containing only molasses. However, yeast produced on alternative substrate possessed a bit higher fermentative activity than the one produced on molasses. By reshaping this production, a biorefinery platform in which waste from one production stream is used as feedstock in another industrial process, could be set up. It would provide not only value-added products but would also generate energy for its own use. Scaling up calculations showed that almost 15 % of global pectin production could be covered by this biorefinery, 15,000 tonnes of alternative raw medium for baker´s yeast production could be produced and 92,400 tonnes of pellets would generate approx. 20.2 GJ per tonne of pellets. One of the problems for setting up a biorefinery concept is the raw material availability. However, apple pomace is widely available and eligible for multiple purposes. Further investigations regarding extraction and distillation process are necessary in order to turn this production into zero discharge biorefinery process.

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7 Summary and perspectives

Presented study demonstrates how baker´s yeast production can be turned into multipurpose bioprocess and sustain in the future industrial environment by following the concept of integrated sustainability. Baker´s yeast manufacture can, besides keeping its main role as supplier of doughs´ leavening agent, supply valuable biomolecules betaine and invertase as well as proteins and amino acids. Close connections can be made with other industries by re-using their waste as feedstock in fed-batch cultivations. Baker´s yeast production can also serve as supplier of energy, i.e. district heat in new future energy systems.

It has been shown in what extent molasses is utilized by baker´s yeast as well as differences in the composition of molasses and corresponding vinasse. Changes made in downstream processing transform vinasse into value-added product. Developed method included two ultrafiltration steps (applying membrane with a MWCO of 30 kDa as well as a membrane with MWCO of 2 kDa) which resulted in food grade invertase and proteins. Natural betaine of high purity was obtained by further processing which included evaporation and ion exchange adsorption. The potential of generating energy for space and water heating by baker´s yeast manufacturer has been confirmed. Problem concerning long term energy storage was addressed. However, further research regarding the capacity and dimensioning of the storage modules needs to be conducted. By reframing apple juice production into the concept of biorefinery, more valuable products were gained: pectin, pellets and pectin free apple pomace extract. In order to cover all the energy demand by pellet combusting, further investigation regarding the optimization of the production processes (extraction and distillation) is necessary. Concentrated pectin free apple pomace extract demonstrated good performance in partly substituting raw material molasses in fed-batch fermentation. Produced yeast was very similar to the one produced on only molasses.

This study should be regarded as an example of multidisciplinary approach; in order to implement these concepts, experts and knowledge from different fields (biotechnology, biochemistry, thermodynamics and material sciences) need to be engaged. Furthermore, the focus of baker´s yeast producing companies needs to be a bit redirected from the main product of yeast in order to see the full potential of new purposes that can be derived from this industrial process.

List of publications

Articles in scientific journals:

Lisičar Vukušić J, Millenautzki T, Sedaghati M, Schallenberg M, Müller P, Hof J, Mösche M, Barbe S. 2018. Fractionation of baker's yeast vinasse via ultrafiltration: Assessment of feasibility. *International Journal of Food Science and Technology*. DOI: 10.1111/ijfs.14080

Lisičar Vukušić J, Kneer A, Mösche M, Barbe S. 2018. Turning industrial aerobic fermentation plants into thermal power stations. *International Journal of Energy Research.* DOI: 10.1002/er.4299

Ivančić Šantek M, Lisičar J, Mušak L, Špoljarić Vrana I, Beluhan S, Šantek B. 2018. Lipid production by yeast *Trichorporon oleaginosus* on the enzymatic hydrolysate of alkaline pretreated corn cobs for biodiesel production. *Energy & Fuels.* DOI: 10.1021/acs.energyfuels.8b02231

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Lisičar J, Scheper T, Barbe S. 2017. Turning industrial baker's yeast manufacture into a powerful zero discharge multipurpose bioprocess. *Industrial Biotechnology*. 13 (4): 184-191. DOI: 10.1089/ind.2017.0018

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Petravić-Tominac V, Mesihović A, Mujadžić S, Lisičar J, Oros D, Velić D, Velić N, Srečec S, Zechner-Krpan V, Petrović Z. 2013. Production of blackberry wine by microfermentation using commercial yeasts Fermol Rouge® and Fermol Mediterranée®. *Agriculturae Conspectus Scientificus.* 78 (1): 49-55.

Conference paper:

Lisicar J, Sedaghati M, Barbe, S. 2018. Looking at baker´s yeast fermentation through new glasses: The neglected potential of vinasse for biotechnological applications. Proceeding of 31st Yeast Conference, Leuven (Belgium), 16.-17.04.2018

Conferences & Congresses:

Lisicar J, Zerhusen C, Schörken U, Barbe S. Yeast fermentation technology: Process optimization and modelling. Poster presentation at BioProcessing Days, Recklinghausen (Germany), 20.-22.02.2017

Lisicar J, Scheper T, Barbe S. Industrial baker's yeast fermentation: from manufacture to integrated sustainability. Chairman/oral presentation at European Biotechnology Congress, Dubrovnik (Croatia), 25.-27.05.2017

Lisicar J, Millenautzki T, Barbe S. Making industrial baker's yeast fermentation great again. Poster presentation at BioProcessing Days, Recklinghausen (Germany), 19.-21.02.2018

Lisičar J, Sedaghati M, Barbe S. Looking at baker´s yeast fermentation through new glasses: The neglected potential of vinasse for biotechnological applications. Oral presentation at 31st VH Yeast Conference, Leuven (Belgium), 16.-17.04.2018

Lisicar J, Millenautzki T, Scheper T, Barbe S. New trends in industrial baker's yeast fermentation: recovery of key biomolecules and low grade heat conversion. Oral presentation at European Biotechnology Congress, Athens (Greece), 26.-28.04.2018

Lisičar J, Millenautzki T, Barbe S. Baker's yeast manufacture as zero discharge multipurpose process. Poster presentation at World´s leading trade fair for water, waste and raw materials management, Munich (Germany), 14.-18.05.2018

Lisicar J, Kneer A, Barbe S. Conversion of low grade heat from industrial aerobic bioprocesses into 4th generation district heat: A case study. Oral presentation at 2nd International Conference on Bioresources, Energy, Environment, and Materials Technology, Hongcheon (Republic of South Korea), 10.-13.06.2018

Lisičar Vukušić J, Millenautzki T, Saaid A. M., Barbe. New feedstocks for bioethanol production. Poster presentation at STEPsCON 2018 International Scientific Conference on Sustainability and Innovation "Arctic Attitude meets German Pragmatism", Leverkusen (Germany), 07.12.2018

Curriculum vitae

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Studium

Okt. 2008 – Jul. 2011	Bachelor Studium an der Fakultät für Lebensmitteltechnologie und Biotechnologie (Universität in Zagreb, Kroatien), Fachbereich Biotechnologie
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Okt. 2011 – 27. Sept. 2013	Master Studium an der Fakultät für Lebensmitteltechnologie und Biotechnologie (Universität in Zagreb, Kroatien), Fachbereich Bioverfahrenstechnik
	Master Thesis: Lipid production by yeast *Trichosporon oleaginosus* on glucose and xylose under nitrogen limitation of growth (auf Kroatisch)
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Berufliche Erfahrung

Nov. 2013 – Apr. 2014	Ingenieur für Umweltschutz bei der Fa. Dalekovod in Zagreb (Kroatien)
Apr. 2014 – Feb. 2016	Ingenieur für Umweltschutz und Qualitätsmanagement bei der Fa. Dalekovod in Zagreb (Kroatien)
Feb. 2016 – Jun. 2016	Doktorandin, Uniferm GmbH & Co. KG, Abteilung Labor, Monheim am Rhein

Seit Feb. 2016	Wissenschaftliche Mitarbeiter bei der TH Köln unter der Leitung von Prof. Dr. S. Barbe, TH Köln und Prof. Dr. T. Scheper, Leibniz Universität Hannover

Auszeichnungen

Jan. 2012	Stipendium für begabte Studenten
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Sprachen

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