Sugar Beet Processing Waste as a Substrate for Yeast Protein Production for Livestock Feed

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This study investigated the optimal yeast strains for producing single cell protein based on waste sugar beet pulp (SBP) under various biomass loads. All tested strains were capable of growing on the waste biomass. *Candida utilis* and *Saccharomyces cerevisiae* Ethanol Red produced the greatest increase in protein on fresh SBP (ΔN 1.84%). *Scheffersomyces stipitis* (ΔN 2.27%) provided the highest increase on dried SBP. All tested strains showed significant assimilation of nitrogenous compounds. Based on the crude fiber content after fermentation, the largest reduction in fiber occurred with *Candida utilis* R6 (10.5%) on fresh SBP, and *Yarrowia lipolytica* (13.1%) on dried SBP. These results demonstrate the potential of SBP as a substrate for the production of single cell protein and highlight the importance of selecting the appropriate strains to optimize the process.

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Keywords: Sugar beet pulp; Waste biomass; Feed; Yeast; Protein; Free amino nitrogen; Crude fiber;

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INTRODUCTION

The main material used for livestock feed is plant biomass that is unsuitable for human consumption. If not used for animal feed, this biomass would most often become waste (Van Zanten *et al.* 2019). The main waste material after white sugar production in Europe is sugar beet pulp (SBP). Despite its low carbohydrate content, SBP can still be used for animal feed as roughage without protein enrichment (Ptak *et al.* 2021). However, simple fermentation with yeast and SBP as a matrix will result in a high value feed additive (Patelski *et al.* 2015). Feed with a sufficient amount of proteins, fats, carbohydrates, and macro and microelements, as well as an appropriate amino acid composition, is necessary to ensure the proper growth of animals and effective meat production (Wu *et al.* 2011; Raspa *et al.* 2019; Pinotti *et al.* 2021). It is necessary to adjust the feed dosage according to life stage of the animal. Dedicated fodder must meet specific standards, especially for young, adult, or lactating animals (McGilliard *et al.* 1983; DeVries *et al.* 2007; Shurson *et al.* 2021).

There are two types of feed for livestock: concentrated feeds, which provide various essential nutrients for proper growth, and roughages with relatively lower nutritional content intended to supplement feed by adding volume and providing satiety (Hunter and Siebert 2007; Galyean and Hubbert 2014). Roughages are less expensive, and they are used to balance the cost of meat production by reducing expenditure on concentrate feed. For example, breeding cows require a special feeding system, the Total Mixed Ration (TMR) system, which provides a proper mixture of concentrated and roughage feed to meet the nutritional needs of the animal for rapid growth (Schingoethe 2017). It is recommended to

enrich feed biomass by using it as a substrate for the biosynthesis of single cell protein (SCP) (Shinya *et al.* 2022). The easiest way to enrich biomass is fermentation with waste biomass as a culture medium, providing a homogenous product. To optimize the production of SCP-enriched feed, it is also necessary to reduce the consumption of utilities—*i.e.*, water, electricity, and heat. This makes it possible to increase financial profit, to reduce water waste, and to provide a higher concentration of carbohydrates in feed (Modenbach and Nokes 2013; Puligundla *et al.* 2019).

The purpose of the present study was to determine the most favorable yeast strains and load of waste biomass for the production of SCP-enriched feed based on sugar beet pulp waste biomass. From the industrial point of view, it is recommended to use the smallest practical amount of water, which is achieved by increasing the portion of waste biomass. The influence of various biomass loads on the process of feed component production was investigated, including microbial growth, protein content, assimilation of nitrogenous compounds, and reduction of crude fiber content.

EXPERIMENTAL

Materials

Waste biomass

Two types of sugar beet pulp were used: fresh and press-dried sugar beet pulp. The fresh pulp was supplied by the sugar factory in Dobrzelin (Poland). The press-dried pulp was supplied by the sugar factory in Werbkowice (Poland).

Yeast strains

Table 1 lists the strains used during the process of fermentation.

Table 1. Yeast Strains

	Strain	Code
A	*Yarrowia lipolytica*	LOCK 0264
B	*Metschnikowia pulcherrima*	NCYC 747
C	*Scheffersomyces stipitis*	NCYC 1541
D	*Kluyveromyces marxianus*	LOCK 0024
E	*Candida humicola*	LOCK 0013
F	*Candida utilis*	LOCK 0021
G	*Candida utilis*	R6
H	*Candida utilis*	R7
I	*Saccharomyces bayanus*	BC S103
J	*Saccharomyces cerevisiae* TT	LOCK 0105
K	*Saccharomyces cerevisiae* Ethanol Red	Leaf/Lesaffre Advanced Fermentation

Culture media

Two culture media were used. For yeast inoculum preparation, medium containing YPG (Yeast extract [10 g/L], Peptone K [20 g/L], and Glucose [20 g/L]) (Merck, Darmstadt, Germany) was applied. For the pour plate method, agar YGC (Yeast extract [5 g/L], glucose [20 g/L], chloramphenicol [0.1 g/L], agar [15 g/L]) (Merck, Darmstadt, Germany) medium was used.

Enzymes

During enzymatic pre-hydrolysis, the following enzymatic preparations were used: Viscozyme® L by Novozymes, which is rich in arabanase, cellulase, β-glucanase, hemicellulase, and xylanase; UltraFlo® Max by Novozymes, which is rich in β-glucanase and an arabinoxylanase (Bagsværd, Dania).

Research Design

Enzymatic depolymerization of sugar beet pulp biomass, with known dry mass (DM) content, was carried out with different biomass loads. Preliminary studies showed the borderline dry mass content for the biological processes (rheological properties appropriate for sample preparation and mixing procedures). Portions of 40 g (I), 45 g (II), or 50 g (III) of fresh sugar beet pulp were placed in conical flasks and hydrated with 60 mL of water. Portions of 10 g (IV), 12.5 g (V), and 15 g (VI) of dried sugar beet pulp were placed in flasks and filled with 90 mL of water (dry mass of prepared samples is shown in Table 2).

Table 2. Determined Dry Mass Content for Each Biomass Sample

Sample	I	II	III	IV	V	VI
Determined dry mass (g/100 g)	9	10	11	9	11	13

The research design considered two stages of enzymatic hydrolysis, as shown in Fig. 1.

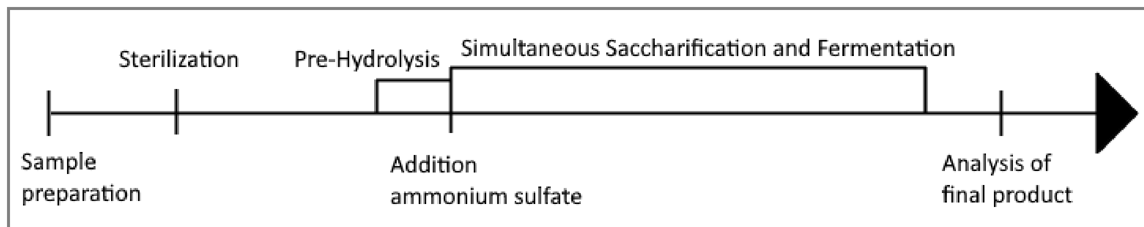

Fig. 1. Sequence of performed research

The sugar beet samples were sterilized at 121 °C for 15 min, then prepared for enzymatic pre-hydrolysis by adding 0.5 mL /10 g DM of both (Viscozyme® L and UltraFlo®) enzymatic preparations and incubated at 50 °C for 4 h. To measure the efficiency of the pre-hydrolyzation processes, the carbohydrate content was measured before and after the process. The process was carried out as simultaneous saccharification and fermentation (SSF), due to the fact that after pre-hydrolysis the enzymes were not deactivated. Further hydrolysis processes could have continued, based on the activity of added enzymes and activity of yeast extracellular enzymes. To provide an inorganic source of nitrogen, 0.3 g of ammonium sulphate was added to each sample. The biomass was inoculated with the tested strains and cultured on an orbital shaker (210 rpm) for 48 h at ambient temperature (approximately 21 °C). Yeast growth was tested using the pour plate method. The biomass was separated from the post culture liquid by centrifugation at 3600 RCF (Eppendorf Centrifuge 5804R, Hamburg, Germany). Before and after fermentation, the biomass was analyzed to determine the protein content (Kjeldahl method) and crude

fiber content. The post-culture liquid was analyzed to measure the content of free amino nitrogen (FAN).

Methods

Enzymatic pre-hydrolysis efficiency control

The sugars used as a primary source of carbon for yeast growth were determined, as a control for the hydrolyzation process. The following sugars were analyzed: D-xylose, D-mannose, D-fructose, and D-glucose. For this purpose, a UV-spectrophotometer (Thermo Scientific Multiskan GO; Thermo Fisher Scientific, Munich, Germany) was used with Megazyme K-MANGL (d-mannose, d-fructose, d-glucose) and K-XYLOSE (d-xylose) test kits (Bray, Ireland).

Microbial growth

The pour plate method was used to determine the ability of yeasts to grow on hydrolyzed waste biomass after 48 h of incubation at ambient temperature (approx. 21 °C). Yeast extract glucose chloramphenicol (YGC) agar medium was used. The post culture liquid was cultured on medium for 48 h at 30 °C. A non-fermented sample was used as the control sample (t = 0 h) (Kodaka *et al.* 2005).

Protein content

To determine the protein increase after fermentation, the Kjeldahl method was applied. A sample of separated biomass was placed in a digestion tube with 5 g of Missouri Tablets as a catalyst (Büchi, Flawil, Switzerland). Sulphuric acid was added, and the sample was heated in a SpeedDigester K-425 (Büchi, Flawil, Switzerland) at 550 °C until complete digestion (when a clean, transparent fluid is obtained). Next, each tube was placed in a KjelFlex K-360 apparatus (Büchi, Flawil, Switzerland), which provided automatic neutralization with sodium hydroxide 30% solution (m/m), steam distillation, and titration to pH 4.5 by the attached SI Analytics TitroLine®5000 (Xylem, Washington DC, USA). Untreated biomass was used a control sample.

Free amino nitrogen determination

Post-culture liquid was tested for free amino nitrogen content, using the ninhydrin method. The full protocol is provided on-line by Eppendorf (Geisler and Weiß 2015). Measurements were carried out using a Spectroquant Prove 300 spectrophotometer (Merck, Darmstadt, Germany). Liquid from a hydrolyzed non-fermented sample was used as a control.

Crude fiber determination

The crude fiber content (including cellulose, hemicellulose, lignins) in the samples was determined using a FOSS Fibertec^{TM} 8000 (Hilleroed, Denmark). Portions of the biomass were weighed with a precision of 0.1 mg and placed in previously prepared crucibles containing 1 g Celite 545 (FOSS, Hilleroed, Denmark). The samples were then washed with acetone to remove possible fat residues in a FOSS FT 121 cold extraction unit (Hilleroed, Denmark). The crucibles were inserted into the apparatus and boiled in a 1.25% (m/m) solution of sulphuric acid, then triple washed with distilled water. The samples were boiled again in a 1.25% (m/m) solution of potassium hydroxide. After acidic and alkaline boiling, the samples were triple washed with distilled water, then transferred to a cold extraction unit for triple acetone washing to remove possible residues of the anti-foaming

agent. The crucibles were dried at 130 °C for 2 h and transferred to a desiccator to cool to room temperature with protection humidity. The crucibles were weighed with precision of 0.1 mg (W_2). The samples were ashed for 3 h at 525 °C in an oven, cooled to room temperature, and weighed again (W_3). The crude fiber content was calculated using Eq. 1,

$$\%CF = (W_2 - W_3 - C) / W_1 \times 100\% \quad (1)$$

where $\%CF$ is the crude fiber content expressed in %(m/m), W_1 is the mass of the sample (g), W_2 is the mass of the crucible after extraction (g), W_3 is the mass of the crucible after ashing (g), and C is the mass of the blank sample with celite (g). Raw waste biomass was used as a control sample.

Statistical Analysis

Statistical analysis (ANOVA, Tukey test, $p<0.05$) was performed for each result using *Statistica* v.14.0.1 (TIBCO Software Inc., Palo Alto, California, USA).

RESULTS AND DISCUSSION

Enzymatic Pre-Hydrolysis Efficiency

The efficiency of pre-hydrolysis conducted before inoculation was determined by measuring the sugar content. Analysis of the obtained hydrolysates was performed for various portions of both fresh and dried sugar beet pulp before fermentation. Non-hydrolyzed biomass was used as a control sample. Table 3 presents the carbohydrate content of the different portions of fresh sugar beet pulp. The results for dried sugar beet pulp are presented in Table 4.

Table 3. Carbohydrate Content of Fresh Sugar Beet Pulp

Sugar (g/L)	I	II	III	I hydrolyzed	II hydrolyzed	III hydrolyzed
D-xylose	0.002	0.024	0.108	1.113	1.212	1.287
D-mannose	0.011	0.027	0.039	0.649	0.456	0.585
D-fructose	0.044	0.064	0.057	0.655	0.730	1.756
D-glucose	0.049	0.030	0.043	16.823	19.456	18.829

Enzymatic pre-hydrolysis releases significant quantities of carbohydrates into the environment, creating optimal conditions for yeast growth. The greatest increase in sugar concentration was registered in the case of D-glucose (up to 16.8 to 19.5 g/L). Increasing the portion of biomass resulted in higher concentrations of D-xylose and D-fructose. However, the amount of D-mannose did not increase. Discrepancies from the linear distribution may be due to the uneven distribution of enzymes in the biomass. During hydrolysis, the flasks where statically incubated, without using an orbitary shaker.

Table 4. Carbohydrate Content of Press-Dried Sugar Beet Pulp

Sugar (g/L)	IV	V	VI	IV hydrolyzed	V hydrolyzed	VI hydrolyzed
D-xylose	0.012	0.015	0.021	1.064	1.112	1.174
D-mannose	0.004	0.009	0.010	0.412	0.483	0.601
D-fructose	0.027	0.031	0.046	0.397	0.451	0.491
D-glucose	0.035	0.045	0.044	15.412	16.95	17.624

Enzymatic pre-hydrolysis of press-dried SBP increased the carbohydrates available in the culture medium. The highest increase was noticed for D-glucose, as high as 15.4 to 17.6 g/L. Increasing the biomass portion increased the concentration of post-hydrolysis carbohydrates. However, lower concentrations of sugars were measured in the press-dried SBP. This may be explained by physio-chemical factors connected with thermal pretreatment. For instance, Maillard reactions may occur between amino acids and reducing sugars during drying (Ellis 1959). At the same time, high temperature can lead to the caramelization of sugars contained in biomass, reducing their availability in the process (Ajandouz *et al.* 2001). Another reason for the reduced sugar content may be the release of inhibitors during the thermal treatment of lignin fractions (Zhai *et al.* 2022). The results for carbohydrate content after pre-hydrolysis do not reflect actual conditions during simultaneous saccharification and fermentation (SSF). The SSF process provides a continuous release of carbon compounds during yeast fermentation (Berłowska *et al.* 2016; Szambelan *et al.* 2018).

Microbial Growth

The pour plate method was used to monitor yeast growth throughout the 48 h fermentation process. A non-fermented sample was used as the control. The results for yeast growth in fresh SBP-based medium are presented in Table 5.

Table 5. Yeast Growth on Fresh Sugar Beet Pulp Based Medium (CFU/mL)

Strain	Full Strain Name	I	II	III	Control Sample
A	*Yarrowia lipolytica*	AV: 1.54×10^7 SD: 5.90×10^6	AV: 3.18×10^7 SD: 8.73×10^6	**AV: 4.36×10^7** SD: 1.28×10^7	AV: 6.50×10^6 SD: 1.29×10^6
B	*Metschnikowia pulcherrima*	**AV: 2.46×10^7** SD: 3.58×10^6	**AV: 2.50×10^7** SD: 6.00×10^6	**AV: 2.40×10^7** SD: 6.43×10^6	AV: 7.05×10^6 SD: 6.86×10^5
C	*Scheffersomyces stipitis*	**AV: 2.82×10^8** SD: 1.31×10^8	**AV: 8.45×10^7** SD: 2.10×10^7	**AV: 3.78×10^8** SD: 4.66×10^7	AV: 3.18×10^7 SD: 8.88×10^6
D	*Kluyveromyces marxianus*	AV: 4.82×10^7 SD: 8.11×10^6	**AV: 6.84×10^7** SD: 1.49×10^7	**AV: 9.08×10^7** SD: 3.66×10^7	AV: 1.34×10^6 SD: 7.33×10^5
E	*Candida humicola*	AV: 1.30×10^7 SD: 3.74×10^6	AV: 4.50×10^7 SD: 1.12×10^7	AV: 5.60×10^7 SD: 2.30×10^7	AV: 4.45×10^6 SD: 4.00×10^5
F	*Candida utilis*	**AV: 2.23×10^8** SD: 8.54×10^7	**AV: 3.89×10^8** SD: 7.09×10^7	**AV: 2.87×10^8** SD: 7.37×10^7	AV: 2.43×10^7 SD: 1.69×10^7
G	*Candida utilis* R6	**AV: 3.74×10^7** SD: 6.91×10^6	AV: 1.36×10^7 SD: 4.16×10^6	AV: 3.28×10^7 SD: 7.40×10^6	AV: 2.34×10^7 SD: 5.29×10^6
H	*Candida utilis* R7	AV: 4.13×10^7 SD: 7.26×10^6	**AV: 1.78×10^7** SD: 7.36×10^6	AV: 4.52×10^7 SD: 1.28×10^7	AV: 5.95×10^6 SD: 2.14×10^6
I	*Saccharomyces bayanus*	**AV: 1.34×10^8** SD: 2.08×10^7	AV: 8.60×10^7 SD: 2.45×10^7	**AV: 2.03×10^8** SD: 5.03×10^7	AV: 3.56×10^7 SD: 1.11×10^7
J	*Saccharomyces cerevisiae* TT	AV: 1.07×10^8 SD: 2.69×10^7	AV: 1.01×10^8 SD: 2.13×10^7	AV: 4.16×10^7 SD: 4.39×10^6	AV: 7.23×10^7 SD: 1.74×10^7
K	*Saccharomyces cerevisiae* Ethanol Red	**AV: 2.16×10^8** SD: 2.29×10^7	AV: 1.20×10^8 SD: 2.42×10^7	**AV: 2.36×10^8** SD: 3.04×10^7	AV: 8.08×10^6 SD: 9.43×10^5

Note: The bolded average values indicate statistically significant differences from the control sample, confidence level less than 0.05

Table 6 shows the results for press-dried SBP-based medium. The bolded average values indicate statistical significant differences from the control sample (Tukey test,

$p<0.05$). All tested strains were capable of growth on the fresh SBP medium. The highest number of cells for portion (I) was registered for *Scheffersomyces stipitis* (C), at 2.82×10^8 CFU/mL. Portion (II) produced the greatest number of cells for *Candida utilis* LOCK 0021 (F), at 3.89×10^8 CFU/mL. A further increase in biomass dose (III) resulted in the highest CFU for *Scheffersomyces stipitis* (C), at 3.78×10^8 CFU/mL. The influence of the biomass portions on the number of yeast cells varied. Increases in the biomass portion did not correlate with a linear increase or decrease in microbial growth.

Table 6. Yeast Growth on Press-Dried Sugar Beet Pulp-Based Medium (CFU/mL)

Strain	Full Strain Name	IV	V	VI	Control Sample
A	*Yarrowia lipolytica*	**AV: 1.34×10^8** SD: 3.12×10^7	AV: 2.34×10^7 SD: 1.06×10^7	AV: 5.36×10^7 SD: 9.68×10^6	AV: 2.46×10^7 SD: 3.92×10^6
B	*Metschnikowia pulcherrima*	**AV: 1.21×10^8** SD: 1.03×10^7	**AV: 1.03×10^8** SD: 9.25×10^6	**AV: 9.10×10^7** SD: 3.00×10^6	AV: 2.46×10^7 SD: 2.08×10^6
C	*Scheffersomyces stipitis*	**AV: 1.27×10^8** SD: 3.42×10^7	**AV: 1.32×10^8** SD: 1.44×10^7	**AV: 1.66×10^8** SD: 3.28×10^7	AV: 3.38×10^7 SD: 1.74×10^7
D	*Kluyveromyces marxianus*	**AV: 1.20×10^8** SD: 2.00×10^7	**AV: 9.44×10^7** SD: 3.30×10^7	**AV: 1.19×10^8** SD: 2.08×10^7	AV: 1.22×10^7 SD: 1.92×10^6
E	*Candida humicola*	**AV: 6.70×10^7** SD: 7.20×10^6	**AV: 7.88×10^7** SD: 7.44×10^6	**AV: 1.02×10^7** SD: 1.04×10^7	AV: 3.62×10^7 SD: 1.10×10^7
F	*Candida utilis*	AV: 1.02×10^8 SD: 7.20×10^6	**AV: 8.16×10^7** SD: 8.32×10^6	**AV: 1.02×10^8** SD: 1.87×10^7	AV: 1.37×10^7 SD: 1.74×10^6
G	*Candida utilis* R6	**AV: 2.14×10^7** SD: 4.48×10^6	**AV: 1.58×10^7** SD: 4.64×10^6	AV: 3.27×10^6 SD: 2.32×10^6	AV: 2.75×10^6 SD: 1.00×10^6
H	*Candida utilis* R7	AV: 5.76×10^6 SD: 2.89×10^6	AV: 7.80×10^6 SD: 1.84×10^6	AV: 1.43×10^7 SD: 5.25×10^6	AV: 3.80×10^6 SD: 4.56×10^5
I	*Saccharomyces bayanus*	**AV: 1.23×10^8** SD: 1.34×10^7	**AV: 7.66×10^7** SD: 1.57×10^7	**AV: 1.06×10^8** SD: 5.28×10^6	AV: 1.27×10^7 SD: 2.26×10^6
J	*Saccharomyces cerevisiae* TT	AV: 4.30×10^7 SD: 1.40×10^7	**AV: 1.20×10^8** SD: 3.60×10^7	AV: 5.22×10^7 SD: 1.78×10^7	AV: 1.14×10^7 SD: 6.25×10^6
K	*Saccharomyces cerevisiae* Ethanol Red	**AV: 1.34×10^8** SD: 1.52×10^7	**AV: 1.58×10^8** SD: 2.64×10^7	**AV: 1.56×10^8** SD: 2.08×10^7	AV: 3.54×10^7 SD: 7.68×10^6

Note: The bolded average values indicate statistically significant differences from the control sample, confidence level less than 0.05

Yeast growth on dried SBP-based medium for sample portion (IV) was highest with two strains of *Yarrowia lipolytica* (A) and *Saccharomyces cerevisiae* Ethanol Red (K), at 1.34×10^8 CFU/mL. In both cases, microbial growth showed statistically significant differences from the control sample. Increasing the biomass dose (V) had a positive influence only on *Saccharomyces cerevisiae* Ethanol Red (K), which had the highest cell count of all the strains, at 1.58×10^8 CFU/mL. However, the highest biomass concentration (VI) created the most favorable conditions for *Scheffersomyces stipitis* (C), resulting in a cell count of 1.66×10^8 CFU/mL.

The number of yeast cells demonstrates the efficient use of sugars released as a result of enzymatic hydrolysis (Tables 2 and 3). Depolymerized polysaccharides provided an excellent source of carbon for the tested strains. The strains were capable of growth on various loads of SBP waste biomass. However, their individual growth performance varied under various biomass loads.

Both the metabolic capabilities of the microorganisms and the conducted method of biomass pretreatment are limiting and decisive factors determining the mode of large-scale production (Basen *et al.* 2014; Frigon 2020). The type of waste biomass and amount of hydrolysed material (high gravity fermentation) also have a significant impact on microbial growth (Xu *et al.* 2019; Debourg 2010).

Protein Enrichment

After fermentation, the biomass and post-culture fluid were separated by centrifugation. The protein content of the biomass was measured using the Kjeldahl method. Waste biomass was used as a control sample. Figures 2 and 3 present the increase in protein after fermentation for fresh and dried waste biomass, respectively, for strains characterized by protein increment over 1.0%.

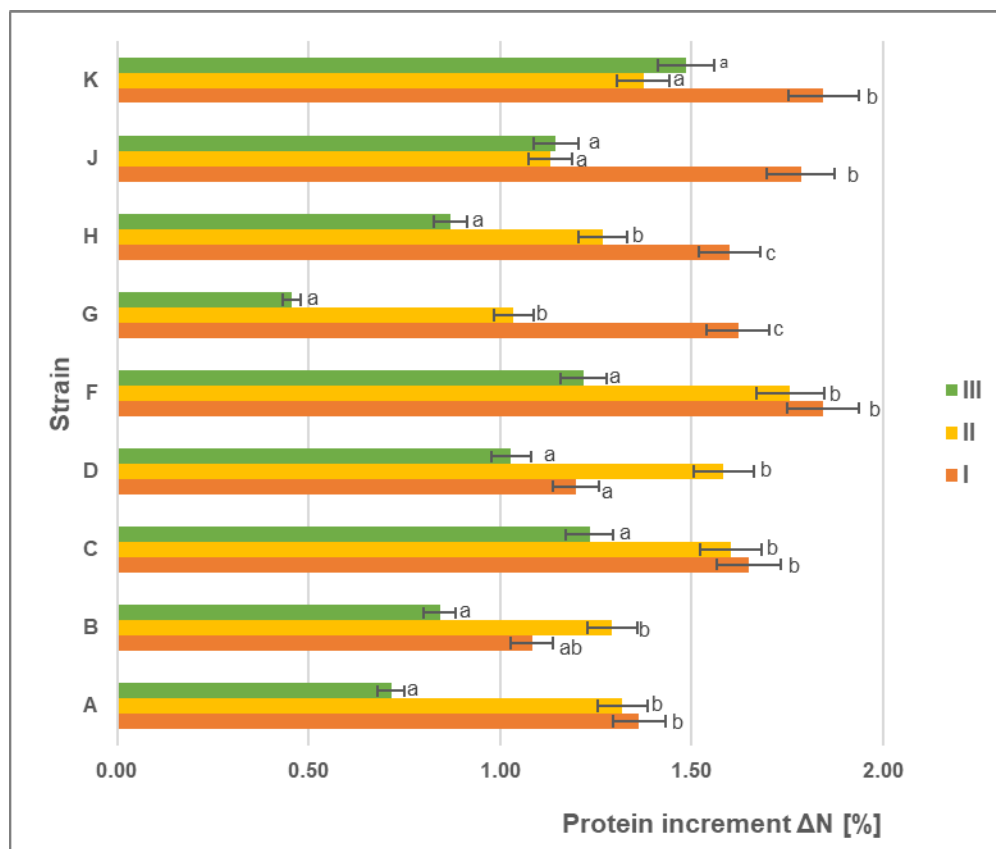

Fig. 2. Protein increase in fresh sugar beet pulp biomass after fermentation with various biomass portions (I – 9 g/100 g, II – 10 g/100g, III – 11 g/100g) [%]. (a,b,c – indicators of statistically significant differences; results for single strains with different letters indicate significant differences; Tukey's test, $p<0.05$).

All the tested strains increased the protein content in SBP. The protein increase for biomass load (I) ranged from ΔN 1.08% to 1.84%, with highest increment for *Candida utilis* and *Saccharomyces cerevisiae* Ethanol Red. Biomass load (II) increased protein content by ΔN 1.03 to 1.76%, with the highest value for *Candida utilis*. Cultivation with portion (III) increased protein growth by ΔN 0.46 to 1.49%, with the most favorable results for *Saccharomyces cerevisiae* Ethanol Red. The greatest increases in protein content were obtained for portion (I).

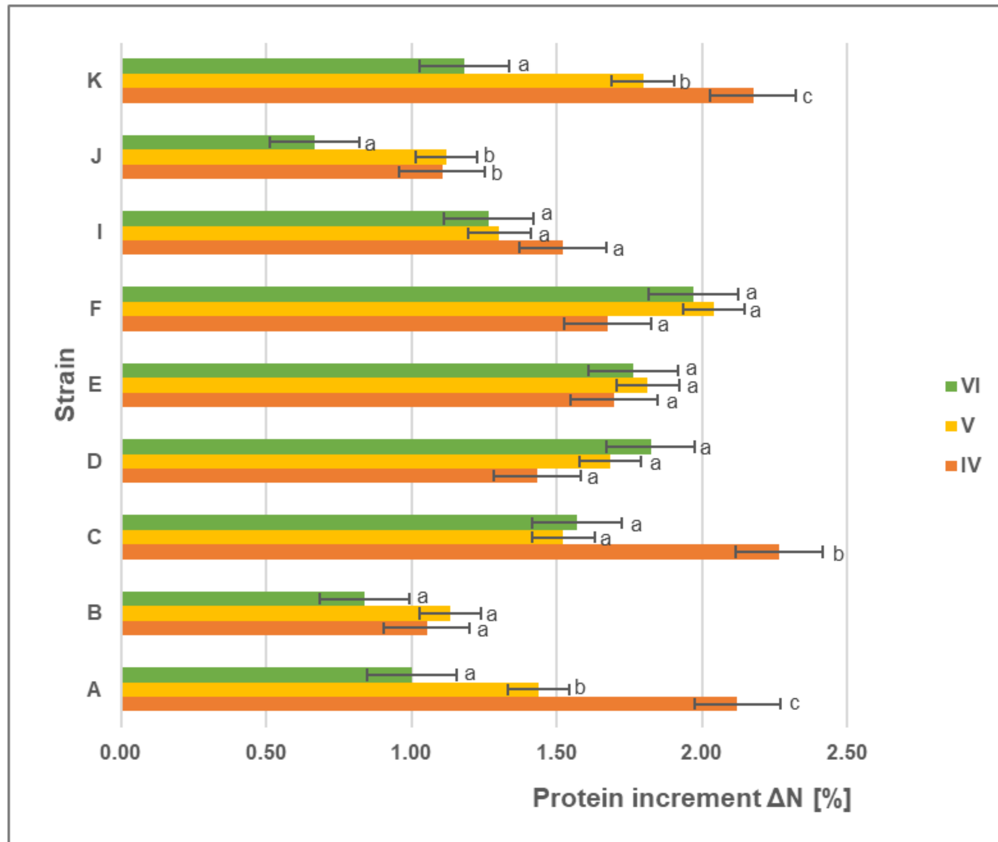

Fig. 3. Protein increase in press-dried sugar beet pulp biomass after fermentation with various biomass portions (IV – 9 g/100g, V – 11 g/100g, VI – 13 g/100g). [%]. (a,b,c – indicators of statistically significant difference; results for single strains with different letters indicate significant differences; Tukey's test, *α*<0.05).

The results for press-dried SBP also showed increases in protein content, ranging from ΔN 1.05% to 2.27%. Fermentation with *Scheffersomyces stipitis* (C) increased protein by 2.27% in biomass portion (IV). The protein increase for portion (V) was lower, between 1.07% and 2.04%, with the highest growth for *Candida utilis* (F). The highest dose, portion (VI), caused the lowest increase of protein, ranging from ΔN 0.67% to 1.97%, with the best result for *Candida utilis* (F). The amount of protein biosynthesized by the strain is an individual feature dependent on the organism, the quality and quantity of the sources of carbon, nitrogen, and phosphorus, and the method of cultivation. The yeast protein content produced from specific waste substrates by different yeast species can vary, from 26.0 to 70.4% of dry mass content (Jach *et al.* 2022; Dever *et al.* 2016). The amount of CFU is not necessarily directly correlated to the protein content.

Candida utilis is commonly used in industry for the production SCP, due to its metabolic capabilities. This strain was used as an alternative source of protein during World War I (Inskeep *et al.* 1951; Buerth *et al.* 2016). Due to its high nutritional value and rich amino acid profile, *Candida utilis* can be used as a nutritional supplement for land and marine animals (Øverland *et al.* 2013). *Saccharomyces cerevisiae* is another strain widely used by industry, including breweries, distilleries, bakeries, and the dairy industry (Parapouli *et al.* 2020). *Saccharomyces cerevisiae* is capable of growing under various environmental conditions, with various carbohydrates as sources of carbon. Its Generally Recognized as Safe (GRAS) status allows applications in feed production (FDA 1998).

Using various types of waste biomass and strains with different metabolic characteristics on an industrial scale requires adapting the developed technology to handle large quantities of the biomass and optimizing process conditions (Nandy and Srivastava 2018). It is necessary to find the optimal combination of protein gain for the largest possible load of waste biomass. Based on the results of this study, the optimal strains for industrial adaptation, in terms of the greatest protein increment, are *Saccharomyces cerevisiae* Ethanol Red for fresh SBP and *Candida utilis* for press-dried SBP.

Free Amino Nitrogen (FAN) Assimilation

The post-culture liquid was separated and analyzed for free amino nitrogen content. Enzymatic hydrolysis results in the release of carbohydrates, which can be used as a source of carbon for yeast growth. It is also necessary to provide a source of nitrogen, in the form of inorganic compounds (ammonium sulphate) and of organic proteins and peptides derived from waste biomass (sugar beet pulp). Figures 4 and 5 show the content of nitrogen compounds [mg/L] in the post-culture liquid, for the same strains presented in the section 'Protein Enrichment' for each type of biomass. The content of nitrogen compounds was determined by the spectrophotometric method. A sample of hydrolyzed non-fermented waste biomass was used as a control.

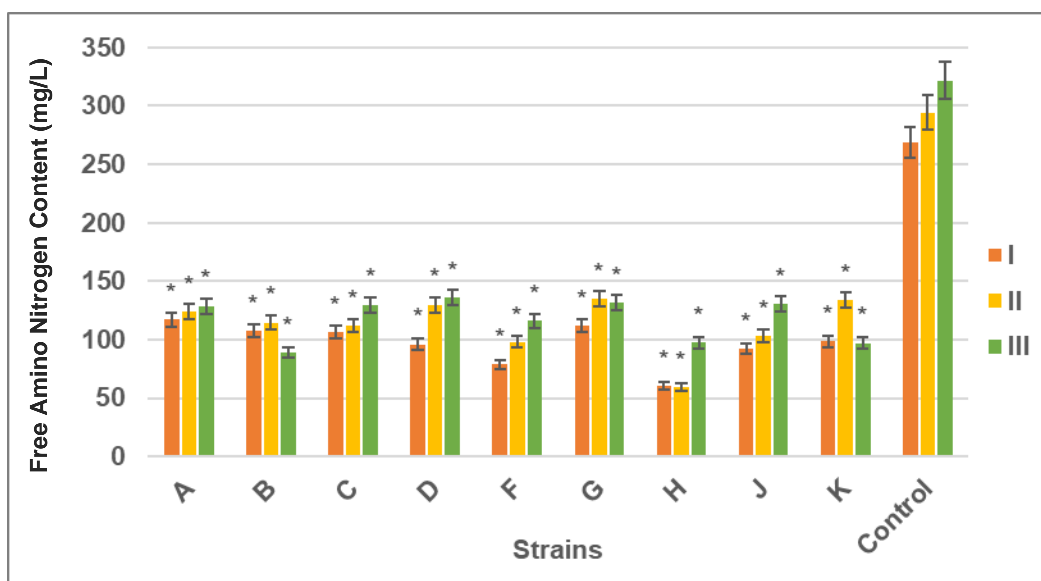

Fig. 4. Free amino nitrogen (FAN) content in fermented fresh sugar beet pulp samples with various biomass portions (I – 9 g/100 g, II – 10 g/100 g, III – 11 g/100g) [%]. * – indicates statistically significant difference relative to the control sample (*p*<0.05).

The measurements of FAN content in samples of fresh sugar beet pulp showed that all the tested strains were capable of assimilating nitrogenous compounds from the environment. The concentrations of FAN in the post-culture liquid from all biomass portions were in range of 59.5 to 136.1 mg/L. Portion (I) of fresh SBP resulted in the highest decrease in FAN with *Candida utilis* R7 (60.7 mg/L; 4.4 times less than the control sample). Dose (II) resulted in a higher concentration FAN after fermentation, with the most efficient assimilation for *Candida utilis* R7 (59.5 mg/L; 4.9 times less than the control). Biomass portion (III) showed the greatest assimilation of FAN with *Saccharomyces*

cerevisiae Ethanol Red (97.3 mg/L; 3.3 times less than the control). In most of the samples, increasing the biomass portion negatively influenced the efficiency of FAN assimilation.

Fermentation of dried sugar beet pulp led to a reduction in FAN content with all tested yeasts (67.8 to 194.5 mg/L). Portion (IV) resulted in the highest FAN reduction with *Metschnikowia pulcherrima* (70.6 mg/L; 3.9 times less than the control). This strain also resulted in the largest FAN reduction with portion (V) (67.8 mg/L; 4.3 times less than the control). The largest biomass portion (VI) resulted in the highest concentration of FAN in the post-culture liquid. The most efficient assimilation was recorded for *Candida humicola* (93.1 mg/L; 3.3 times less than the control). Increasing the biomass portion resulted in a greater concentration of residual nitrogenous compounds in the post-culture liquid.

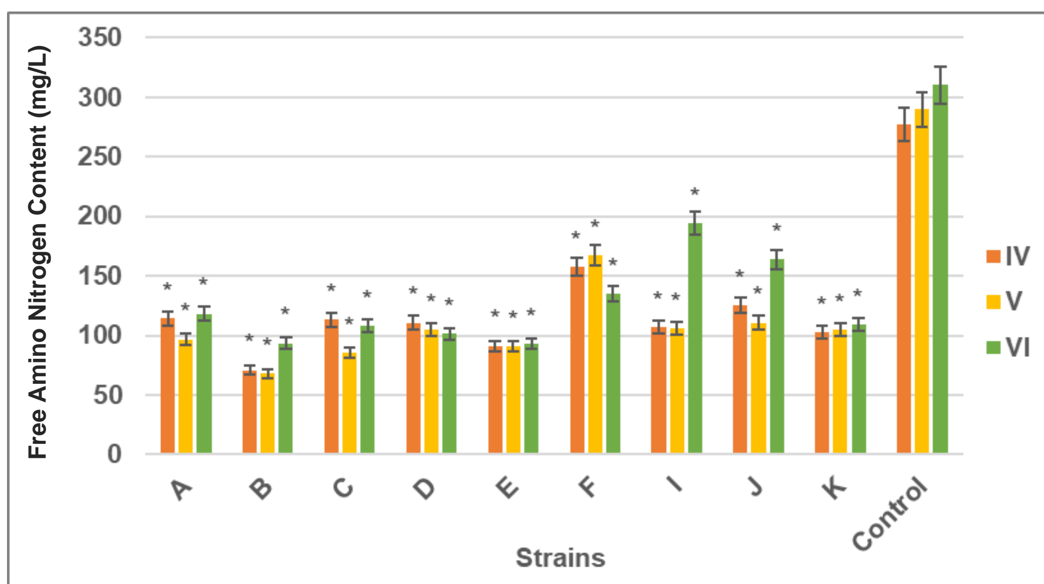

Fig. 5. Free amino nitrogen (FAN) content in fermented press-dried sugar beet pulp samples with various biomass portions (IV – 9 g/100 g, V – 11 g/100 g, VI – 13 g/100g) [%]. * – indicates statistical significant difference relative to control sample (p<0.05).

Free amino nitrogen (FAN) content is very useful parameter that is commonly used in the brewery industry. It measures the compounds in wort that affect the fermentation process and the final aroma of the beer (Hill and Stewart 2019). Measuring the FAN in post-culture liquid reveals the amounts of nitrogenous compounds that were absorbed by yeasts throughout the process of bioconversion into single cell protein, and of any residual nitrogenous compounds (Nasseri *et al.* 2011). Determination of FAN enables accurate control of the bio-conversion of inorganic nitrogen compounds to organic forms of proteins. It also enables estimation of the efficiency of assimilation and utilization of nitrogen by yeast during fermentation. All the tested strains significantly absorbed nitrogen present in the culture medium.

Crude Fiber Content

Non-hydrolyzed, non-fermented waste biomass served as a control sample. Six yeast strains producing the most favorable protein increases for each type of biomass were chosen for CF analysis (for fresh SBP: *Scheffersomyces stipitis* – C; *Candida utilis* – F; *Candida utilis* R6 – G; *Candida utilis* R7 – H; *Saccharomyces cerevisiae* TT – J; *Saccharomyces cerevisiae* Ethanol Red – K; for press dried SBP: *Yarrowia lipolytica* – A;

Scheffersomyces stipitis – C; *Kluyveromyces marxianus* – D; *Candida humicola* – E; *Candida utilis* – F; *Saccharomyces cerevisiae* Ethanol Red – K). Figure 6 shows the CF content for the fresh and dried sugar beet pulp after fermentation with the selected strains.

The selected yeasts decreased the CF content in fresh SBP biomass within the range of 10.5 to 15.0 g/100 g, compared to the control sample CF content of 20.4 g/100 g. The largest reduction in fiber for (I) was registered for *Saccharomyces cerevisiae* Ethanol Red (11.5 g/100 g). A higher dose (II) resulted in a favorable result for *Candida utilis* R6 (10.6 g/100 g). However, further increasing the biomass portion (III) resulted in an even lower CF content for the same strain (10.5 g/100 g). Increasing the biomass portion resulted in lower CF content with *Scheffersomyces stipitis*, *Candida utilis*, *Candida utilis* R6 and R7, but lower CF reduction with the two *Saccharomyces* strains.

All the tested strains were capable of reducing CF content in press-dried SBP biomass during fermentation. Various biomass portions delivered different CF concentrations. The greatest reduction with portion (IV) was registered for *Candida utilis* at 14.0 g/100 g CF, compared to the control sample at 29.0 g/100 g CF. Fermentation with portion (V) also resulted in the largest decrease in CF with *Candida utilis* (15.0 g/100 g). However, portion (VI) showed the most significant reduction in CF with *Yarrowia lipolytica* (13.1 g/100 g). Overall, increasing the biomass load resulted in greater reductions in CF content with all the tested strains.

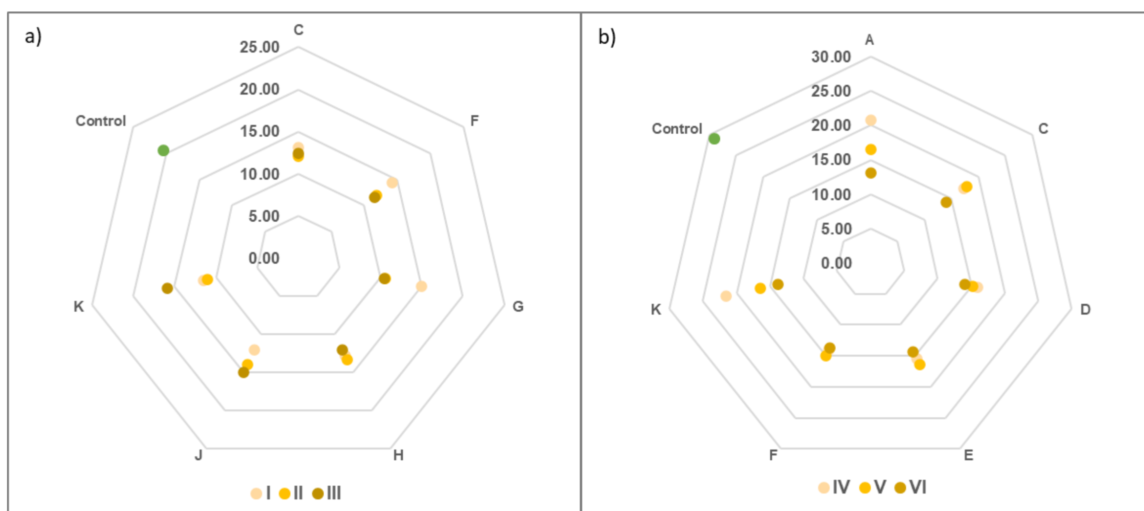

Fig. 6. Crude fiber content in (a) fresh and (b) press-dried SBP after fermentation with selected strains (g/100 g) under various portions of biomass (I – 9 g/100g, II – 10 g/100g, III – 11 g/100g, IV – 9 g/100g, V – 11 g/100g, VI – 13 g/100g).

One of the important parameters for feed is the CF content of the biomass. The fiber content determines its usefulness as food for particular animals (Chuang *et al.* 2021). Since CF has a high content of cellulose, hemicellulose, and lignin, it may prevent the product from being used as feed, due to possible digestive complications leading to poor growth (Singh and Kim 2021). Based on the literature, the fiber fraction content in SBP is composed of 22 to 30% cellulose, 24 to 32% hemicellulose, and 3 to 6% lignin (Grahovac and Rončević 2021). On an industrial scale, the largest reduction in fiber is from the cellulose fraction, due to the simplicity and low financial cost of the hydrolysis process, compared with hydrolysis of the hemicellulose fraction (Houfani e*t al.* 2020). Nevertheless, problems with cellulose hydrolysis may be caused by cellulase inhibitors

present in SBP, including xylose (Qing *et al.* 2010). Another way to liquify of sugar beet pulp biomass and reduce other CF and pectin fractions is to carry out enzymatic processes with peroxidase and laccase. However, gelation occurs, which is not expected in the present study (Bonnin *et al.* 2009).

Microorganisms that can decompose fiber are particularly useful for regulating fiber content in feed biomass, simultaneously improving its nutritional value (Shi *et al.* 2020). The studied strains show the ability to reduce CF content with the simultaneous production of SCP.

CONCLUSIONS

1. All of the tested strains were found to be capable of growing on various biomass loads of sugar beet pulp (SBP) waste biomass. However, they showed different growth performance under various biomass loads. The metabolic capability of each strain was not dependent on the environmental conditions, but rather on different factors such as biomass loads, the composition of the biomass, and the form of the biomass (fresh or dry).
2. The greatest increase in protein content for both tested forms of SBP was registered for the lowest biomass loads. A low biomass portion means a higher share of water in the sample, which means better conditions for microbial growth.
3. Taking into consideration industrial requirements, sustainability goals, and the maximum possible biomass loads, *Saccharomyces cerevisiae* Ethanol Red should be used with fresh SBP, and *Candida utilis* with press-dried SBP.
4. Yeast fermentation is effective at reducing the crude fiber content of SBP biomass. Depending on the animal for which the feed is intended, different crude fiber contents are acceptable. At the maximum biomass load, the greatest reductions in crude fiber content were achieved with *Candida utilis* R6 (10.48 g/100 g) on fresh SBP and *Yarrowia lipolytica* (13.14 g/100 g) on press-dried SBP.

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