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Distillery wastewater decolorization by *Lactobacillus plantarum* MiLAB393

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Abstract: Sugar beet molasses vinasse is a high-strength distillery wastewater. It contains colored substances which significantly affect the degree of pollution and toxicity of vinasse. This study aimed to optimize the medium composition and the process condition of sugar beet molasses vinasse decolorization by *Lactobacillus plantarum* MiLAB393. The research was conducted in two stages: the shake-flask stage in the 250 cm^3 Erlenmeyer flasks and the batch experiments in the 5 dm^3 working volume stirred-tank bioreactor. During the study, the concentrations of glucose and yeast extract were optimized using experimental design of experiments (DOE). The influences of the initial value of pH and pH control, temperature, stirrer speed and glucose concentration on decolorization were tested. The highest color reduction of 24.1% was achieved for an experiment in which 24.93 g/dm^3 of glucose was added to the medium and stirrer speed was 200 rpm. This efficiency of 30% v/v sugar beet molasses vinasse decolorization was obtained at non-controlled pH 6.0 and at 35.8°C. It was found that pH control determines vinasse decolorization. When the pH was controlled, decolorization did not exceed 9%. The glucose and yeast extract concentration and the stirrer speed have a great influence on the process. Changes in these parameters may increase biomass growth while decreasing the decolorization.

Introduction

Beet molasses is the only raw material in Polish distilleries which does not compete with food production. Sugar beet molasses vinasse (BMV) is a byproduct of processing molasses for ethanol. Its characteristic feature, in addition to the high loads of organic pollutions expressed by the chemical and biological oxygen demand, is a low pH and a dark brown color (Ryznar-Luty et al. 2015, Wilk et al. 2019). Melanoidins, caramel compounds and hexoses alkaline degradation products (HADP) contribute to the intense color of the vinasse and have a huge influence on the degree of its pollution and toxicity (Agnihotri 2015, Arimi et al. 2014, Chowdhary et al. 2018). Because of the vinasse color it is not recommended to drain this wastewater into water surfaces, even after lowering indicators of pollution, because it would limit the penetration of sunlight and disrupt the functioning of aquatic flora and fauna (Agarwal et al. 2010, Chandra et al. 2008). Furthermore, because of its chemical composition, sugar beet molasses vinasse can be used as feed additive in only a few percent. For this reason, it is more likely to use the grain and corn vinasse. BMV could be used as a partial supplement of agricultural fertilizer on fields located near the distillery. It should be noted, however, that on such fields only crops not intended for food production can be cultivated. Another problem is the time delay between the production of the vinasse and the point at which it could be used as fertilizer on fields (Szoege and Wiśniewski 2013).

Although the chemical and physicochemical methods allow effective decolorization of distillery vinasse, they are criticized for their high costs and excessive pollutant load in the form of undesirable products which are toxic or in the form of a large amount of sludge as secondary pollutant (Sridevi et al. 2011, Chowdhary et al. 2018). The alternative seems to be the method of microbial decolorization. Researchers used fungi (Agnihotri 2015, Shukla et al. 2014), yeast (Tiwari et al. 2014, Mahgoub et al. 2016) and bacteria (Santal et al. 2016, Boopathy and Senthilkumar 2014) for color removing. Because of high pollution loads, many researchers treat vinasse before decolorization. The vinasse is centrifuged, filtered or anaerobically treated (Tiwari and Gaur 2014, Boopathy and Senthilkumar 2014, Tondee and Sirianuntapiboon 2008, Ravikumar et al. 2013). Other researchers did not use wastewater from distilleries but synthetic melanoidins, which are easier to decolorize (Agnihotri 2015, Santal et al. 2016). In our study we used sugar beet molasses vinasse without pretreatment. Moreover, this kind of wastewater has not been tested by other researchers because they have focused on cane vinasse (Espana-Gamboa 2015, Shukla 2014).

The aim of our study was to optimize the medium composition and the process conditions of sugar beet molasses vinasse decolorization by *Lactobacillus plantarum* MiLAB393. The optimization of the concentrations of glucose and yeast extract using experimental design (DOE) was made at the stage of shake-flask cultures. During the batch experiment, the

influence of the initial value and adjusting pH, temperature, stirrer speed and glucose concentration on the degree of decolorization were estimated.

Materials and methods

Vinasse

In the study one batch of the sugar beet molasses vinasse (BMV) was used. It was collected from the CHECO Manufacturing Plant, Ltd., Włocławek, Poland. The vinasse was stored in a sealed container, at -20°C. Chemical oxygen demand COD, biochemical oxygen demand BOD_5 and total organic carbon TOC of BMV were [g/dm^3]: 89.5; 261.7 and 35.5, respectively. The pH and density were 5.0 and 22°Blg, respectively. The wastewater absorbance measured at 475 nm was 10.48. BMV detailed characteristics are shown in Table 1.

Microorganism

Lactobacillus plantarum MiLAB393 was obtained from the Department of Microbiology of the Swedish University of Agricultural Sciences in Uppsala. The strain was stored in the MRS medium (de Man, Rogosa and Sharpe; Biocorp, Poland) with 10% v/v glycerol, at -65°C.

Inoculum

The thawed bacterial cell suspension of a volume of 0.1 cm^3 was activated in 100 cm^3 of sterile MRS medium. The culture was incubated at 37°C for 72 hours under static conditions. After this time, the culture medium was inoculated with 1 cm^3 of a bacteria suspension in MRS medium, which corresponded to 1.5 g/dm^3 of bacteria dry weight. Dry weight of cell mass was measured after drying at 105°C to constant weight.

Process Condition

Shake-Flask Processes

In the first step of this research, the rotatable central composite experimental design (N_0=4, α=1.4142, N=10) was used to optimize the glucose (X_1) and yeast extract (X_2) content in the BMV during its decolorization by *Lactobacillus plantarum* MiLAB393 (Table 2). Coded variables have been assigned to the applied experimental plan as given in Table 3.

Table 1. Sugar beet molasses vinasse characterization

Parameter	Value [g/dm^3]
Glycerol	3.9 ± 0.19
Glucose	1.42 ± 0.07
Total nitrogen	5.075 ± 0.205
Total phosphorus	0.1 ± 0.02
Lactic acid	20.4 ± 1.02
Acetic acid	2.22 ± 0.11
Pyroglutamic acid	8.51 ± 0.43
Succinic acid	11.65 ± 0.58
Isobutyric acid	21.07 ±1.05
Tartaric acid	1.04 ± 0.05
Gluconic acid	2.17 ± 0.11
Hexoses alkaline degradation products (HADP)	20.07 ± 1
Caramels	1.75 ± 0.09
Melanoidins	2.91 ± 0.15

Table 2. Coded variables in the matrix of the experiment

Run	Factor	
	X_1	X_2
1	-1	-1
2	-1	1
3	1	-1
4	1	1
5	0	0
6	-2	0
7	2	0
8	0	-2
9	0	2
10	0	0

Table 3. Values assigned to the plan

Level	Factor	
	X_1	X_2
-2	0.25	0.64
-1	5.0	1.0
0	25.0	7.0
1	40.0	10.0
2	49.75	13.37

Two factors were coded on five levels. The mathematical model of the experimental plan was expressed by the following equation:

$$Y = \beta_0 + \Sigma\beta_1 X_1 + \Sigma\beta_2 X_2 + \Sigma\beta_{1i} X_1^2 + \Sigma\beta_{2i} X_2^2 + \Sigma\beta_{12} X_1 X_2$$

Where:
Y– criterion of optimization (response function, the degree of decolorization [%]);
X_1, X_2 – coded variables;
β_0, β_1, β_2, β_{1i}, β_{2i}, β_{12} – model coefficients.

Fit of the model is expressed by a coefficient of determination R^2. The statistical significance was tested using the Fisher test (F). The statistical significance of the regression coefficients was evaluated by Student test (t). The results of the experimental design were analyzed and interpreted using STATISTICA version 10 (StatSoft, Inc., 2011, USA) statistical software.

Experiments were carried out in triplicate (10 experimental runs with three repetitions) for 4 days in the Erlenmeyer flasks (250 cm^3) at 35.78°C and 120 rpm. Glucose (GLU) and yeast extract (YE) were added to the culture medium (100 cm^3, BMV 30% v/v) in amounts according to the experimental plan. The initial pH of the medium was adjusted by 33% NaOH to the level 6.5. The pH, temperature and vinasse concentration values were adopted from our previous studies (Wilk et al. 2017) in which the statistical optimization was performed.

Bioreactor Experiments

The experiments were performed in a 5 dm^3 working volume stirred-tank bioreactor (Biostat_B, B. Braun Biotech International) with a stirrer speed of 200 rpm and no aeration. When investigating the effect of stirrer speed ("*Batch Experiments*" section) the experiments were carried out at stirrer speed of 100, 200 and 300 rpm and no aeration. Experiments were carried out for 84 hours, at 35.8°C. When investigating the effect of temperature ("*Batch Experiments*" section) the experiments were performed at 30, 32, 34, and 35.8°C. The medium contained 30% v/v vinasse, yeast extract (7.19 g/dm^3) and glucose (24.93 g/dm^3). When investigating the effect of carbon source addition ("*Batch Experiments*" section), the addition of glucose at five dosages [g/dm^3]: 6.23, 12.47, 24.93, 49.86 and 99.72 was tested. The carbon source (glucose dissolved in sterile distilled water) was introduced into the medium after sterilization through a membrane filter with a pore diameter of 0.45 µm. The inoculum was added in an amount of 20 cm^3. The pH value was maintained automatically by using 2M H_2SO_4 and 33% NaOH, if the experiment plan required.

The effects of initial pH and its control, temperature, stirrer speed and glucose concentration on decolorizing of vinasse were examined during batch experiments.

Analytical Methods

The decolorization effectiveness and biomass growth were evaluated spectrophotometrically (475 nm and 620 nm, respectively). Melanoidins, caramels and HADP content were determined using the Ivanov-Sapronov method (Krzywonos et al. 2016, Sapronov 1963). The concentrations of glucose, glycerol, and organic acids (lactic, acetic, pyroglutamic, succinic, isobutyric, tartaric and gluconic) were determined by HPLC (Knauer; UV-VIS and RI detectors; column type, Phenomenex ROA organic acids; column size, 7.8 mm i.d.×300 mm; effluent, 5 mM H_2SO_4; flow rate, 0.5 cm^3/min; temperature, 40ºC; wavelength, 210 nm).

Decolorization Yield

The sample was centrifuged (9000 *g*) and the supernatant was analyzed for color intensity at 475 nm with a UV-VIS spectrophotometer. The effectiveness of decolorization (%A) was calculated using the formula: $\%A=(A_0-A_t)/A_0 \cdot 100\%$, where: *A0* – the initial value of the absorbance, *At* – value of the absorbance in the time *t*.

Results and discussion

Shake-Flask Processes

The rotatable central composite experimental design was used to optimize two factors (glucose and yeast extract concentration) on BMV decolorization by *Lactobacillus plantarum* MiLAB393. The factors were coded on five levels (Table 2 and 3). Statistically, the most significant effect ($p \leq 0.05$) of the decolorization process was the yeast extract concentration (Table 4 and 5).

However, it should be taken into account that in the previous studies, it was found that the addition of glucose determined the decolorization process (Wilk et al. 2015). On the basis of the results (Table 4 and 5) a model has been created (R^2=0.81) describing the dependence of the decolorization (Y%) of input variables. After maximizing the value obtained an objective function, the optimum values of factors (X_1 and X_2) were set, which amounted to 24.93 and 7.19 g/dm^3. For these values, a model has been developed that describes interdependence, and the obtained equation is given a form: $Y = 24.622 + 0.91854X_2 - 1.0996X_2^2$.

The effect of glucose and yeast extract concentration on decolorization is shown in Fig. 1.

Maximum decolorization was achieved on the 3rd day of the process. The highest decolorization was 31.32%. In any runs, the degree of HADP and caramel removal was not higher than 20.5% and 23.1%, respectively, and in some runs, a total removal of melanoidins was observed (data not shown). Agnihotri (2015) has investigated decolorization of biomethanated distillery effluent by using spore inoculum of *Aspergillus oryzae* JSA-1. As in our study, the medium contained 30% v/v distillery effluent including: a similar initial concentration of melanoidins (2.91 g/dm^3 in our study and 2.7 g/dm^3 in cited authors study), ten times higher initial concentration of caramel and two times lower initial concentration of HADP. The cited authors achieved 39.07%, 26.47% and 27.35% reduction of melanoidin, caramel and HADP, respectively. If we take into account the initial concentration of the colorants, we have achieved a higher reduction of melanoidins and HADP. Seruga and Krzywonos (2015) have also studied the effect of nutrients' addition on the effectiveness of BWM decolorization by *L. plantarum*. They found that wheat stillage, yeast extract, glucose, peptone, and $(NH_4)_2SO_4$ were significant factors for

Table 4. Regression model coefficient data

Factor	Effect	Standard error	t	p	-95% Confidence level	+95% Confidence level	Regression coefficient
Constants	**24.622**	**0.5895**	**41.767**	**0.000**	**22.986**	**26.259**	**24.622**
X_1	0.907	0.6347	1.430	0.226	-0.855	2.670	0.454
X_1^2	-1.510	0.7895	-1.913	0.128	-3.702	0.682	-0.755
X_2	**1.837**	**0.6432**	**2.857**	**0.046**	**0.051**	**3.623**	**0.919**
X_2^2	**-2.199**	**0.7439**	**-2.957**	**0.042**	**-4.265**	**-0.134**	**-1.100**
$X_1 X_2$	-1.292	0.8432	-1.533	0.200	-3.633	1.049	-0.646

Bolded values p≤0.05

Table 5. Analysis of variance for the fitted model

Factor	Sum of squares	df	Mean sum of squares	F – value	p
X_1	1.542	1	1.542	2.044	0.226
X_1^2	2.760	1	2.760	3.659	0.128
X_2	**6.153**	**1**	**6.153**	**8.159**	**0.046**
X_2^2	**6.593**	**1**	**6.593**	**8.741**	**0.042**
$X_1 X_2$	1.772	1	1.772	2.349	0.200
Error	3.017	4	0.75419		
Total SS	15.600	9			

Bolded values p≤0.05

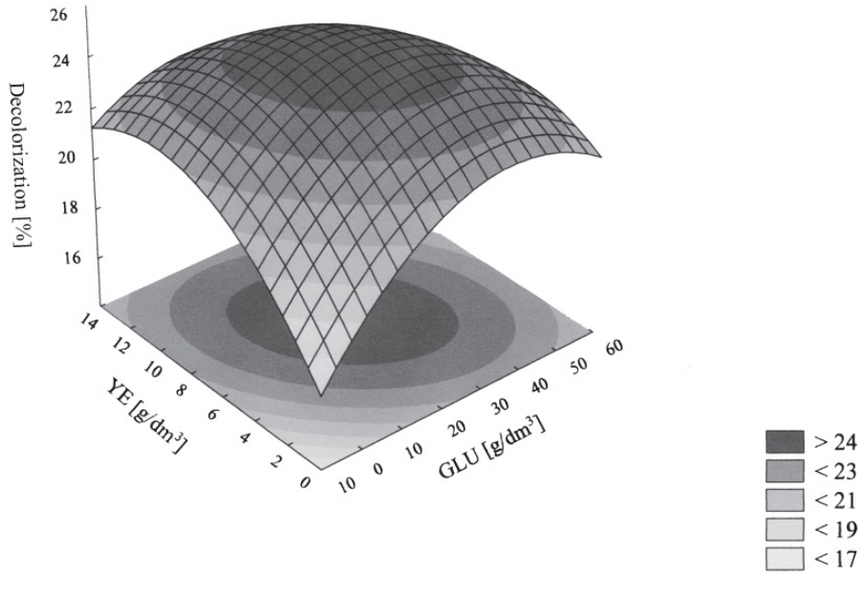

Fig. 1. Effect of glucose (GLU) and yeast extract (YE) concentration on vinasse decolorization

the decolorization process. The cited authors noted a 67% efficiency of decolorization of sugar beet molasses vinasse by *L. plantarum*. It is noteworthy that the achievement of such a result requires use of 150 g/dm^3 of external carbon source (glucose, fructose and sucrose at the same concentration of 50 g/dm^3) and 55 g/dm^3 of nitrogen source (5 g/dm^3 of yeast extract and 50 g/dm^3 of peptone). Besides, the medium contained about 5% of the vinasse less than used in our study. It can be concluded that the composition of the medium and the concentration of the vinasse have a large impact on the efficiency of the process. The role of colorant concentration on decolorization was described by Santal et al. (2016). They used *Paracoccus pantotrophus* in the decolorization of a medium which contained 5 to 25% v/v (at the interval of 5% v/v) distillery effluent. It was observed that the maximum decolorization activity was at the lowest concentration of the distillery effluent. As the distillery effluent concentration increased, the decolorization decreased and a concentration higher than 25% inhibited the growth of bacteria. It is worth recalling that in our study, the concentration of sugar beet molasses vinasse in medium was 30% v/v, so *Lactobacillus plantarum* MiLAB393 showed potential for growth and decolorization of higher concentration of distillery effluent than *Paracoccus pantotrophus.* Tondee and Sirianuntapiboon (2008) in their research used similar amounts of glucose and yeast extract as in the present study. They noted 76.6% decolorization of cane vinasse which was previously subjected to a treated anaerobic. It is worth noting that the vinasse before decolorization was significantly diluted – the absorbance, measured at a wavelength of 475 nm, before dilution amounted to 81.4, and afterwards 3.5. Ravikumar et al. (2013) optimized the parameters of the process using Response Surface Methodology. They investigated decolorization of anaerobically treated distillery spent wash after biomethanation from anaerobic digester by *Cladosporium cladosporioides*. In 30 runs, four factors: pH, fructose, peptone, and inoculum concentration were tested. The cited authors concluded that carbon concentration and pH were highly significant and play a major role compared to the concentrations of inoculum and nitrogen. They also noted the highest integration effect of carbon and pH on decolorization in response. This was explained as acquiring a sufficient carbon source from the spent wash by the organisms in an optimum pH level, leading to an increase in decolorization.

Batch Experiments

Medium pH

It was found that pH control determines vinasse decolorization. When the pH was controlled, decolorization did not exceed 9% (Fig. 2a). Similarly, in our previous study (Wilk et al. 2019), when the consortium of *Lactobacillus casei*, *L. plantarum* and *Pediococcus parvulus* was used at a controlled pH, the decolorization was two times lower than at non-controlled pH.

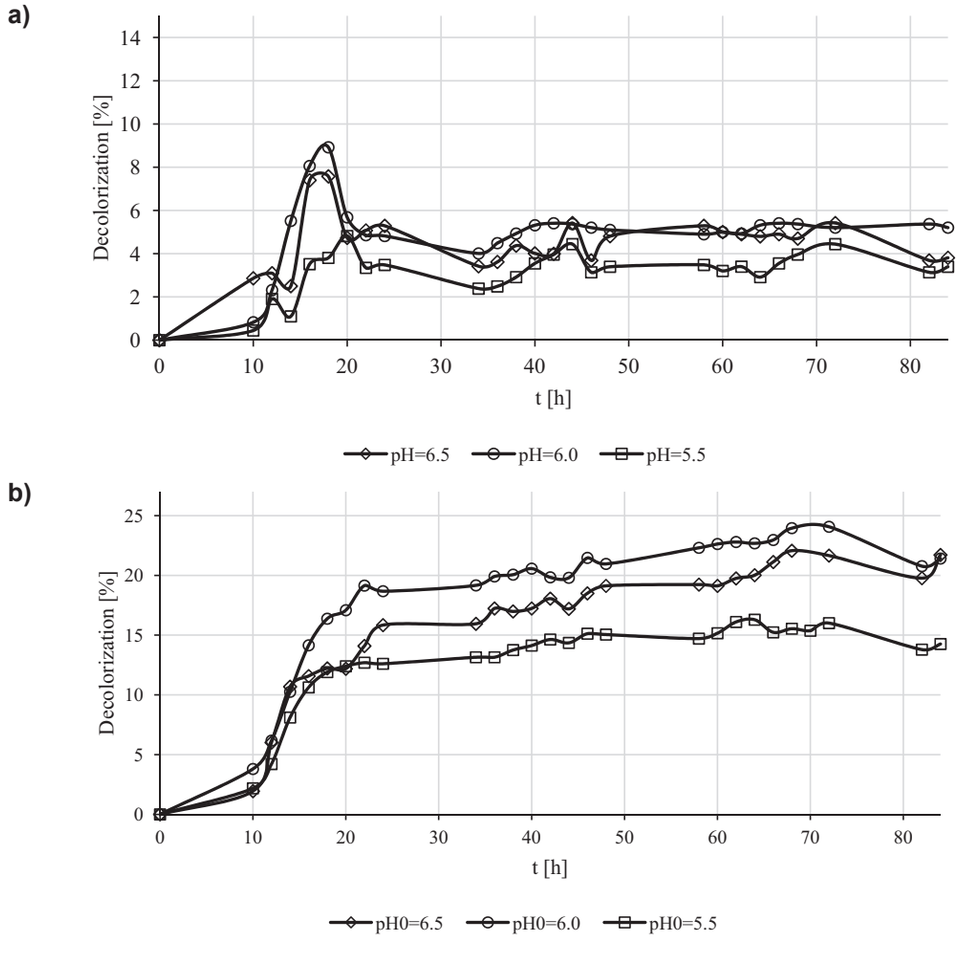

Fig. 2. Effect of controlled (pH) (a) and non-controlled (pH0) (b) pH medium on decolorization process

The highest decolorization (24.01%) was obtained for the experiment when pH_0 was 6.0 (Fig. 2b). In this variant, HADP and melanoidins removal amounting to 19.39% and 100%, respectively was observed, but with an increase of caramel. It is interesting that a reduction in the concentration of caramels was observed only in variants with controlled pH.

Limkhuansuwan and Chaiprasert (2010) achieved 60.91% efficiency during decolorization of molasses melanoidins solution by *L. plantarum* SF5.6. The initial pH was the same as in this work (6.0) but the medium they used included only colorants of molecular weight less than 10,000 Da.

Effect of temperature

The experiments were performed at 30, 32, 34, and 35.8°C. For most runs decolorization did not exceed 20%; the highest removal efficiency, 24.07%, was achieved at 35.8°C (Fig. 3). During our previous study (Wilk et al. 2017) in shake flask we optimized i.a. the temperature value. This factor was statistically significant and also amounted to 35.8°C.

The biggest removal of melanoidins, approx. 94%, was achieved at 30°C. Caramels content increased in all of the conducted experiments. The removal of hexoses alkaline degradation products did not exceed 20% at 32, 34, and 35.8°C. At 30°C the content in the medium hexoses alkaline degradation products increased (data not shown). Colorants removal during the process at 35.8°C is shown in Figure 4.

The optimum temperature for the process is not simultaneously optimal for the growth of the bacteria. In other runs of the test the biomass growth was almost 3-times higher (data not shown). Different observations were made by Zuraida et al. (2013) who used *Lactobacillus delbruckii* in the decolorization of Batik wastewater. The cited authors noted that the optimum temperature for decolorization as well as for bacteria growth was 37°C.

Kaushik and Thakur (2009) optimized the decolorization of vinasse by *Bacillus sp*. They determined the optimum conditions using the Taguchi method. Parameters were coded on two levels and for the temperature it was 30 and 35°C. They, unlike the results that were obtained in our work (the highest color removal efficiency was achieved at 35.8°C), found the optimal temperature to be 30°C.

Effect of carbon source addition

An external carbon source is often necessary in the process of vinasse decolorization using bacteria (Santal et al. 2011). Maltose, sucrose, galactose and fructose are sometimes used as additives to a medium but most often glucose is added (Santal

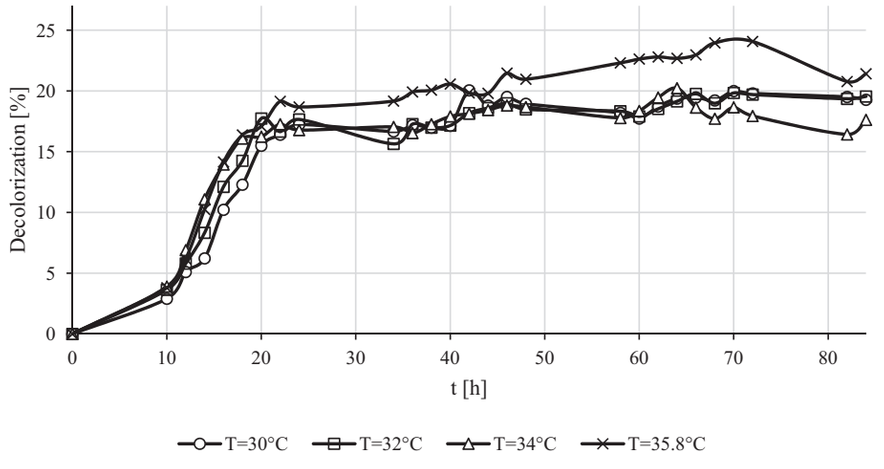

Fig. 3. Effect of temperature on decolorization

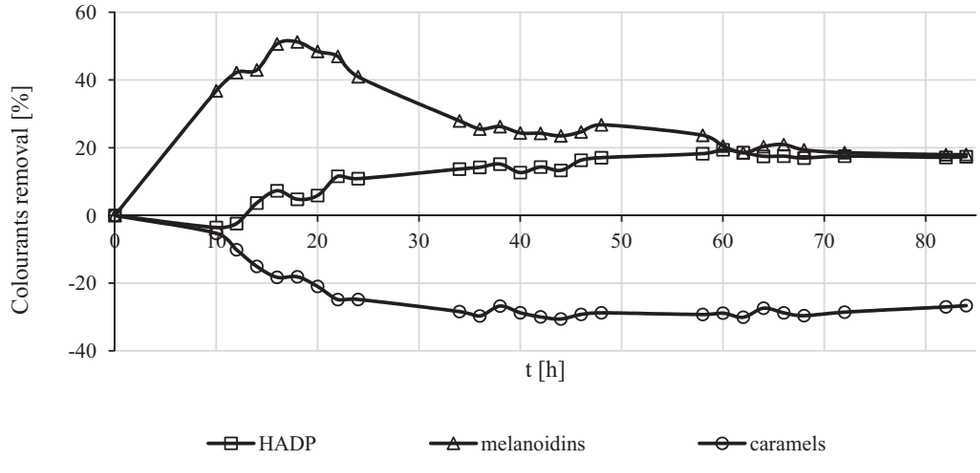

Fig. 4. Decolorization of BMV at 35.8°C

et al. 2016, Boopathy and Senthilkumar 2014, Santal et al. 2011, Gupta et al. 2011, Tiwari et al. 2012). Yadav and Chandra (2012) tested the influence of adding glucose in the range of 1–15 g/dm^3 during decolorization of molasses colorants by bacterial consortium. They noted that the decolorization increased with an increase in glucose concentration from 5 to 10 g/dm^3. It is worth noting that any further increase in the dose of glucose had an inhibitory effect on decolorization but improved the bacterial growth and biomass production. In our work, the addition of glucose at five dosages [g/dm^3]: 6.23, 12.47, 24.93, 49.86 and 99.72 was tested. It was found that the glucose concentration determines the degree of vinasse decolorization. The maximal color removal in experiments with glucose addition of 6.23 g/dm^3 and 12.46 g/dm^3 were respectively 14.83% and 13.1% (Fig. 5).

The highest reduction of color, 24.1%, was achieved for an experiment in which 24.93 g/dm^3 of glucose was added to the medium. Despite the two- and four-fold increases in the dose of glucose in subsequent experiments, the decrease of glucose (in g/dm^3) during the process was almost twice lower than when 24.93 g/dm^3 of carbon source was added to the medium (data not shown). In the experiments with 49.86 and 99.72 g/dm^3 addition of glucose, the maximum degree of decolorization and lactic acid concentration were at a similar level, respectively 21.22%, 20.61% and 18.43 g/dm^3, 18.47 g/dm^3 (data not shown). Interestingly, in the study with the highest dose of glucose, a two times higher increase in biomass was observed than in the other experiments. Because of similar color removal in these experiments we can suppose that the decolorization process occurs through biodegradation or biotransformation of colored compounds, rather than through absorption by the cells of bacteria. Some bacteria, e.g. *Pseudomonas putida,* use glucose oxidase in the extracellular conversion of glucose into gluconic acid and hydrogen peroxide. This product of enzymatic oxidation decolorized vinasse by oxidizing melanoidins (Ghosh et al. 2002).

Effect of stirrer speed

Three stirrer speeds were tested: 100, 200 and 400 rpm. The Figure 6 shows that increasing the speed from 200 to 400 rpm does not improve the ability of the bacteria to effect decolorization of the vinasse. Under these conditions biomass growth was about 104%. On the other hand, when the stirrer speed was 100 rmp, the decolorization was only 14.93% but the biomass growth was more than 3-times higher (352%). It can be inferred that the low stirrer speed, 100 rpm, is a good condition for *L. plantarum* MiLAB393 growth, but not for decolorization, so the best stirrer speed for color removal was 200 rpm. Kaushik and Thakur (2009) also recognized a stirrer speed of 200 rpm as being the most suitable for the decolorization process. On the other hand, Santal et al. (2016) studied decolorization of the synthetic melanoidins by *Paracoccus pantotrophus*. They examined the effect of shaking (180 rpm) and non-shaking conditions. They achieved maximum decolorization in the static condition. When Krzywonos et al. (2017) optimized parameters of sugar beet molasses vinasse decolorization, they found that both static and agitated bacterial culture did not have any effect on the efficiency of decolorization. Tiwari et al. (2014) investigated the effect of static and shaking (20 to 140 rpm, at the interval of 20 rpm) conditions on the melanoidin decolorization. They concluded that bacteria *Pediococcus acidilactici* showed significant decolorization at both process conditions, but for yeast *Candida tropicalis* they noticed maximum decolorization under static condition.

Conclusions

It can be concluded that optimal doses of glucose and yeast extract were essential for sugar beet molasses vinasse decolorization by *Lactobacillus plantarum* MiLAB393. In this study we also revealed that controlled pH was a decolorization inhibitor and that optimal temperature for decolorization was not simultaneously optimal for bacterial growth.

Future study should be concentrated on a continuous processes in a bioreactor. The proposed process, because of microbial color removal, could provide an opportunity for an additional way to wastewater treatment and protection of the environment.

Acknowledgement

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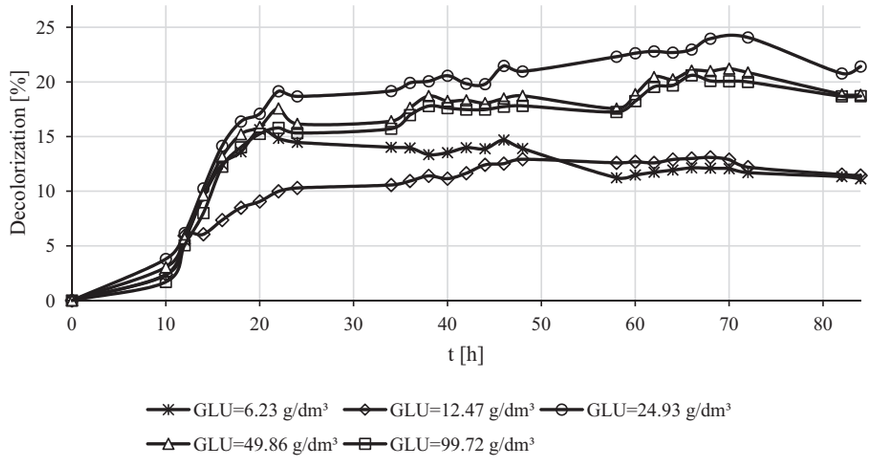

Fig. 5. Effect of various glucose concentrations on decolorization

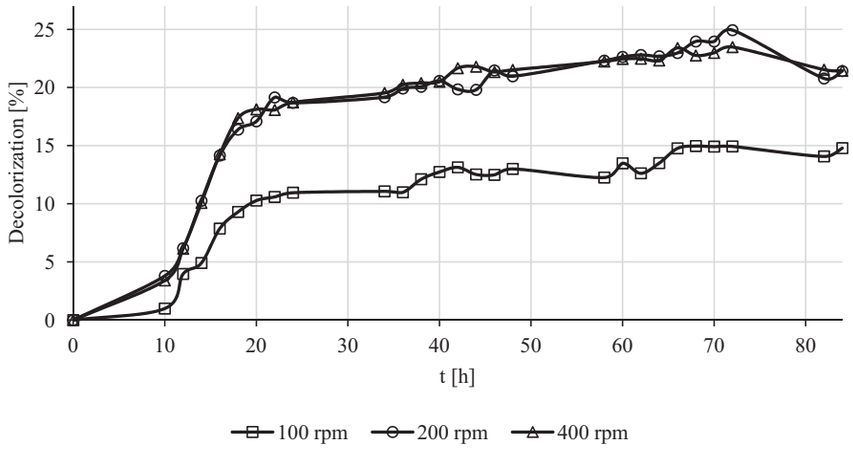

Fig. 6. Effect of stirrer speed on decolorization

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Dekoloryzacja wywaru gorzelniczego przez *Lactobacillus plantarum* MiLAB393

Streszczenie: Buraczany wywar melasowy to gorzelniany produkt uboczny charakteryzujący się wysokim ładunkiem zanieczyszczeń. Zawiera substancje barwne, które znacząco wpływają na jego toksyczność i stopień zanieczyszczenia. Celem pracy było zoptymalizowanie składu podłoża i parametrów procesu dekoloryzacji buraczanego wywaru melasowego przez *Lactobacillus plantarum* MiLAB393.

Badania przeprowadzono w dwóch etapach: w hodowli wytrząsanej w kolbach Erlenmeyera o pojemności 250 cm^3 oraz w eksperymentach okresowych w bioreaktorze o pojemności roboczej 5 dm^3, z mieszaniem. Badania przeprowadzono z wykorzystaniem metody planowania eksperymentu (DOE), dzięki której zoptymalizowano stężenia glukozy i ekstraktu drożdżowego. Badano również wpływ regulacji i początkowej wartości pH oraz wpływ temperatury, prędkości mieszania i stężenia glukozy na stopień usunięcia związków barwnych.

Największą redukcję barwy uzyskano dla doświadczenia, w którym do pożywki dodano 24,93 g/dm^3 glukozy, a szybkość mieszadła wynosiła 200 obrotów na minutę. 24,1% stopień dekoloryzacji 30% v/v buraczanego wywaru melasowego otrzymano przy nieregulowanym pH równym 6,0 i temperaturze 35,8°C.

Stwierdzono, że regulacja pH determinuje proces odbarwiania wywaru. Gdy pH było regulowane, dekoloryzacja nie przekraczała 9%. Stężenie glukozy i ekstraktu drożdżowego oraz prędkość mieszania mają duży wpływ na proces. Zmiany tych parametrów mogą zwiększać wzrost biomasy przy jednoczesnym zmniejszeniu odbarwienia.