Two-stage lactic acid fermentation of distillery stillage

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Lactic acid (LA) has recently achieved considerable importance and growing demand in the global market because of a wide range of applications in chemical, food, pharmaceutical, cosmetic and polymer industries. The world lactic acid consumption is continually increasing, mostly due to the expansion of the application range of biodegradable polymers such as poly-lactides. Economic feasibility is of prime importance in choosing the raw material for fermentative LA production. Utilization of industrial distillery stillage, wastewater from bioethanol production, as a cheap and abundant substrate could be sustainable and environmentally friendly approach. However, since the lactic acid bacteria are nutritionally rather demanding strains, especially regarding the sources of nitrogen, sugars, minerals and vitamins, the cheap and sustainable lactic acid production on the agro-industrial wastes is not an easy task. This is mostly because the agro-industrial wastes are usually nutritionally poor and/or of a complex chemical structure. Therefore, various strategies have been assessed in order to enhance the lactic acid productivity on these substrates. Some of them are: a combination of complementary waste substrates (Mladenović et al., 2016a); the cell adaptation to substrate (Mladenović et al., 2017); selection of the most appropriate fermentation mode (fed batch fermentation, fermentation with immobilized cells), fermentation by a mixed bacterial culture etc. (Mladenović et al., 2016b).

The aim of this study was to assess the effects of a two-stage lactic acid fermentation of distillery stillage. In the first stage the stillage was pretreated with a proteolytic bacteria *Bacillus licheniformis* in order to increase free α-amino nitrogen content in the waste substrate, while in the subsequent stage the lactic acid fermentation with *Lactobacillus paracasei* strain was performed.

The study was performed on an industrial distillery stillage from bioethanol production on waste bread (obtained from Reahem Ethanol Plant, Srbobran, Serbia). After adjustment of the pH in the stillage to 7 with 30% NaOH solution, it was sterilized at 121 °C for 15 minutes and inoculated with 2% (v/v) of *Bacillus licheniformis*-TMFB in order to perform the proteolytic pretreatment in the first fermentation stage. After the pretreatment, the stillage was centrifuged and the liquid part was subsequently subjected to lactic acid fermentation by *Lactobacillus paracasei* NRRL B-4564 (second stage fermentation). At the beginning of the LA fermentation, the sugar content of the stillage media was adjusted to 20 g/L (by addition of a concentrated glucose solution) and inoculated with 5 % (v/v) of the *L. paracasei* inoculum. During the batch fermentation the pH value of fermentation media was adjusted to 6.5 by the addition of sterile 30% NaOH solution (w/w) in 4 h intervals. During both stages the samples were withdrawn to analyze: α-amino nitrogen content, sugar consumption, bacterial growth and lactic acid concentration. The results of the fermentation with proteolytically pretreated stillage (two stage fermentation) were compared with untreated stillage (one stage fermentation).

The results have shown that the stillage which was pretreated by *B. licheniformis* contained significantly higher amount of free α-amino nitrogen, released due to the hydrolysis of the proteins present in stillage by the activity of *B. licheniformis.* The sugar content of the pretreated stillage was slightly lower. The final concentration of the LA obtained in the second stage by *L. paracasei* was 48% higher than in untreated stillage. In addition, a number of viable cells (CFU) achieved in the two stage LA fermentation was around one log units higher compared to that obtained in one stage fermentation. The results are suggesting that the assessed two stage fermentation mode could be more effective option for the lactic acid and probiotic biomass production on the waste distillery stillage. The process enables economical and sufficient supply of assimilative nitrogen sources needed for lactic acid bacteria, thus avoiding a common addition of expensive yeast extracts or other costly sources of assimilative nitrogen.

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