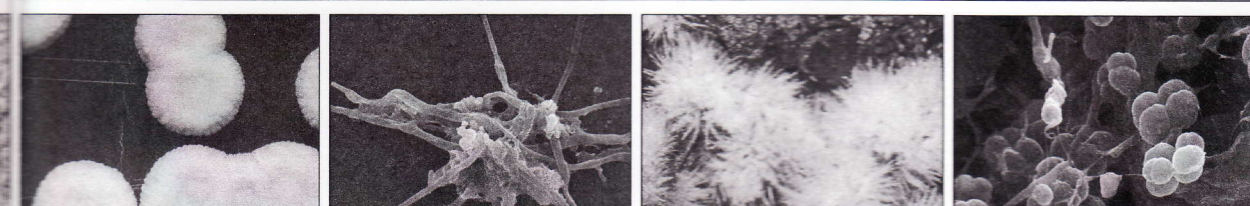


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the higher content of degradable substrates and the greater microbial activities in them. At pig slurry and corn silage mixtures the fermentation time was shorter (2 days) in contrast with the pig litter and manure mix, where the fermentation time was 5 days. The chopped silage can be better for the bacterial communities than the pig litter manure, due to the bigger surface areas, and therefore the shorter fermentation time. Results related to 1 gram of substrate mixtures, however showed 3,5 times higher methane amount at the pig litter manure mixtures in comparison with the corn silage mix. Up-scaling of results needed for the agricultural scale of utilization.

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EFFECTS OF SUBSTRATE CONCENTRATION AND ORGANIC-AQUEOUS BIPHASIC MEDIA ON FRUCTOSYLTRANSFERASE ACTIVITY OF PECTINEX ULTRA

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Oligosaccharides are a major class of naturally occurring carbohydrates consisting of 3 to 10 monosaccharides. These biomolecules play a fundamental role in many important biological processes and are commonly found in nature as glycoconjugates. Although there are some oligosaccharides, which have received particular attention because of their favourable features, being low in calories and noncariogenic, and acting as selective energy sources for beneficial microorganisms in the intestinal flora. Recently, oligosaccharides can be produced by transglycosyl activity of enzymes. Due to hydrolytic activity of these enzymes, the yield of synthesis process may be enhanced by decrease of the water activity that can be done by several methods such as increasing of substrate concentration or by adding of organic solvent. In this work, the effects of substrate concentration and some organic agents on transfructosyl activity of Pectinex ultra from *A. aculeatus* were studied. The transfer reaction was carried out at 60°C and pH 5.5 with about 1U hydrolytic activity of enzyme preparation. Based on our previous studies maltose and sucrose were selected as acceptor and donor, respectively. The ratio of maltose and sucrose was 1:9. Samples were taken at time intervals and boiled for 10 minutes for stop the reaction before analysis. Bioconversion was monitored by measurement of amount of carbohydrates using HPLC technique. Effects of carbohydrate concentration on transferase activity were investigated in range from 20 to 70 (w/v)%. The highest oligosaccharide content (4% DP3) was achieved on the 5th day of bioconversion with 60 % carbohydrate content and 9:1 ratio of sucrose and maltose. This value was two times higher than in case of control sample. Effects of organic-aqueous biphasic media on synthesis of oligosaccharides were also studied. Different organic solvents (acetone, acetonitrile, tri-ethyl-phosphate, ethanol, ethyl-decanoate and glycerol) were applied in combination with buffer system in ratio 1:2. Ethyl-decanoate and acetone induced the oligosaccharide synthesis. In the cases of ethyl-decanoate and acetone, the yields were increase in 203% and 102%, respectively, comparing with control sample. Generally, about 72 hours are needed to form the highest contents of oligosaccharides. Adding of acetonitrile, tri-ethyl-phosphate, ethanol and glycerol strongly inhibited the transfer reaction. Summarising, the optimal substrate concentration was 60 (w/v)% for transferase activity of Pectinex ultra. Organic-aqueous biphasic media with acetone and ethyl-decanoate induced the fructosyl transferase activity of the enzyme. These results are very promising for development of technology for synthesis of certain oligosaccharides.

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PURIFICATION AND PARTIAL CHARACTERIZATION OF INULINASE FROM *THERMOMYCES LANUGINOSUS*

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Inulinases are carbohydrases (β -fructan fructanohydrolases, EC 3.2.1.7, EC 3.2.1.80) which have two types that depolymerase their substrate, the inulin polysaccharide. Chemically, inulin is a fructan and consists of linear β (2,1) linked polyfructose chains displaying a terminal glucose unit. One type is exo-inulinase (EC 3.2.1.80), which hydrolyses inulin to produce high fructose syrup (full hydrolysis). This product is usable as sweeteners and can be used for energy drinks and therapy. Exo-inulinases can be used for bioethanol production from artichoke as well. The other type is endo-inulinase (EC 3.2.1.7), which acts randomly at the internal glycosidic bonds of inulin producing fructooligosaccharides (partial hydrolysis). These products are usable as sweeteners and prebiotics. Inulinase preparations are commercially available and all of them are from mesophilic *Aspergillus*. *Thermomyces lanuginosus* is real thermophilic fungus and none pathogen as well as secretes numerous thermostable extracellular enzymes. Hence very few data are available in literature dealing with inulinase, in this study, purification and partial characterization of extracellular inulinase from *Thermomyces lanuginosus* was focused. As a result of an earlier selection procedure and medium optimisation by Response Surface Method (RSM) - *Thermomyces lanuginosus* IMI 140524 strain was found to be the most promising one to produce inulinase. After fermentation in shaken flasks the mycelia were removed by filtration. Extracellular proteins were precipitated by iso-propanol and collected by centrifugation at 8000 rpm for 15 minutes. The precipitated prorotheins then were dissolved in minimal amount of sodium acetate buffer pH 5.5 and the isopropanol was removed by ultrafiltration. Enzyme purification was executed by combination of numerous chromatographic steps. Ion exchange chromatography (DEAE-Sepharose, Q-Sepharose) followed by gel filtration (Superose 12). All chromatographic steps were performed with fast performance liquid chromatography system (FPLC) at 4 °C. Protein contents were monitored by determination of absorbance at 280 nm, meanwhile the inulinase activity was assayed by monitoring released reducing sugars by bicinchoninic acid method (BCA). Purified inulinase enzyme exhibits