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Research Article

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Preparation and Characterization of Microbial Growth Media and its Application for the Production of Microbial Feed Protein

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Abstract

Cereal and grains have been using as a source of food molecules for centuries. However, the preparation of microbial growth media from a particular cereal and grain for the growth of microorganisms has not been considered. In this study, preparation of microbial growth media for the cultivation of Bacillus subtilis strain st-103 was done using rye pellets. The rye pellets, obtained after the hydrolysis of rye grains were enriched using Bacillus subtilis strain st-103 producer of protein derived by the ecological selection.The cultivation process of Bacillus subtilis strain st-103 was carried out under aerobic conditions in an incubator shaker (ES-20) at 28 °C at 250 rpm. The total viable microorganisms are grown on the media were determined according to standard test method for automated colony forming unit (CFU) assay. According to the result of the experiment, the total viable count of Bacillus subtilis strain st-103 was 1.2 X 108 CFU/ml. Based on the result obtained in the course of scientific work suggest the prospects for further steps of the enrichment of rye grain (pellets) with Bacillus subtilis strain st-103 to produce microbial feed protein.

Keywords: Rye Pellet, Bacillus Subtilis, Microbial Protein, Grains Hydrolysis.

Introduction

Cereal and grains have been using as a source of food molecules for centuries. However, the potential application as growth media for the growth of microorganisms has not been considered. Rye grain is mainly composed of starch, dietary fiber, protein, and mineral matter. The starch content of rye grain is between 57.1 and 65.6 g/100 g of dry matter [1]. Starch is present mainly in the endosperm part of the rye grain. The dietary fiber components are found as cell-wall constituents in all parts of the kernel, and the total dietary fiber content of rye grain is between 14.7 and 20.9 g/100 g of dry matter [2]. The protein and ash content of rye grain grown in different countries is between 9.0 and 15.4 g/100 g of dry matter, and 1.61 and 2.24 g/100 g of dry matter respectively [3].

Microorganisms need sugars as sources of energy and minerals such as nitrogen and phosphorus for metabolism [4]. Experts can fulfill the requirement of minerals during growth by adding rye wort filtrate. The feed protein content is directly related to the amount of filtrate added to the grain (pellets). To prepare the nutrient medium for a pure culture of a strain of protein producers and cultivation, adding more than 30 % of the filtrate to the grains [5].

Microorganisms are an excellent source of single-cell protein, vitamins, and contain beneficial lipids [6]. Microbial protein refers to the dried microbial cell or total protein extracted from pure microbial cell culture of algae, bacteria, filamentous fungi, yeast [7].

Bacillus subtilis is an anaerobic, non-pathogenic, endospore-forming, rod-shaped, Gram-positive bacterium [8]. Bacillus subtilis can grow in minimal media containing only essential salts and carbon, nitrogen, and phosphorus sources [9]. Escherichia coli Nissle 1917 is a nonpathogenic Gram-negative strain used in many gastrointestinal disorders including diarrhea [10]. Recently, Scholars have tried to produce single-cell protein from Escherichia coli strain Nissle 1917 using rye pellet as growth media in a mild laboratory condition [11].

Protein is a necessary key ingredient for growth, body maintenance, the fertility of Animals, and directly proportional to the output of milk, meat, eggs, and other products of Animals [12]. Rye (Secale cereale) is a great animal fodder cereal, with high energy, but the protein content is low. Thus, additional feed protein sources are required to meet the animals' needs (Olstorpe, 2008).

This study aims to formulate a microbial growth media and to obtain protein from Bacillus subtilus strain st-103 using rye grain (pellets). Moreover, the determination of colony-forming unit (CFU) of biomass with the cultivation of the strain of Bacillus subtilus strain st-103 was carried out in this study.

Materials AND Methods

The study sought to produce microbial protein from *Bacillus subtilis* strain st-103 using rye pellets. The whole production process was divided into three main stages. These stages included the preparation of microbial growth medium, inoculation of the protein producer microorganisms (Bacillus subtilis strain st-103), and production of microbial protein through the cultivation of microorganism.

Preparation of the Rye Grain

The rye was received as completely grains and ground into a fine powder and sieved using1mm-diameter sieving. 500g of the rye grain was weighed using the weighing machine for further analysis.

Grinding

A knife grinder was used for crushing the rye grain into fine particles for further analysis. The size of the crushed rye flour 80 % particles could pass through the sieve with a diameter of 1mm). The powdered rye was then weighed using a Weighing Scale (Shimadzu-UW2200H). Initially, 5 kilograms of the rye grain was used for the study. Then 500gram of rye flour was used from the grounded flour.

Mixing and Kneading

500g of rye flour was mixed with 1500ml tap water in a jar (1: 3 flour to water ratio). Then the mixed and kneaded rye mash was transferred into the water bath (LOIP LB-163 Estonia) at 70oc for hydrolysis, saccharification, and proteolysis.

Hydrolysis and Saccharification

The kneaded rye mash was placed in a water bath (LOIP LB-163, Estonia) at a heating rate of 1°C/min with constant stirring at a temperature of 70°C. The enzyme preparation 'AmiloLux-ATS was added.The hydrolysis of the starch process was carried out for 1 hour. For saccharification, the enzyme preparation "GlucoLux-A" was added. The process was carried out for another 1.5 hours.

Proteolysis and Biomass Separation

The temperature of the saccharified biomass was reduced to 55°C, and the enzyme preparation of the bacterial protease 'Protosubtilin GZx A-120' was added. After 1 hour, the acid protease 'Pro100L' enzyme preparation was added, and the proteolysis of wort was carried out for 1 hour. All the unit operations (hydrolysis, saccharification, and proteolysis) were carried out using a water bath (LOIP LB-163 Estonia).

The sample was subjected to centrifugation using the centrifuge machine (Hettuch Zentrifugen, Tuttligen , Germany) for 1hr at 4600 rpm.The total soluble solid of the filtrate was 24.9 %. The pellet was dried at 60 °C to 6 % moisture content.

Microbial growth media preparation and inoculation

100 g of dried rye solid sediment (grains), 58 % moisture content, was dissolved in 800 ml of water in a conical flask. Then, 50 % (50 ml) of rye wort (70 % dry matter) were added to the mixture and sterilized at 121°C at 1 atmosphere for 30 min. After sterilization, the mixture was cooled down, and Bacillus subtilis strain st-103 was inoculated into the mix.

The inoculation of strains of Bacillus subtilis strain st-103 was carried out in sterile conditions in the laminar cabinet. The growth of strains of *Bacillus subtilis* strain st-103 was carried out under aerobic conditions in an incubator-shaker (ES-20) at 28 o C at 250 RPM. After that, the sample was incubated for two days (48 hours) in a small glass container /fermenter equipped with an air supply compressor at 28°C. After incubation, the samples were subjected to centrifugation using the centrifuge machine (Hettuch Zentrifugen, Tuttligen, Germany). After centrifugation, the filtrate was separated from solid biomass. The biomass was dried at 60°C LOIP-LF-25/350-VS2 (Russia) to a moisture content of 10 % .The total viable microorganisms are grown on the media were determined according to standard test method for automated colony forming unit (CFU) Assay F2944 – 12.

Data generated were subjected to analysis of variance (ANOVA) using Origin statistical software (version 6.1) at 5% significance. Measurements were made in triplicate. Results were reported as means ± standard deviations.

Results AND Discussion

In this work, rye wort and pellet preparation for the microbial growth was made by hydrolysis of the grain using liquifying and proteolytic enzymes. Before the hydrolysis, the physicochemical parameters of rye flour were analyzed, and the results are presented in Table 1.

Table 1: Physicochemical parameters of rye flour used for hydrolysis

Physicochemical parameters	Contents, %
Moisture content	8.87 ± 0.13
Starch content	56.01 ± 0.09
Trash impurities	>1

The used rye flour contains 8.87 ± 0.13% moisture content, 56.01 ± 0.098% starch content, and trash impurities up to 1%. The starch is essential during enzymatic hydrolysis. Usually, the rye flour contains 56–70% starch. The moisture content of rye flour should not exceed 14, the less water in the flour, the better its storage ability.

The concentration of Dry solids (oBrix) and free amino nitrogen (FAN) of the filtrate obtained after centrifugation of the hydrolysate were measured, and the results are presented in Table 2.

Table 2: The nutrient composition of rye filtrate

Parameters	Results
Dry solids, (o Brix)	22.0 ± 0.2
Free Amino Nitrogen, (mg/L)	528.74 ± 1.28

The concentration of dry matter (sugar) in rye filtrate was 22.0± 0.2 (o Brix), whereas Free Amino Nitrogen content was 528.74 ± 1.28 mg/L. Sugars in the filtrate are the primary carbon and energy sources for food microorganisms, and free amino nitrogen is the source of nitrogen for microbes.
The physicochemical parameters of rye grains (pellets) were determined before and after inoculation. The results are presented in Table 3.

Table 3: Physicochemical parameters of rye Grain (pellets) before and after the inoculation of Bacillus subtilus strain st-103.

Parameters	Before inoculation	After inoculation
Moisture content before drying, %	67.8 ± 2.1	-
Moisture content after drying, %	9.96 ± 0.03	11 ± 0.2
Color	Light Brown	Brown

The results of the total soluble solids (TSS)obtained during wort preparation from rye grain in the study are shown in figure 1.

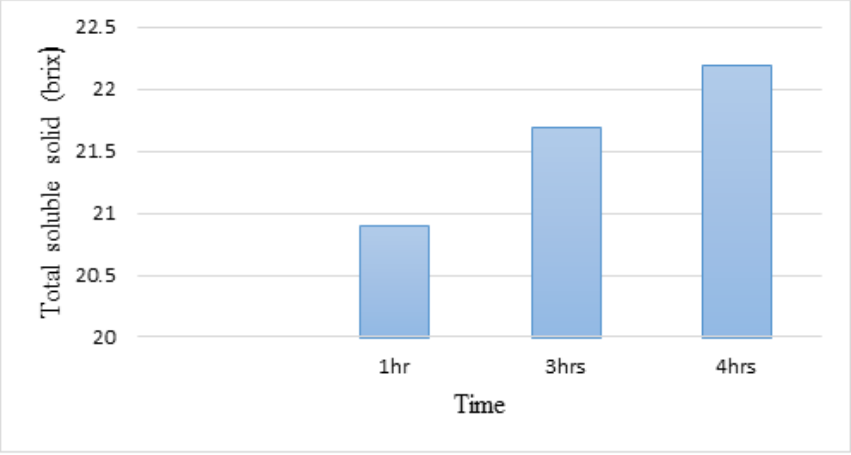

Figure 1: The total soluble solid of rye wort preparation.

The total soluble solids (TSS) contents of rye wort during hydrolysis (Fig 1) increase as the time of hydrolysis increases.
The viscosity of rye grain wort during the liquefication saccharification process was measured and presented in figure 2.

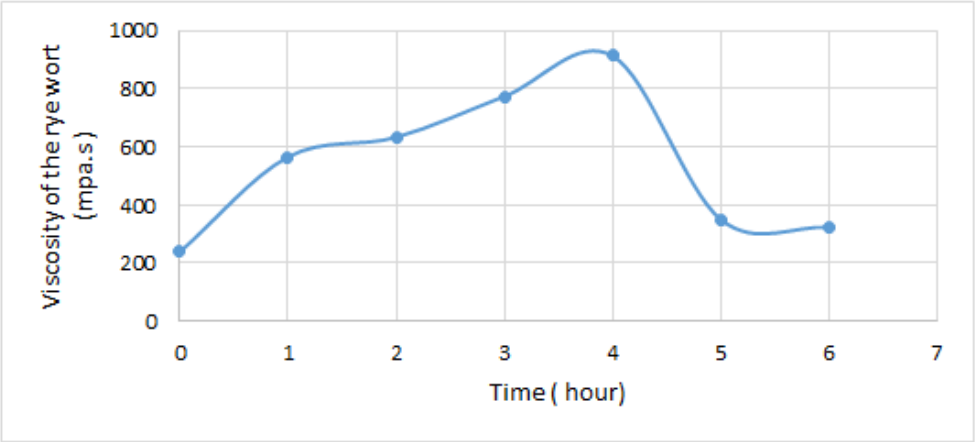

Figure 2: Viscosity versus time profile of rye wort during liquefication and saccharification unit operation.

The viscosity of rye wort increases as the time of heating increase and decrease after the addition of proteolytic enzymes. The reduction in viscosity of the rye wort was related to the extent of hydrolysis of high molecular mass protein.

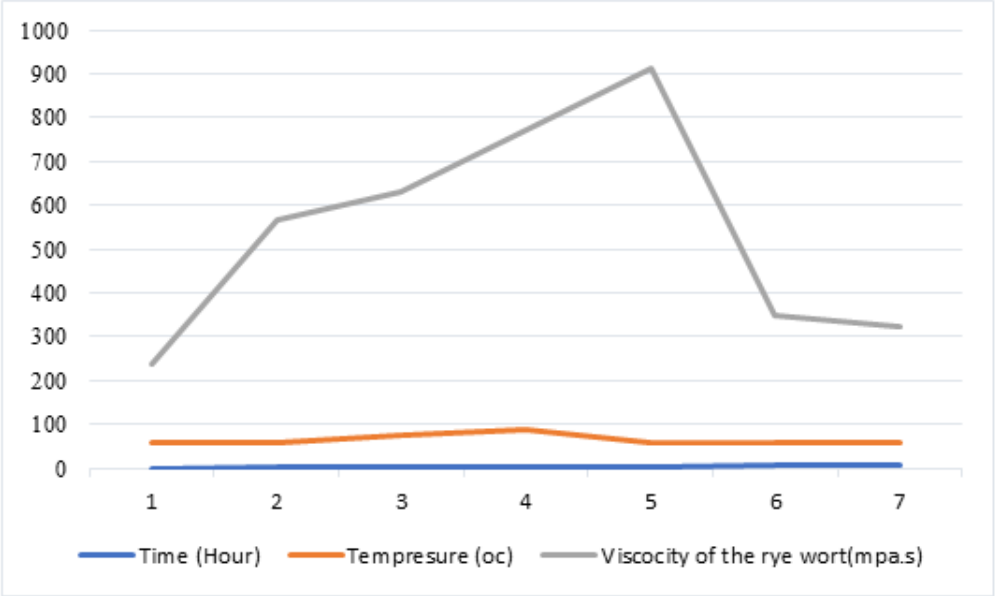

Figure 3: Time, temperature, and viscosity profile of the rye wort preparation.

The viscosity of the rye wort(Fig 3.) increases as the temperature increase. The viscosity of rye wort was decreased as the temperature of saccharification was low and the addition of proteolytic enzymes. The results obtained show that the concentration of total soluble solid of the rye wort was increased during liquefaction and saccharification unit operations. Based on the result complex organic compound (starch and protein) content of the rye grain has been effectively degraded to simple sugar and amino acids by liquifying and proteolytic enzymes respectively.

Conclusions

The objective of the research was to preparare and characterize microbial growth media for the production of microbial feed protein. In this study, the preparation of microbial growth media for the cultivation of *Bacillus subtilis* strain st-103 was done using rye pellets. The rye pellets, obtained after the hydrolysis of rye grains were enriched using the strain of *Bacillus subtilis* strain st-103 producers of protein derived by the ecological selection.The cultivation process of *Bacillus subtilis* st-103 was carried out under aerobic conditions in an incubator shaker (ES-20) at 28 ℃ at 250 rpm. According to the result the experiment the total viable count of Bacillus subtilis strain st-103 was 1.2 X 108 CFU/ml. Based on the results obtained in the course of scientific work suggest the prospects for the further steps of the enrichment of rye grain (pellets) with *Bacillus subtilis* strain st-103 to produce microbial feed protein.

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Conflict of interest

The author declares that there are no conflicts of interests regarding the publication of this article.

Data availability

No datasets were generated or analyzed during the current study.

Fund

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