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Research Article

THE PRODUCTION OF SINGLE-CELL PROTEIN FROM MOLASSES RELEASED AS A RESULT OF SUGAR PRODUCTION IN THE FACTORY

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ABSTRACT. Proteins are the most important nutrients required in daily life for the healthy nutrition of humans and animals. In countries with relatively weak economies and developing countries, health problems due to inadequate and unbalanced nutrition are exacerbated by the rapidly increasing population. As a result of the desire to use alternative protein sources containing protein close to animal sources in food and feed, it has been focused on the production of single-cell protein (SCP). SCP is a biomass product formed by microorganisms grown under optimal conditions in the medium. Some of the microorganisms, used as an SCP source, can grow using waste materials as carbon or energy source. At the end of this bioprocess, the SCP is obtained by eliminating the environmental pollutant. The SCP may be originated from algae, bacteria, or fungi. SCP prepared by the large-scale production of yeasts or fungi are called mycoproteins. This study aims to investigate the possibility of economically producing SCP (mycoprotein) using molasses, which is sugar beet factory waste. For this purpose, *Saccharomyces cerevisiae*, which is a wild species, and molasses produced by sugar mill production were used. The effect of parameters such as pH, amount of sucrose, temperature, and amount of inoculum required for mycoprotein production was examined. According to the results of this study, the necessary conditions for the best mycoprotein production in molasses medium were determined as pH 6 at 30°C, and single-cell protein production was provided in a low-cost way. The augmentation in the microbial SCP production was calculated as 100% at determining optimal conditions. The results obtained from this study contribute to the solution of the increasing nutritional need problem, which is one of the most important problems of our world.

Keywords: *S.cerevisiae, Molasses, Single Cell Protein*

INTRODUCTION

In today's world, improvements have been made in agricultural production around the world to meet the meat needs of the rapidly increasing population. Universal food production doubles with a 10% increase in agricultural land use [1]. With this strategy, changes in living standards, famine, population pressure, and urbanization affect human nutrition and health very effectively [2]. The food industry has an important place in terms of local and universal environmental impact and resource use [3]. Many studies are showing the effects of food production on the loss of biodiversity and the deterioration of the ecosystem [4, 5]. It has been reported that 70-85% of water pollution is caused by human activity as a result of agricultural activities [6]. Moreover, 30% of the greenhouse effect comes from the agricultural production sector, of which more than half is associated with meat production [6].

The world population reaching 9 billion by the middle of this century is putting extra pressure on the food system [7]. For these reasons, it poses a risk in terms of future food and food safety, and as a result, it is expected that public health will be affected. The problems that arise as a result of not providing food safety not only create environmental problems but also cause serious health problems [8]. The World Food and Agriculture Organization (FAO) stated that the definition of primitive smart agriculture consists of the concepts of combating climate change while trying to ensure food safety, and stated that this can be achieved with a diet that reduces the need for meat [1]. Livestock production causes extensive land use, land degradation, and afforestation, as well as abundant methane emissions [1]. For this reason, the production of foods that provide similar nutritional benefits to meat and are less harmful to the environment has gained importance. Products that overlap with these definitions are known as meat analogs or meat alternatives [9] and can be products of vegetables (such as soy and beans), animal (such as milk and kidney), or microbial (such as mycoprotein or single-cell protein) products [6]. In addition to these, in recent years, ethical and environmental concerns have been encouraging the production of meat-like products and causing growth in market share [7], which is expected to increase to six billion dollars in 2022 [10]. The most important reason for the tendency to meat-like products is that these products can meet the nutritional benefits that meat can provide.

Many published studies have proven that red meat consumption increases the mortality rate [11, 12]. On the other hand, the suggestion of not using meat in the diet is also discussed [13]. Elzerman et al. (2011) showed in the results of their study that there is no problem in using meat-like food instead of meat in the diet of non-vegetarians if it is compatible with the meat content [14]. Accordingly, while the smell or taste of the meat alternative product does not need to be similar to meat, its appearance should resemble meat. In this case, the meat alternative product obtained from single-cell protein (SCP) is a more realistic approach than the protein-rich plants. Because according to consumer reviews, SCP resembles meat [15]. Most of the organisms such as algae, bacteria, and fungi are used to produce SCP.

Mycoproteins are foods with high protein content obtained from fungal biomass (SCP of Fungi) that can be consumed by humans. It has been reported that many edible mushroom species in the fungal kingdom contribute to human nutrition in terms of both taste and nutritional value [16]. On the other hand, there are limited studies on its use as a meat substitute in terms of protein content. Some studies indicate that it is important for mushrooms to be consumed by both animals and humans as protein foods due to their rapid development and richness in protein content [17].

Yeasts in the fungal kingdom have been used as animal feed additives in agricultural studies for many years due to their high vitamin B12 content. Today, it has been determined that yeasts, whose nutritional value is determined to be more than 50% protein, can be used as a protein source in both animal and human nutrition under the name of mycoprotein. Among the yeasts recommended as SCP sources, there is *S. cerevisiae, S. fragilis, S. pasteurianus, Torulopsis utilis, Brettanomyces, Candida tropicalis, C. utilis, C. lipolytica, C. maltosa,* and *C. intermedia* species [18]. In commercial production, *Candida utilis* species, which is commonly called 'Torula yeast', is used as *Torulopsis utilis*. Reducing the production cost of mycoproteins encourages consumers to use cheaper protein source foods as meat alternatives. In addition, a more suitable meat alternative can be produced both in terms of health and the environment.

For this purpose, the use of some industrial wastes for SCP production has been suggested in some recent studies [19].

In the literature, there are studies on the usability of cellulosic waste [20], paper waste [21], sulphite waste liquid [22], and lignin waste [19] for SCP production. Gao et al. (2011) [23] used the soybean waste molasses in the production of *Candida tropicalis* species and suggested a successful SCP production method. Different fungi and yeast strains for SCP production have been studied in the literature. However, the studies on the determination and optimization of the capacity of these species to be a source of SCP in the medium using molasses as a cheap medium component are limited. This study aims to determine the optimal conditions for SCP production with the yeast *Saccharomyces cerevisiae* using molasses, a waste product of the Sugar Factory. For this purpose, the effects of pH, inoculation amount, temperature, and the amount of sucrose on SCP production by using molasses, which is suggested as a cheap food source, were investigated. The results indicated that appropriate and easy production ways are being reached to meet the increasing nutritional needs of the increasing population around the world.

MATERIALS AND METHODS

Microorganism

In this study, *Saccharomyces cerevisiae*, which belongs to the Ascomycetes class, was used as the SCP resource. The species was taken from Ankara University Science Faculty Biotechnology Laboratory Culture Collection.

Culture Conditions

Molasses, which was sugarcane residue, was used as an inexpensive substrate for microbial growth, and the content of the molasses medium was explained in [24]. In this study, molasses obtained from Eskişehir Sugar Factory for previous studies were used.

Activation of yeast cells

S. cerevisiae (+ 4°C) was stocked in the refrigerator inoculated into a Sabouraud dextrose broth medium and activated for 24 hours at 25°C. Activated yeast cells were inoculated into the molasses medium and the yeast was acclimated to the molasses medium. This process was repeated two times.

Single Cell Protein (SCP) Production

Molasses medium containing sucrose was used for SCP production. The activated *S. cerevisiae* yeast was inoculated into the molasses medium, and the culture was incubated. The microbial growth was determined spectrophotometrically. The 1 ml samples were taken from the culture medium and measured at 600 nm. The increment of the optical density of the samples showed microbial growth and was also accepted as SCP production.

At the end of the incubation period, the concentration of microbial biomass concentration formed in the test tubes was determined and the percentage of microbial growth was calculated with Equation 1.

$$\% \text{ B (The percentage increase in biomass)} = C_i - C_f/C_i$$

Eqn. 1

In this equation; C_i (mg/L) is the initial biomass concentration and C_f (mg/L) is the final biomass concentration.

Optimization of SCP Production

Optimization experiments were carried out to determine the best SCP production working conditions. For this, sucrose concentration (10, 20, and 40 g/L), optimum temperature (25, 30 and 35°C), appropriate pH (3, 6, and 9), and optimal initial inoculum amount (1, 2 and, 4 %) were used to determine the conditions under which the best production of SCP takes place. The optimization studies were carried out in 250 mL flaks containing 100 mL molasses medium.

RESULTS AND DISCUSSION

Determining the Optimal pH Level

pH is one of the important factors affecting mycoprotein production. Since the medium pH affecting cell division has a role in biomass augment. To determine the optimal pH, the pHs of the media was adjusted to 4, 5 and, 6. Among these values, the optimal pH was determined in the production of mycoprotein. The most appropriate pH for mycoprotein production was determined as pH 6 (Fig.1).

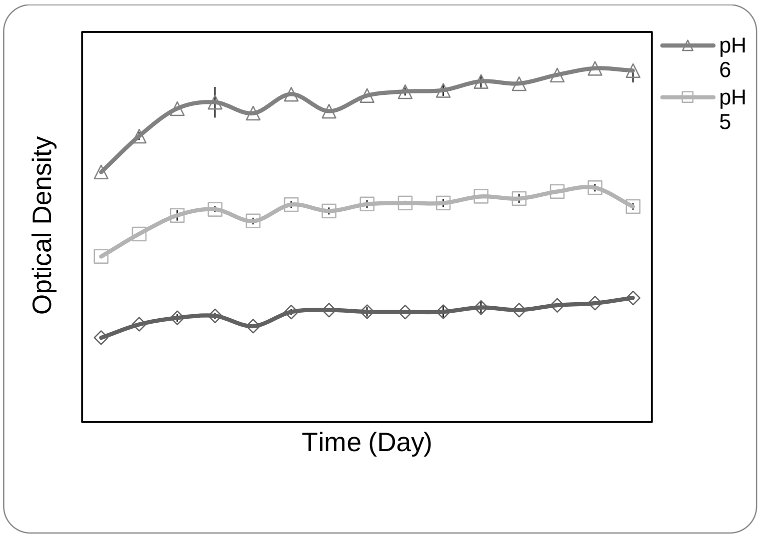

Fig. 1. *The effect of pH on mycoprotein production*

Determination of Optimal Inoculation Amount

The amount of microorganisms inoculated to the medium is another important factor affecting the rate of mycoprotein production. To determine the optimal inoculation amount, the molasses medium was prepared at pH 6. The variety of inoculation amounts were examined as 1%, 2%, and 4%. The most suitable inoculation was determined in the production of mycoprotein. Figure 2 shows the effect of the inoculation amount. The increment of the amount of inoculum increased microbial growth [25] and, also mycoprotein production. As seen in Fig. 2, the increasing inoculation amount resulted in the augmentation of microbial growth, and the optimal inoculation amount was determined as 4%.

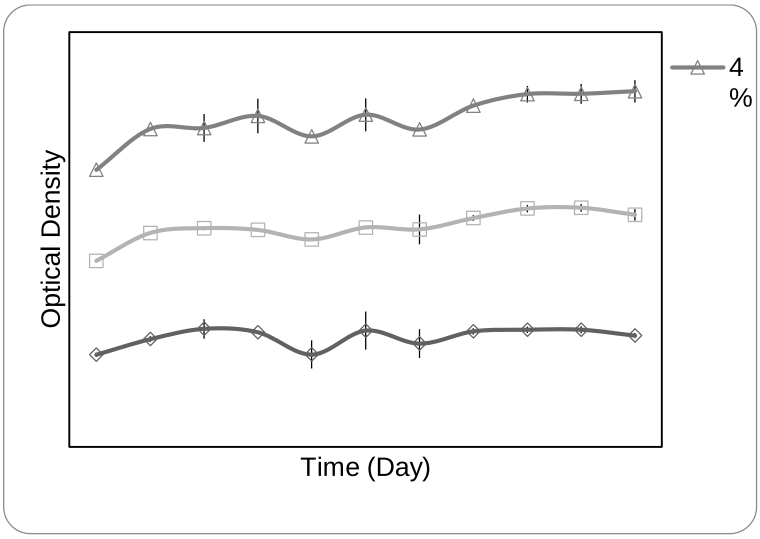

Fig. 2. *The effect of inoculation amount on mycoprotein production*

Determination of Optimal Temperature

To investigate the optimal temperature, the effect of different temperature values such as 25, 30, and 35°C were tested. Among these values, the most suitable temperature was determined. Previously, it was reported that the optimal temperature for mycoprotein production was 28- 30 [26]. Similarly in this study, the optimum temperature for mycoprotein production was determined as 30°C (Fig.3).

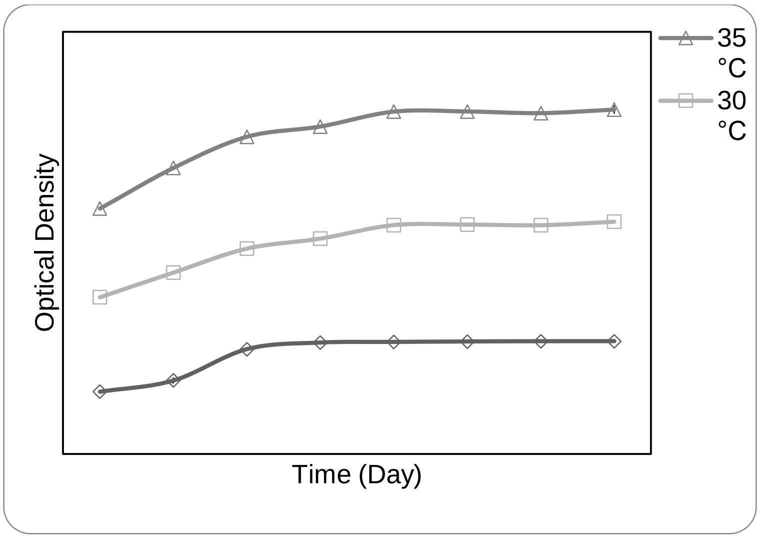

Fig. 3. *The effect of temperature on mycoprotein production*

Determination of the Optimal Sucrose Concentration

To determine the optimal sucrose concentration, the concentration of sucrose in the molasses medium was adjusted as 10, 20, and 40 g/L. Determination of the optimal sucrose concentration is given in Figure 4. Augmentation of carbon source dosages caused an increase in fungal growth [27]. The findings of this study are consistent with those in the literature. The raising sucrose concentration caused the increase in fungal biomass (Fig. 4).

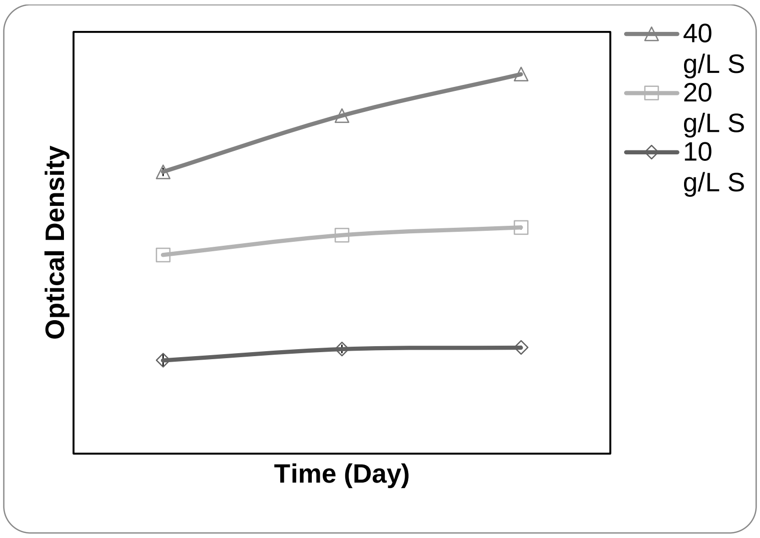

Fig. 4. *The effect of sucrose amount (10, 20 and, 40 g/L S) on mycoprotein production*

In this study, it has been demonstrated that SCP can be obtained for use in animal nutrition by inoculating activated yeast cells on molasses media prepared at different concentrations. According to the results of the study, the best improvement was seen in the medium containing 40 g/L sucrose concentration at pH 6 and 30 °C with 4% inoculation concentration. The maximum increase in the biomass of the yeast was calculated as 100.00% at optimal conditions (Table 1).

Table 1. *The effect of sucrose concentration on the percentage increase in biomass at optimal conditions*

Sucrose Concentration (g/L)	B%
10	99.93
20	99.96
40	100.00

CONCLUSIONS

SCP provides a lot of opportunities for meeting the protein needs of living things for the future. To meet the rapidly increasing needs of the world population, the production of single-cell protein takes place based on the necessity of finding conventional protein sources. The micro-organisms used as a single-cell protein source can also reproduce by using waste materials that will cause environmental pollution as a substrate source, then both environmental pollution is eliminated and protein for the elimination of hunger problem is obtained.

SCP is of such great importance for all living things, it should be preferred because its production has a low-cost way, and studies should be carried out to obtain SCP by using different organic wastes. SCP has advantages that will provide unlimited opportunities in meeting the protein needs of living things in the future. In this study, the usability of molasses in SCP production has been demonstrated. As a result, the use of molasses in

SCP production will both contribute to the economy, and environmental pollution will be prevented by using molasses, which is sugar factory waste.

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