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ARAŞTIRMA MAKALESİ/RESEARCH ARTICLE

INFLUENCE OF CORN STEEP LIQUOR IN NUTRIENT MEDIUM OVER PRODUCTIVITY OF BIOPULPING FUNGUS *Ceriporiopsis subvermispora*

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ABSTRACT

In biopulping researches fungal inoculum preparation have a significant role. In this study addition of corn steep liquor (CSL) as a nutrient in inoculum growth medium was investigated. Flasks and petri plates containing potato dextrose yeast (PDY) and PDY+CSL were inoculated with two strains of white rot fungus *Ceriporiopsis subvermispora* and maintained at 27°C. Productivity of PDY+CSL medium was higher than PDY medium in both dish flasks and petri plates. The highest productivity of 15 mg/mL and 17 mg/mL for FP 90031-sp and CZ-3 strains respectively was observed in PDY+CSL petri plate culture. Small 4.5 mm inoculum plugs also indicated high productivity for both strains.

Key Words: Fungal nutrient medium, Corn steep liquor, Biopulping, *Ceriporiopsis subvermispora.*

BİYOLOJİK KAĞIT HAMURU ÜRETİMİNDE KULLANILAN *Ceriporiopsis subvermispora*'NIN GELİŞTİRİLMESİ ÜZERİNE MISIR MASERASYON SUYUNUN ETKİSİ

ÖZ

Biyolojik yöntemler ile kağıt hamuru üretiminde aşılanacak mantarın hazırlanması büyük önem taşımaktadır. Bu çalışmada mantar yetişme ortamına besleyici olarak mısır maserasyon suyunun (CSL) ilave edilmesinin etkisi incelenmiştir. Patates dekstroz yeast (PDY) ve PDY+CSL besi ortamı içeren kaplar beyaz çürüklük oluşturan *Ceriporiopsis subvermispora* mantarı ile aşılanmıştır ve 27°C sıcaklıkta 10 gün bekletilmiştir. Mısır maserasyon suyu içeren besi ortamlarında mantarın miseliyum verimi daha yüksek olduğu belirlenmiştir. Her iki izolasyon için de petri kaplarında en yüksek verim elde edilmiştir, FP 90031-sp için 15 mg/mL ve CZ-3 için 17 mg/mL olarak belirlenmiştir. Ayrıca 8 mm yerine 4.5 mm çapında parçacıklar ile aşılama yapıldığında daha yüksek verim elde edildiği belirlenmiştir.

Anahtar Kelimeler: Mantar besi ortamı, Mısır maserasyon suyu, Biyolojik yöntemler ile kağıt hamuru üretimi, *Ceripiriopsis subvermispora.*

1. INTRODUCTION

The pulp and paper industry utilises basically chemical or mechanical pulping methods or a combination of these, to produce pulps of desired characteristics from wood or any other fibrous raw material. The rather new method, biopulping, defined as the pre-treatment of wood chips with lignin degrading fungi prior to pulping, appears to have the potential to overcome some problems associated with conventional chemical and mechanical pulping methods. This offers both economic and environmental benefits through reducing electrical energy consumption, which is a major cost in mechanical pulping, improving paper strength, increasing pitch removal on machinery (Fischer et al., 1994, Scott et al., 1995), and lowering effluent toxicity (Akhtar et al., 1997a).

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The use of white-rot fungi for the biological delignification of wood was perhaps first seriously considered in 1950's (Akhtar at al. 1998a). Based on vitro screening procedure *Phellinus pini, Ceriporiopsis subvermispora, Phelebia tremellosa, Phelebia brevispora, Dichomitus squalens, Phanerochaete chrysosporum* were found to be the best in preferential degradation of lignin, among the several species of fungi (Otjen et al., 1987, Akhtar et al., 1998b). After a long period of investigation a lot research are focused on the white-rot fungus *Ceriporiopsis subvermispora,* because this fungus is effective on both hardwood and softwood species (Akhtar et al., 1992, 1996, 1997b, 1998b, Scott et al., 1998, Blanchette et al., 1992).

Cheep and fast contamination of wood chips with selected white rot fungus have an important role over the success of pre-treatment. A spore solution usually is used for contamination of cheeps with fungus. Since the fungus *Ceriporiopsis subvermispora* does not produce spores, fragmented mycelium was used as inoculum with acceptable result (Fischer et al., 1994). However, the amount of required inoculum to achieve good result was reported to be too high to be practical. In order to reduce the required amount of fungal inoculum for wood chips inoculation, addition of low cost nutrition is believed to be essential (Akhtar et al., 1997b). Meanwhile, the nutrient stimulates initial fungal growth and establishment in the chips. Akhtar (1997b) successfully utilised corn steep liquor (CSL) as a fungal nutrition in wood chips. They concluded that the amount of *C subvermispora* inoculum was reduced as low as <5 g/ton of wood with inexpensive CSL amendment (0.5%).

Addition of protein-based nutrient as CLS to liquid media will stimulate mycelial growth and required amount of mycelium for inoculation can be produced in less time and medium. Therefore, in this study the effect of CSL addition to fungal grown medium, over the mycelium productivity is experimentally investigated.

2. MATERIALS AND METHODS

Mycelium productivity of the white-rot fungus *Ceriporiopsis subvermispora* (Pilát) Gilb & Ryvarden (synonym *Poria ceriporiopsis*) in different mediums was investigated in this study. Two strains (CZ-3 and FP 90031-sp) of the fungus based on their greater lignin degrading ability compared with other strains (Blanchette et al., 1992, Akhtar et al., 1997b) were selected. They were supplied by the Centre for Forest Mycology Research of the USDA Forest Products Laboratory in Madison, WI. Cultures were maintained on potato dextrose agar (PDA) slants at 4°C until used. PDA plates were inoculated from these slants and incubated at 27 ± 1°C for 10 days.

CSL utilised as a nutrient in mycelium medium in this study was supplied from Pendik Nişasta – Istanbul. It is a light brown or brown coloured semi liquid derived while steeping the corn in dilute sulphur dioxide. Spent steeping water, containing 6-7% dry substance, is continually drawn off for subsequent concentration. The steep water is condensed to an auto-sterile product - a valuable nutrient in the fermentation industry - or concentrated to approximate 48% dry matter. CSL is an excellent source of lactic acid, which accounts for about 25% of the solids content. Lactic acid is a suitable carbon source for fermentation microorganisms. In addition, corn steep liquor contains amino acids, peptides, proteins, carbohydrates, vitamins, trace metals, minerals, and several complex growth factors.

Table 1 exhibits the compositions of three CSL, which were produced by various mills. Properties of CSL supplied by Pendik Nişasta, which is used in this study do not differ much from CSL produced by the other foreign companies.

The control culture liquid medium (1 L potato dextrose yeast extract (PDY)) contained potato broth from 200 g peeled potatoes, 20 g dextrose (Riedel-de Haen) 7.27 yeast extract (Merck). The nutrified medium was prepared as first with addition of 5 g (o.d.) CSL. Flasks with medium were autoclaved for 20 min at 121°C and cooled to room temperature. Flask containing 100 mL of medium were inoculated with 7 plugs cut with a 8 mm diameter cork bore from 10 day old PDA plate cultures. For laboratory biopulping researches small amounts of mycelium will be enough, therefore sterile petri plates with less liquid media were prepared. These plates (90 mm petri dishes) containing 25 mL of medium were inoculated with one plug cut with an 8 mm diameter or 4.5 mm diameter cork bore from 10 day old

Table 1. Composition of CSL From Various Sources.

Composition	Pendik Nisasta A.S. Istanbul-Turkey	American Maize Products Co. USA*	CPC International Inc. USA*
Dry substance (%)	50	42	56
pH	4	4	4
Protein (%)	46	45	41

* Source AKHTAR et al. (1997)

PDA plate cultures. Flask and petri dish incubation was maintained at 27± 1°C for 10 days without agitation. Procedure was carried out in triplicate.

At the end of the incubation period the spent medium was decanted and mycelial mats were rinsed with sterile water. To determine the dry content of fungus, mycelial mat was dried at 65°C to constant weight.

3. RESULTS AND DISCUSION

For good contamination of growing medium, distribution of inoculum plugs in dish play an important role. In the liquid medium of flask, inoculum plugs cannot be distributed uniformly and make steady, therefore plugs become grouped at the side of the flask. In spite of this petri dishes were inoculated with only one plug in the centre of the dish, and at the end of the incubation period mycelial mat covers the whole surface of the medium in the plate with more uniform mycelial mat.

Meanwhile, petri dishes are stackable and occupy less volume in the incubator while provide much surface area for the some medium volume.

The mycelium production results of investigated two strains are reported in Table 2 as average values with standard deviations.

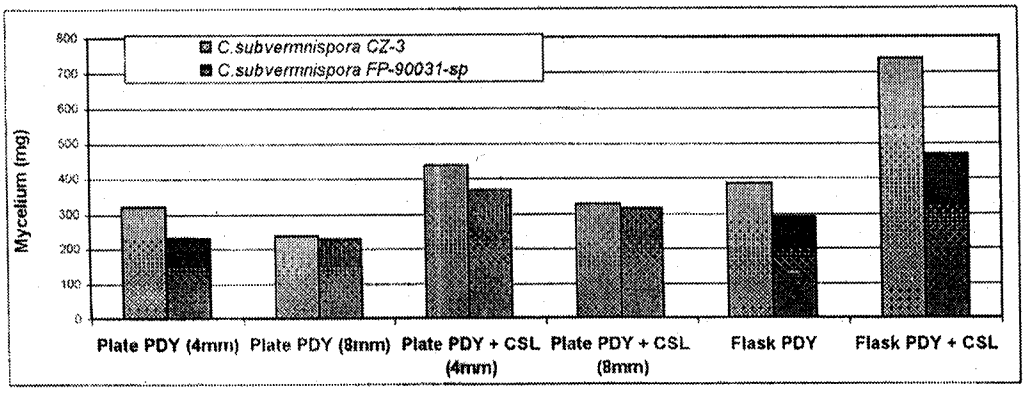
Figure 1 shows an increasing of mycelium production with addition of CSL in medium, increase are from 1.3 to 1.5 times in plates and from 1.6 to 1.9 times in flasks.

Productivity of *C. subvermispora* CZ-3 in plates is 2.5-3.3 times higher for PDY medium and 1.8-3.4 times higher for PDY+CSL medium. Productivity of *C. subvermispora* FP 90031-sp in plates is 3.2 times higher for PDY medium and 2.7-3.1 times higher for PDY+CSL medium.

Table 2. Dry Mycelial Content of *C. subvermispora* Strains in Different Mediums.

Strain	Plug diam. (mm)	Dish	Medium	Mycelium (mg)	Standard deviation	Productivity (mg/ml)
C. subvermispora CZ-3	4	Plate	PDY	319	0.039	12.76
	8	Plate	PDY	236	0.066	9.44
	4	Plate	PDY-CSL	435	0.096	17.41
	8	Plate	PDY-CSL	327	0.086	13.10
	8	Flask	PDY	383	0.070	3.83
	8	Flask	PDY-CSL	736	0.098	7.36
C. subvermispora FP-90031-sp	4	Plate	PDY	230	0.083	9.20
	8	Plate	PDY	228	0.066	9.14
	4	Plate	PDY-CSL	365	0.015	14.59
	8	Plate	PDY-CSL	313	0.060	12.51
	8	Flask	PDY	290	0.076	2.90
	8	Flask	PDY-CSL	468	0.068	4.68

Figure 1. Mycelium Production of Fungi in Different Mediums and Dishes.



For both two strains CZ-3 and FP 90031-sp, plates with PDY +CSL medium indicate the highest productivity 17.4 mg/mL and 14.6 mg/mL respectively (Table 1).

Results show that big inoculum plugs does not means bigger mycelium production, in both two strains and two mediums the smallest 4.5 mm plugs gave the higher mycelium yield. However, bigger inoculum plugs cause relatively higher amount of big particles in blended mycelium solution used for inoculation of wood chips.

For laboratory scale biopulping researches where the small amount of chips are inoculated mycelium from one petri plate may be enough.

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