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Effect of Different Media on Growth Kinetics Parameters of A*spergillus ochraceus:* an Approach Towards Production of Fungal Biomass

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Abstract

The importance of fungi derived polymers and their characterization have provided an impetus polymer industry. The role of culture conditions in biomass production, cannot be ruled out. Media is one such abiotic parameter that play a significant role in growth of fungal biomass. In current study,*Aspergillus ochraceus* was maintained in three different culture media *viz.,* Potato Dextrose Broth medium (PDB) ,Yeast Potato Dextrose Broth medium (YPD),and Sabourd's Dextrose Broth (SDB) for 8 days. The results showed around two fold higher biomass production in Sabourd's Dextrose Broth. The specific growth rate was higher and doubling time was relatively lower in Potato Dextrose Broth medium (PDB). The results suggest that the biomass may be relatively higher in Sabourd's Dextrose Broth. The study may be extended to compare the yield of chitosan and their physico-chemical properties.

Keywords Chitosan, Growth kinetics, *Aspergillus ochraceus*, media, yield

Introduction

The growing interests to explore new or modified polymers have provided an impetus to scientific community to explore various sources of their production and extraction. Chitin is one such polymer of N-acetyl-glucosamine and glucosamine monomers, linked together with β-(1- 4) glycosidic linkage [1]. Previous studies have categorized Chitin and deacetylated derivative chitosan, based on the degree of deacetylation, primarily regulated by N-acetyl-glucosamine [2]. Chitin and chitosan are derived from seafood waste and different extensively in chain length, degree of deacetylation and the associated applications [3]. The polymers with less than40% degree of deacetylation is categorized as chitin while those with40% or more are categorized as chitosan [4]. The chitosan can be obtained from chitin by chemical or biochemical deacetylation of chitin [2]. Chitosan is environmental friendly biopolymer having unique property like nontoxic, polycationic, biodegradable as well as antimicrobial all these property make chitosan an acceptance in different areas include pharm, agriculture, food industry, cosmetic and biotechnology. Degree of deacetylation can be range of 60% - 95% to 70% -95% according to use and application on which chitosan is used [5]. The chitosan find vast applications in various sectors, viz. food industry and as packaging material [6], waste water treatment and in heavy metal removal [7], in membrane for purification [8], in agriculture for controlled release of agrochemicals [9], paper and pulp industry [10], cosmetics [11], in wound healing and drug delivery [12,13] and gene delivery [14]. The vast application of the polymer are attributed to diversities in degree of deacetylation, molecular mass and size [15]. Among various natural sources of chitosan, fungal chitosan obtrudes as one of the most well accepted polymer due to consistent yield of low molecular weight chitosan [16]. Fungi are a rich source of diverse primary and secondary metabolite of commercial and therapeutic importance [17]. The biomass, left after harvesting of fungal fermentation broth and their processing, possess a serious decomposition problem leaving incineration as a common method, posing challenge for environment. Alternatively, the biomass may be used for extraction of chitosan, often present as a structural polymer in the wall of fungi, with relatively higher proportion in the phylum Ascomycete [18].

The degree of deacetylation and molecular weight of chitosan play significant role in determining the industrial application of chitosan. Studies have shown different fungal systems, being explored for chitosan yield under different culture conditions (Table (1)). The data in Table 1 showed the importance of culture conditions and organism on chitosan yield, their molecular mass and degree of deacetylation. Arcidiacono and Kaplan (1992) observed that incubation period and composition of culture media are the two important parameters that affect the growth and production of fungal biomass. Moreover, the molecular weight of chitosan was also affected [19].

Table 1: Different fungal strains, reported for chitosan extraction and he properties of chitosan obtained.*

Fungi	Culturemedia/ Fermentation type	Chitosan yield/ Molecular Weight	Degree of acetylation/ Deacetylation*	Reference
Mucror rouxii	Synthetic Medium/ SMF	5% to 10% of total biomass dry weight 200-1400 kDa	8% - 13%**	[19]
Rhizopus oryzae	PDS/ SMF	138 mg/g dry weight 6.9×10^{4} Da	87.9 ± 2.1**	[20]
Gongronella butteri	Sweet potato/ SSF	12.7% of total biomass 25.38 kDa	92% to 96%*	[21]
Absidia coerulea	Potato pieces/ SSF	6.12 g/kg Substrate 6.4 kDa	85%*	[22]
Absidia coerulea	Synthetic Medium/ SMF	9.4% dry mycelia 140 kDa	35%**	[23]

SSF: Solid Sate Fermentation; ** SMF: Submerged Fermentation

The quest to explore new chitosan sources had provided an

impetus to explore less or unexplored organisms as potential source of the versatile polymer, chitosan. Current study envisaged to explore the fungal strain, *Aspergillus ochraceus* a potential alternative organism for the production of chitosan polymer. The fungal strain is not so well explored so far for metabolite production but may obtrude as a potential source of chitosan. In the study, the role of different media on growth and biomass yield, from submerged culture of *Aspergillus ochraceus* was compared.

Material and Methods

Fungal Culture

In this study fungal strain *Aspergillus ochraceus* ((MTCC1877) was used which belong to phylum Ascomycete.

Media used for Cultivation

Different media used in the study, *viz.* Potato Dextrose Broth medium(PBD),Yeast Potato Dextrose Broth medium (YPD), and Sabourd's Dextrose Broth (SDB) were purchased from HiMedia Laboratories Pvt. Ltd., India. All other chemicals used in the study were of analytical grade, unless specified

Revival and growth studies

The fungal strain, preserved as cryostock in the institute repository, was revived by growing on solidified PDB agar (2% w/v) plate. The spore suspension 1 ml was prepared from 7 days old PDB agar plates and it was inoculated into 100 ml of PBD, YPD and SDB. Flasks were incubated at 30C, 150 rpm for next seven days. The flasks were harvested and fungal biomass was separated from liquid by centrifuging the suspension at 5000 rpm for 30 minute.

Estimation of dry weight

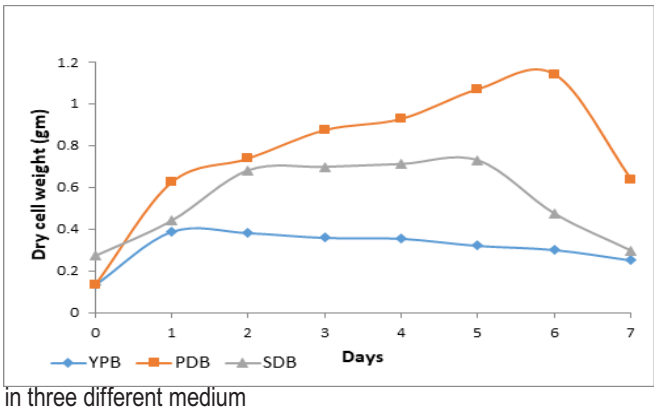
The sample was collected from the flasks after every 24 hours for 8 days. The harvest was centrifuges at 9000 rpm at room for 20 minutes. The pellet was air dried for 24 hours and the pellet was weighed.

Result and discussion

Aspergillus ochraceus had attracted scientific interest for being a source of value added products. In a recent study, Aracri et al., (2019) reported the production of enzyme tannase from the submerged culture of the fungi [24]. However, scarce reports on its role as potential source of polymers, especially chitosan had been available. The yield of chitosan depends on the media and biomass yield. The growth and biomass yield of the fungal strain in three different media, *viz.* Potato dextrose broth (PBD),Sabourd's Dextrose broth (SDB), Yeast Potato Dextrose broth (YPD). Results showed that higher biomass was obtained in Sabourd's Dextrose broth (SDB) on fifth day of fermentation (Table 2). The analysis of biomass increase with time (Fig. 1) showed that the culture showed highest increase change in biomass during the first day of the study. However, the secondary growth was differing in different media.

Table 2: Time dependent biomass estimation of *Aspergillus ochraceus*

Day	Potato dextrose broth (PDB)Dry weight cell(g)	Sabourd's Dextrose broth (SDB)Dry weight cell(g)	Yeast Potato Dextrose broth(YPD) Dry weight cell(g)
0	0.132	0.273	0.132
1	0.388	0.681	0.388
2	0.382	0.441	0.382
3	0.359	0.697	0.359
4	0.251	0.682	0.251
5	0.301	0.728	0.301
6	0.355	0.474	0.355
7	0.321	0.298	0.321



in three different medium

Fig. 1: Growth of *Aspergillus ochraceus* in three different culture media

Table 3: Kinetic parameters of growth and generation time of

Submerged fermentation in culture media	Specific growth rate μ (day^{-1})	Doubling time t^d (day)
Potato dextrose broth (PDB)	1.016	0.6
Sabourd's Dextrose broth (SDB)	0.559	1.2
Yeast Potato Dextrose broth (YPD)	0.255	2.7

Aspergillus ochraceus in three different media.

The kinetic parameter estimation had shown comparatively higher growth in Potato dextrose broth (PDB). However, the highest biomass was obtained in Sabourd's Dextrose broth (SDB). The results showed that through the doubling time of fungal biomass is higher in Sabourd's Dextrose broth (SDB), compared to Potato dextrose broth (PDB), the overall biomass yield, obtrude Sabourd's Dextrose broth (SDB) as a better media. The study may be further extended toward analysing the role of solid state fermentation as a cost effective alternative for improving biomass yield and chitosan yield thereof. Moreover, the role of culture conditions on chitosan yield, molecular mass and degree of deacetylation may be a fascinating parameter to be explored.

Conclusion

The growing global cues toward wide acceptance of natural or biological polymers, provided an impetus to researchers to explore

alternative organisms as potential source of such polymers. Current study explored the growth response of *Aspergillus ochraceus*, a potential organism that may be explored for its role as an alternative source of chitosan. The yield of chitosan, which is an integral component of the fungal cell wall can be regulated by altering the fungal biomass yield. It may be proposed that variation in growth response and biomass yield of fungi under different culture conditions may affect the yield of chitosan, thereof. The study will paved the way for designing a robust strategy to study the effect of abiotic parameters on chitosan yield in other organisms too.

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