Research in Biotechnology, 4(6): 01-07, 2013 ISSN: 2229-791X
www.researchinbiotechnology.com

Regular Article

Effective utilization of *Salvinia molesta* D. Mitch. for the production of cellulase enzyme by using *Aspergillus* species and reuse of fungal biomass for dye degradation

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The present study reveals *Salvinia molesta* D. Mitch.in various concentration is used as carbon source for the production of cellulase enzyme using *Aspergillus* species by submerged fermentation. The basal medium supplemented with *Salvinia molesta* blend in the proportion of 1:0.5 as carbon source showed maximum cellulase production after 6 days of incubation at room temperature with agitation speed of 150 rpm in rotary shaker, were liberated 3.2mg/ml glucose. Methylene blue dye was completely decolourized within 6 days of incubation at room temperature with agitation speed of 150 rpm. This demonstrates the reuse of fungal biomass for dye degradation after enzyme production.

Key Words: *Salvinia molesta* D. Mitch., Aquatic weed, *Aspergillus* sps., Fungal biomass

Rivers, Streams, Dams, Lakes, Paddy fields both natural and manmade ecosystems play an important role in the cultural and economic status of the nations. But most of these aquatic systems are infested with different kinds of weeds. One of the important aquatic weed is *Salvinia molesta* D. Mitch., a free floating aquatic fern, also called as *Kariba weed* or *African payal* secondly *Eichornia crassipes*. They are rapidly spreading throughout the water body(Holm *et al*., 1977; Farrell, 1978). The excessive growth of these aquatic weeds prevents light penetration in the water column and it forms dense floating mats. It can shade out favourable vegetation and also degrade habitat of both aquatic flora and fauna (Room, 1986; Owens *et al*., 2004).

Over the past 70 years *Salvinia molesta* D. Mitch. has spread from its native range in south America to tropical and subtropical regions around the world (Nagendra Prabhu, 2001). Its explosive growth has devasting socio-economic impacts in parts Africa, Sri-Lanka, Guniea, Philippines, Australia and India(Douglas and Oliver, 1993).

Salvinia molesta D. Mitch.was thought to be used as cheap source of cellulose for the production of cellulase by fungal isolates. These enzyme has novel application in the production of fermentable sugars, ethanol, organicacids, detergents and other chemicals. They have been used in the pulp industry, paper industry etc. Cellulose is the world's most natural biopolymer and has a great significance for the production of industrially valuable materials such as fuels and chemicals (Lynd *et al*., 2002).

Cellulose contains simple repeating units of glucose, but it has complex structure because of the long chains of glucose subunits joined together by β-1,4 linkages (Levy *et al*., 2002; Lone *et al*., 2012). Some fugal genera like *Aspergillus, Trichoderma, Myrothecium*, *Cheatomium* and *Neocallimastics* are the decomposers of cellulose. However, *Trichoderma* has been found to produce an enzyme termed 'swollenin' which breaks hydrogen bonds and it leads to the degradation of cellulose (Tomme *et al*., 1995; Usama *et al*., 2008).

The coloured industrial effluent from the dyeing industries is one of the major pollutant to the aquatic systems which leads major environmental problems to both aquatic flora and fauna. Such effluents in water bodies also increase Biochemical Oxygen Demand (BOD) in these aquatic ecosystems (Jothimani and Prabakaran, 2003). Now days a number of biotechnological approaches have been suggested by recent researchers to solve this pollution in an eco-efficient manner by using bacteria or fungi (Singh *et al*., 2012).Bio-treatment of dye is cheaper and environmentally friendlier alternative for colour removal in textile effluent. Such eco-friendly approach is a good method to solve many environmental problems (Lin and Tanaka, 2006; Vishal *et al*., 2010).

So present study focus on the production and characterisation of cellulase by local fungal isolates using *Salvinia molesta* as carbon source and reuse the fungal biomass for dye degradation after cellulase enzyme production and there by production cost of cellulose enzyme (cellulase) is reduced gradually. The efficiency of *Aspergillus* species for producing cellulose was also measured.

Materials and Methods

Sample collection

The plant source such as *Salvinia molesta* D. Mitch. for the present study was collected from the upper surface of pond situated in kuravilangad (Kottayam district, Kerala, India).

Isolation of fungal microflora from *Salvinia molesta* D. Mitch.

Isolation of fungal microflora done by the technique such as serial dilution method. About 10 g of *Salvinia molesta* weighed and taken into flask containing distilled water (90 ml) and shake vigorously for a number of times to remove the fungal flora from the surface of plant and then it is deposited in to the water. The serial dilutions 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} respectively. Then spread on potato dextrose agar medium (Sherman, 2004).

The spread plate technique is used for the separation of mixed population of microorganisms.Finally it is spread over the solidified medium with a sterile L-shaped glass rod.These plates are incubated in an inverted position for 3-7 days at 25^0C. After 7 days of incubation count the number of colonies were observed (Mandels and Reese, 1964).

Identification of fungal microflora

The identification is done by tease mount method. Taking a bit of the mycelial growth from the agar surface and transferring it on a glass slide in Lactophenol-cotton bluestain and then remove a small amount of hyphal mass by the help of flame sterilized stiff needles in to the slide and mounded it with coverslip. Finally examine it under the microscope (Mahdishahriarinour *et al*., 2011).

Screening of cellulose producing fungi

20ml of carboxy methyl cellulose agar medium, melted and cooled at 45^0 C. The plates were inoculated with spore suspension of pure culture and incubated at 30^0 C for screening of cellulase producing fungi. After 3 days, the plates were flooded with congo-red solution for 15 minutes and it is de-

stained with 1M NaCl solution for 15 minutes. The fungal colony in which largest zone of decolorization was selected for cellulose production (Kusmanova *et al*., 1991; Sherman, 2004).

Pure culture of *Aspergillus*

Aspergillus colonies were cultured and the stock cultures are maintained on potato dextrose agar at 4^0 C.

Biochemical analysis

The 100 gms of fresh leaves *Salvinia molesta* blended by mixer with equal volumes of water. The whole blend was used as carbon source for the production of cellulose throughout. The basal medium supplemented with different volumes of *Salvinia molesta* blend was prepared in the ratios of 1:0.1, 1:0.2, 1:0.3, 1:0.4, 1:0.5, 1:0.6 and 1:0.7 respectively.It is transferred to the 250 ml Erlenmeyer flask, which is incubated with 2ml of fungal spore suspension containing 10^4spores per ml. It is incubated at room temperature with agitation speed 150 rpm in rotary shaker for seven days. Then culture was centrifuged at 4000 rpm for 20 minutes and cellulose activity of each supernatant was measured using DNS method (Sadasivam and Manickam, 2005).The total protein can be estimated by Lowry's method (Lowry, *et al*., 1951).

Reuse of fungal biomass for dye degradation

After cellulase production, fungal biomass recovered by centrifugation and inoculated into 250 ml Erlenmeyer flask containing 100 ml basal medium with 3% sucrose ascarbon source and 40 mg of filter sterilized methylene blue and incubated at 30^0 C with agitation speed of 200 rpm for 6 days (Jothimani and Prabakaran, 2003). The decolorization of methylene blue was observed by visual observation of flask**.**

Result and Discussion

Salviniamolesta D. Mitch.,the plant source utilized for present study (**Fig.1**). The present study results that, various fungal micro-flora obtained on dextrose agar medium, by tease mount method (**Tables 1**& **2**)**.** The cellulose producing fungi was screened by using carboxy methyl cellulose agar medium. Among these, *Aspergillus sp.* shows largest zone of decolourization(**Fig.2**). Amount of protein present in various concentrations of *Salvinia molesta* associated with *Aspergillus* after fermentation. It indicates that the amount of protein present with increased with increasing the concentration of *Salvinia molesta* (**Fig.3** & **Table 3**).

Figure 1. Habit of ***Salvinia molesta*** D. Mitch.

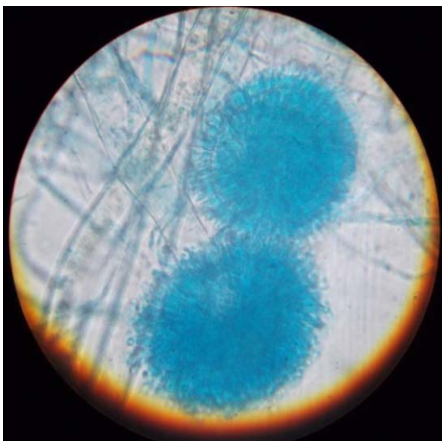

Figure 2.Microscopic view of *Aspergillus* sps. with its fruiting body

Table 1. Amount of fungal cells present in different dilutions isolated from *Salvinia molesta* D. Mitch.

Sl. No.	Dilution	Number of colonies	Number of Cells/mL
1.	10^{-3}	13	1300Cells/mL
2.	10^{-4}	8	8000Cells/mL
3.	10^{-5}	6	60000Cells/mL

Table 2.Microflora isolated from the surface of *Salvinia molesta* D. Mitch.

Sl. No.	Type of Fungi	Number of Colonies
1.	*Penicillium*	2
2.	*Trichoderma*	2
3.	*Rhizopus*	8
4.	*Yeast*	6
5.	*Aspergillus*	4
6.	*Mucor*	5

Table 3.Effect of basal medium /*Salvinia molesta* D. Mitch. **on protein production**

Sl.No.	Concentration	Optical Density	Protien (mg/ml)
1.	**1:0.1**	**0.18**	**0.1**
2.	**1:0.2**	**0.22**	**0.12**
3.	**1:0.3**	**0.28**	**0.155**
4.	**1:0.4**	**0.30**	**0.165**
5.	**1:0.5**	**0.35**	**0.195**
6.	**1:0.6**	**0.40**	**0.22**
7.	**1:0.7**	**0.52**	**0.29**

Table 4. Concentration of glucose with respective OD value

Sl.No.	Concentration of glucose (mg/ml)	OD value
1.	**1.6 mg/ml**	**0.010**
2.	**2.0 mg/ml**	**0.15**
3.	**2.5 mg/ml**	**0.21**
4.	**3.3 mg/ml**	**0.31**
5.	**4.0 mg/ml**	**0.39**
6.	**5.0 mg/ml**	**0.51**
7.	**6.7 mg/ml**	**0.72**

Amount of cellulase producing in various concentrations are obtained by DNS method. The basal medium supplemented with *Salvinia molesta* blend in the proportion of 1:0.5 as carbon source showed maximum cellulase production after 6 days of incubation at room temperature with agitation speed of 150 rpm in rotary shaker, were liberated 3.2mg/ml glucose (**Fig. 4** & **5 and Tables 4**& **5**).

Table 5.Effect of basal medium/ *Salvinia molesta* D. Mitch. **on cellulase production**

Sl. No.	Concentration	Optical Density	Glucose Liberated mg/mL
1.	1:0.1	0.016	1.05
2.	1:0.2	0.012	0.9
3.	1:0.3	0.010	0.8
4.	1:0.4	0.015	1
5.	1:0.5	0.030	1.6
6.	1:0.6	0.012	0.9
7.	1:0.7	0.010	0.8

Active cellulase producers were selected among isolates of fungi belonging to the genera *Aspergillus* species. After the cellulase production, de-colourisation of methylene blue was observed by visual observation of flask. This results indicates that, the dye de-colourization is more effective due to the activity of *Aspergillus species* (**Fig.-6**).

Conclusion

The present study concluded that the association of *Aspergillus* sps. with *Salvinia molesta,* can be utilized for production of useful enzymes like cellulase by submerged fermentation**.** The leaves of *Salvinia molesta* is a good carbon source for the production of cellulase enzyme. The decolorization of methylene blue dye by fungal biomas (*Aspergillus* sp.) is also gives clues for the biodegradation of industrial coloured effluents and thereby it minimizes environmental pollution. It indicates that, *Aspergillus* species have both the capacity for the production of cellulase enzyme and also for dye degradation. So that, the industrial

effluents and other environmental pollutants could be treated with biological sources before it is discharged into the environment. It would be an eco-friendly approach for balancing the equilibrium of our ecosystem by minimizing environmental pollution.

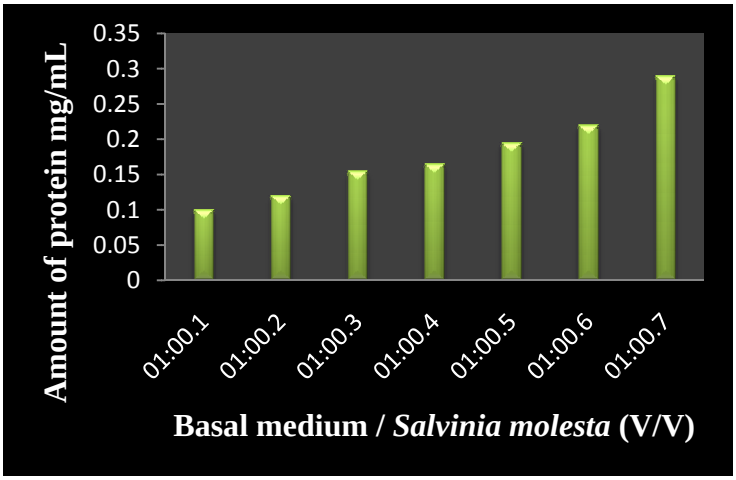

Figure 3.Effect of basal medium /*Salvinia molesta* D. Mitch. on protein production

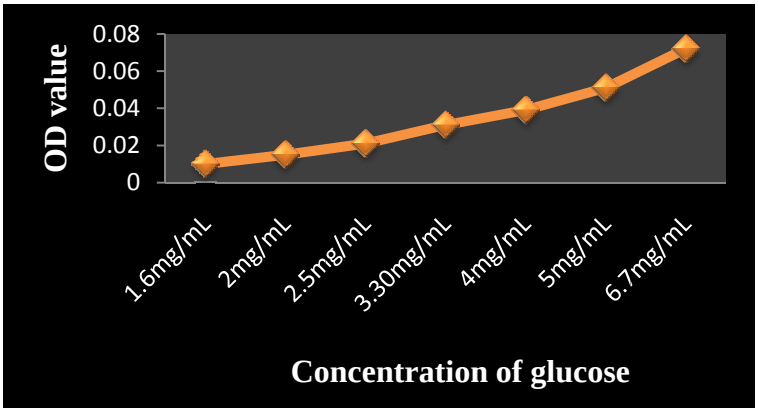

Figure 4. Concentration of glucose with respective OD value

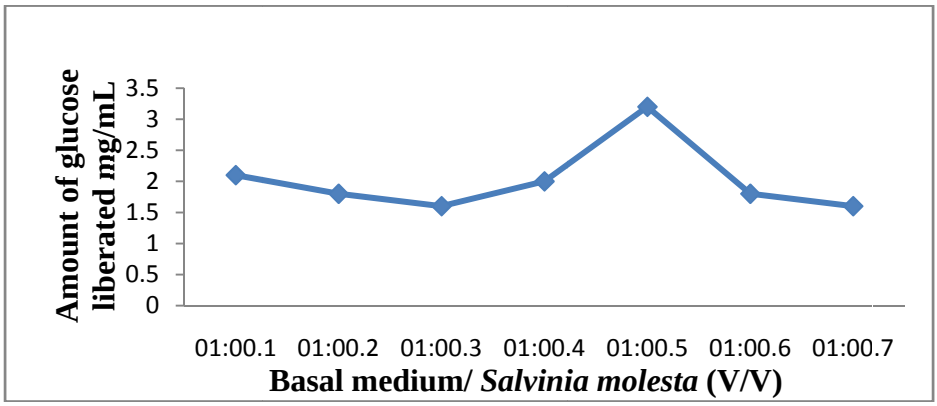

Figure 5.Effect of basal medium /*Salvinia molesta* D. Mitch. on cellulase production

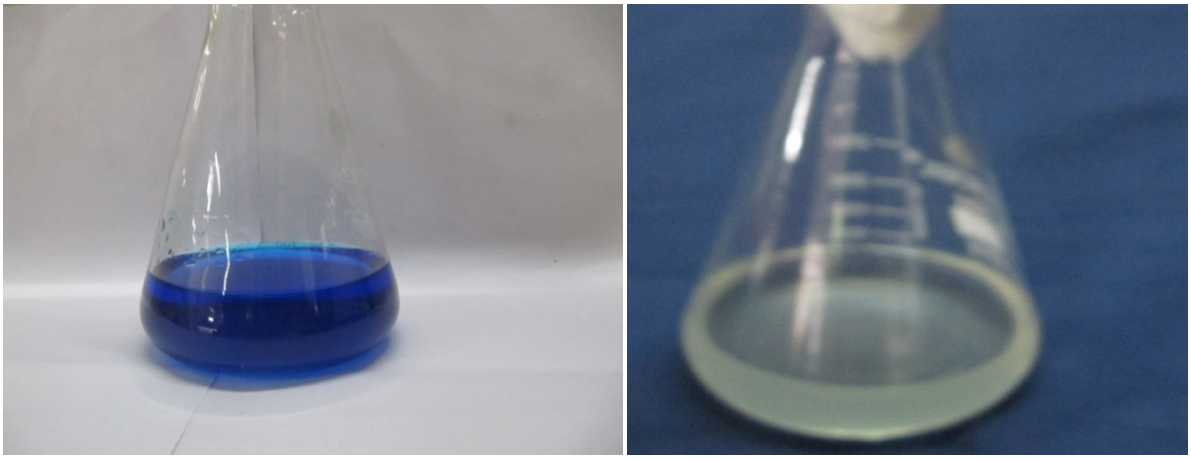

Figure 6. De-colorization of methylene blue by *Aspergillus* sps. biomass

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