OPTIMIZATION OF CULTURAL CONDITIONS FOR THE PROPAGATION OF *GLIOCLADIUM VIRIDE* ZIC$_{2063}$ AS POTENTIAL BIOSORBENT

Arifa Tahir[1], Sidra Zahid[1], Bushra Mateen[1], Tasnim Farasat[1], Tahira Mughal[2]

1. Environmental Science Department, Lahore College for Women University Lahore, Pakistan
2. Botany Department, Lahore College for Women University Lahore, Pakistan

ABSTRACT

In the present study, the propagation of a newly isolated *Gliocladium viride* ZIC$_{2063}$ for maximum mycelium formation is checked. This is the first report on the optimization of *Gliocladium viride* ZIC$_{2063}$ cultural conditions to get high mycelium concentrations that is to be used in further biosorption process. The mould mycelium was exploited as biosorbent. The present study was designed to optimize culture medium and other cultural conditions (pH, temperature, incubation time, inoculum age and size) for the growth of fungal culture as biosorbent. The amount of mycelium was doubled due to optimization. The *Gliocladium viride* ZIC$_{2063}$, being high chromium resistant, thermostable and acid stable, can find application in the treatment of tanning effluent of leather industry and combating

Key Words: *Gliocladium viride*, Biosorbant, Culture

Address for Correspondence: Arifa Tahir, Environmental Science Department, Lahore College for Women University Lahore, Pakistan. E-mail: arifa.tahir@yahoo.com

INTRODUCTION

Fungi are well known for their metal binding abilities. Fungi exhibit marked tolerance towards metals and other adverse conditions (Faryal *et al*., 2006). The metal binding capacities of fungi depends on the presence of functional groups on cell wall (Luef et al., 1991). These functional groups can be blocked or damaged by surrounding environments of fungi (Ewan and Pamphlett, 1996). One of studies investigated that chemical composition of cell wall is very sensitive to growth conditions (Yang and Illman, 1999). This is how the composition of growth medium and other cultural conditions effects the present study. The cultural conditions were optimized to get high biomass concentration that can be used in further biosorption experiments. The conditions in which microorganisms grow affect its cell surface phenotype which in turn affects its biosorption potential (Gadd, 1990). *Aspergillus niger* grown in the presence of potassium hexacyanoferate exhibited high biosorption (Luef *et al.,* 1991). Gram-positive bacteria show increased biosorption by adding nutrients in 2h of incubation, while Gram-negative bacteria didn't show the same trend (Gourdon *et al.,* 1997). *Phormidium laminosum*, biosorption increased with addition of sufficient nitrogen source (Sampedro *et al.,* 1995). Detailed work was done on the optimization of pH, temperature, inoculum size, agitation and carbon nitrogen sources on fungal culture. This is the first report on the optimization of cultural conditions of *Gliocladium viride* ZIC$_{2063}$ on potato dextrose medium . In this

study, an attempt has been made to formulate a suitable and cost-effective medium for the propagation of *Gliocladium viride* ZIC_{2063} as biosorbent

MATERIAL AND METHODS

Organism

More than fifty fungal cultures from tanning unit effluent were isolated using Potato Dextrose Agar medium. After screening the mould culture *Gliocladium viride* ZIC_{2063} was selected for further investigation. The selected culture was transferred in glycerol at −20°C and also at 4°C.

Biomass Analysis

Fungal cell biomass was estimated gravimetrically by filtering the culture through a pre-weighed dry Whatman No. 1 filter paper. The mycelium was thoroughly washed with distilled water and then weighed.

Propagation of biomass

To achieve maximum amount of *Gliocladium viride* $ZIC_{2063,}$ cultural conditions were optimized. Four culture medias, Potato dextrose broth (M_1), yeast peptone sucrose medium (M_2), liquid medium (M_3) and Czapek Dox medium (M_4) were used for the propagation of fungal growth (Table1). Selected medium was further supplemented with different carbon sources (Sucrose, dextrose and Sodium acetate) and nitrogen sources (Sodium nitrate, urea and yeast extract) to evaluate their effect on the growth of fungi. These carbon and nitrogen sources were added at the concentration of 1%**.** In order to investigate the effect of initial pH of growth medium the pH was varied from 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5 and 6.0. Phosphoric acid (1M) was used to adjust the pH. Incubation temperature ranged from 20 ^{o}C to 35 ^{o}C for different time periods i.e., 1, 2, 3 and 4 days to optimize incubation temperature as well as incubation time. Inoculum age (3, 5 and 7 days old) and size (2%, 4%, 6%, 8% and 10%) were optimized. Role of agitation on mycelium growth was also recorded.

RESULTS & DISCUSSION

Screening of culture Medium

Four different culture media (M_1, M_2, M_3 and M_4) were evaluated for the propagation of *Gliocladium viride* ZIC_{2063} for mycelium formation (Table 1). Of all the media testedM_1 (Potato Dextrose Broth) gave best results (4.9029 g wet weight) and was used in further propagation process. While in M_2, M_3 and M_4 medium the biomass growth was 3.6945 g wet weight, 3.0246 g wet weight and 2.8301 g wet weight respectively.

Potato broth was supplemented with different carbon and nitrogen sources to study the effect on the growth of fungi. It was found that type and concentration of carbon and nitrogen sources had a significant effect on growth. Among carbon sources dextrose gave the maximum results (4.7813 g wet weight) (Table 2). All nitrogen sources were found effective for fungal growth but Sodium Nitrate was found to be best nitrogen source (4.9508 g wet weight) for the propagation of *Gliocladium viride* ZIC_{2063} (Table 3). Fungi use nitrogen in the form of ammonium and nitrate nitrogen as nitrogen sources (Srivastava and Thakur, 2006). Potatoes Dextrose Broth (PDB) is the most commonly used media for fungi (Nourisepehr *et al.,* 2005; Srivastava and Thakur, 2006; Congeevaram *et al.,* 2007). These results are in agreement with previous work (Srivastava and Thakur, 2006).

Effect of initial pH

It has identified that pH can change the charge of fungal cell surface and its attached functional groups (Yan and Viraraghavan, 2003). It was observed that the initial pH of medium affected the growth rate during log phase with a maximum biomass growth (3.8303 g wet weight) observed at pH 4.0. The biomass growth was poor at pH above or below 4.0 as shown in Table 4.

Effect of incubation time

Gliocladium viride ZIC_{2063} was incubated for 24, 48, 72 and 96 h (Table 5). Fungal growth was found maximum (4.6967 g wet weight) after 72 h of incubation. Thick mass of brown to black mycelial pellets appeared after 72 h incubation. Further increase in the time of incubation did not cause considerable increase in mycelium concentration.

Effect of incubation temperature

Incubation temperature was varied from 20, 25, 30 and 35 ^{o}C. The data of table 6 shows that maximum mycelium growth (4.6847 g wet weight) was obtained at 30 ^{o}C. Decreasing temperature below 25 ^{o}C decreased the fungal mycelium growth. Further increase in temperature above 30 ^{o}C also decreased the mycelium concentration. Temperature of growth media is much important because growth is energy dependent mechanism (Srivastava and Thakur, 2006). The optimal temperature for *Gliocladium viride* ZIC_{2063} growth is 30 ^{o}C. Similar observations have also been reported by other researchers (Congeevaram *et al.,* 2007).

Effect of inoculum age & inoculum size (%)

Inoculum must be in healthy and active state to minimize the length of lag phase. Inoculum of different age (3, 5 and 7 days) was used to check the effect of inoculum age on the propagation of *Gliocladium viride* ZIC_{2063}. It was observed that maximum mycelial pellet (4.8905g wet weight) was obtained with 5 days old inoculum (Table 7). Inoculum size has profound effect on fungal morphology (Foster, 1949). Similarly to investigate the effect of inoculum size the growth medium (M_1) was incubated with 5 days old inoculum of

different inoculum sizes (2%, 4%, 6%, 8% and 10%) mycelium concentration for 2%, 4%, 6%, 8% and 10% inoculum was 3.7794 g, 3.7802 g, 3.7813 g, 4.0023 g and 4.0132 g wet weight respectively (Table 8). Hence, in further experiments 8% inoculum size was used.

Effect of agitation

To see the effect of static and agitated conditions, the culture was incubated for 48 h at 122 rpm in orbital shaker for agitated conditions and flasks were incubated at 30 ^{o}C in static conditions. It was interesting to observe that agitation affects the biomass yield and it was about three times greater when after 48h shaking the cultural conditions were shifted to static conditions. Kirk et al, 1986, Yang Illman, 1999 wee also recorded the similar trend. Uniform agitation for 72 h gave 4.7813 g wet weight mycelial pellet and biomass growth was very poor when it was incubated under static conditions (2.7194 g wet weight). Maximum thick black mycelium (5.2802 g wet weight) was obtained, when after 48 h shaking the cultural conditions were shifted to static conditions (Table 9).

Table – 1 Effect of different growth media (along with composition) on the propagation of *Gliocladium viride* ZIC$_{2063}$

Sr. No.	Growth media		Wet weight of mycelial pellet(g)	composition of growth medias	Growth rate
1	M_1	PDB Medium (Atlas, 1997)	4.9029	Potatoes 300g/500ml Dextrose sugar 1.5g/100ml	++++
2	M_2	YPS Medium (Li *et al.,*2007)	3.6945	Yeast extract 3.0 g/l, Peptone 10g/l Sucrose 20g/l pH 4.5	+++
3	M_3	Liquid Medium (Mungasavalli *et al.,* 2007)	3.0246	Dextrose 20g/l, Peptone 10g/l, Yeast extract 3g/l pH 5	++
4	M_4	Czapek Dox Medium (Atlas, 1997)	2.8301	Sucrose 30g/l, $NaNO_3$ 3g/l, $MgSO_4$ $7H_2O$ 0.5g/l, KCl 0.5g/l, K_2HPO_4 1g/l, pH 6.2	+

Note: *(+) = very slow growth (++) = slow growth (+++) = moderate growth (+++) = fast growth*
Cultural conditions*: Incubation time72 h, incubation temperature 30^{o}C, inoculum size 2%, Volume of media 50ml, Shaking velocity 122 rpm*

Table – 2 Effect of carbon sources on the propagation of *Gliocladium viride* ZIC$_{2063}$

Sr. No.	Carbon Sources	Wet weight of mycelial pellet (g)	Growth rate
1	Sucrose	3.7194	++
2	Dextrose	4.7813	++++
3	Sodium acetate	4.7802	+++
4	Sodium Citrate	4.0123	++

Note: *(+) = very slow growth (++) = slow growth (+++) = moderate growth (+++) = fast growth*
Cultural conditions*: Incubation time 72 h, pH of media 4, incubation temperature 30^{o}C, inoculum size 8%, Volume of medium 50ml, Shaking velocity 122 rpm*

Table – 3 Effect of Nitrogen sources on the propagation of *Gliocladium viride* ZIC$_{2063}$

Sr. No.	Nitrogen Sources	Wet weight of mycelial pellet (g)	Growth rate
1	Yeast Extract	4.0014	++
2	Urea	4.1022	+++
3	Sodium Nitrate	4.9508	++++

Note: *(+) = very slow growth (++) = slow growth (+++) = moderate growth (+++) = fast growth*
Cultural conditions*: Incubation time 72 h, pH of media 4, incubation temperature 30^{o}C, inoculum size 8%, Volume of medium 50ml, Shaking velocity 122 rpm*

Table – 4 Effect of initial pH growth medium on the propagation of *Gliocladium viride* ZIC$_{2063}$

Sr. No.	Initial pH	Wet weight of mycelial pellet (g)	Growth rate
1	2.0	3.0153	+
2	2.5	3.0499	+
3	3.0	3.2360	++
4	3.5	3.6450	++++
5	4.0	3.8303	+++
6	4.5	3.3266	++
7	5.0	3.1704	++
8	5.5	3.0732	+
9	6.0	2.3832	+

Note: *(+) = very slow growth (++) = slow growth (+++) = moderate growth (+++) = fast growth*
Cultural conditions*: Incubation time 5 days, incubation temperature 30^{o}C, inoculum size 2%, Volume of medium 50ml, shaking velocity 122 rpm*

Table – 5 Effect of incubation time on the propagation of *Gliocladium viride* ZIC$_{2063}$

Sr. No.	Incubation time (h)	Wet weight of mycelial pellet (g)	Growth rate
1	24	3.0245	+
2	48	3.7793	++
3	72	4.7002	++++
4	96	4.6967	++++

Note: *(+) = very slow growth (++) = slow growth (+++) = moderate growth (+++) = fast growth*
Cultural conditions*: pH of media 4, incubation temperature 30^{o}C, inoculum size 2%, Volume of medium 50ml, shaking velocity 122 rpm*

Table – 6 Effect of incubation temperature onto the propagation of *Gliocladium viride* ZIC$_{2063}$

Sr. No.	Incubation temperature (^{o}C)	Wet weight of mycelial pellet (g)	Growth rate
1	20	3.0734	+
2	25	3.8979	+++
3	30	4.6847	++++
4	35	3.0245	++

Note: *(+) = very slow growth (++) = slow growth (+++) = moderate growth (+++) = fast growth*
Cultural conditions*: Incubation time 72 h, pH of media 4, inoculum size 2%, Volume of medium 50ml, Shaking velocity 122 rpm*

Table – 7 Effect of inoculum age on biomass production of *Gliocladium viride* ZIC$_{2063}$

Sr. No.	Age of inoculum (days)	Wet weight of mycelial pellet (g)	Growth rate
1	3	4.1122	+++
2	5	4.8905	++++
3	7	4.0054	++

Note: *(+) = very slow growth (++) = slow growth (+++) = moderate growth (+++) = fast growth*
Cultural conditions*: Incubation time 72 h , pH of media 4, incubation temperature 30^{o}C, inoculum size 8%, Volume of medium 50ml, Shaking velocity 122 rpm*

Table – 8 Effect of inoculum size (%) on biomass production of *Gliocladium viride* ZIC$_{2063}$

Sr. No.	Inoculum size (%)	Wet weight of mycelial pellet (g)	Growth rate
1	2	3.7794	++
2	4	3.7802	++
3	6	3.7813	++
4	8	4.0023	++++
5	10	4.0132	+++

Note: *(+) = very slow growth (++) = slow growth (+++) = moderate growth (+++) = fast growth*
Cultural conditions*: Incubation time 72 h , pH of media 4, incubation temperature 30^{o}C, Volume of medium 50ml, Shaking velocity 122 rpm*

Table – 9 Effect of agitation onto the propagation of *Gliocladium viride* ZIC$_{2063}$

Sr. No.	Agitation (rmp)	Wet weight of mycelial pellet (g)	Growth rate
1	Agitation 3 days	4.7813	+++
2	Without agitation 3 days	2.7194	++
3	2 days agitation followed by 2 days static conditions	5.2802	++++

Note: *(+) = very slow growth (++) = slow growth (+++) = moderate growth (++++) = fast growth*
Cultural conditions*: pH of media 4, incubation temperature 30^{o}C, inoculum size 8%, Volume of medium 50ml.*

REFERENCES

1. Atlas, R.M. (1997). Handbook of Microbiological media. 2nd ed. Lawrence C. parks.

2. Congeevaram, S., Dhanarani, S., Park, J., Dexilin, M. and Thamaraiselvi, K. (2007). Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. Journal of Hazardous Materials, 146: 270–277.

3. Ewan,K.B. and Pamphlett,R. (1996) .increased inorganic mercury in spinal motor neurons following chelating agents .Neurotox.17: 343:349

4. Faryal, R., Lodhi, A. and Hameed, A. (2006). Isolation, characterization and biosorption of zinc by indigenous fungal strains Aspergillus fumigatus RH05 and Aspergillus flavus RH07, Pak. J. Bot., 38: 817-832.

5. Foster,J.W.(1949)Chemical activites of fungi,p.62.Academic Press,New York.

6. Gadd, G.M. (1990). Heavy metal accumulation by bacteria and other microorganism. Experientia, 46: 834–840.

7. Gordon, M.K. and Porter, J.F. (1997). Equilibrium parameters for the sorption of copper, cadmium and zinc ions onto peat. Journal of Chemical technology and Biotechnolgy, 69: 309-320.

8. Kirk, T.K., Croan, S., Tien, M., Murtagh, K.E. and Farrell, R.L. (1986). Production of multiple ligninases by Phanerochaete chrysosporium:effect of selected growth conditions and use of a mutant strain. Enzyme Microb. Technol., 8: 27-32.

9. Li , H., Liu , T., Li , Z. and Deng, L. (2007). Low-cost supports used to immobilize fungi and reliable technique for removal hexavalent chromium in wastewater. Bioresource Technology

10. Luef, E., Prey, T. and Kubicek, C. P. (1991). Biosorption of Zinc by fungal mycelia wastes. Appl. Microbiol. Biotechnol., 34: 688–692.

11. Mungasavalli, D.P., Viraraghavan T. and Jin, Y.C. (2007). Biosorption of chromium from aqueous solutions by pretreated Aspergillus niger: Batch and column studies. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 301:214-223.

12. Nourisepehr, M.., Nasseri, S., Mazaheri M., Assadi and Yaghmaian K. (2005). Chromium bioremoval from tannery industrial effluent by Aspergillus oryzae, Iran. J. Environ. Health. Sci. Eng., 2: 273-279.

13. Sampedro, M.A., Blanco, A., Llama, M.J. and Serra, J.L. (1995). Sorption of heavy metals to Phormidium laminosum biomass. Biotechnol. Appl. Biochem., 22: 355–366.

14. Srivastava, S. and Thakur, I.S. (2006). Isolation and process parameter optimization of Aspergillus sp. for removal of chromium from tannery effluent. Bioresource Technology, 97:1167-1173.

15. Yan, G. and Viraraghavan, T. (2000). Effect of pretreatment on the bioadsorption of heavy metals on Mucor rouxii. Water SA, 26: ISSN 0378-4738.

16. Yang, V.W. and Illman, B.L. (1999). Optimum Growth Conditions for the Metal-Tolerant Wood Decay Fungus, Meruliporia incrassata TFFH 294, Prepared for the 30th Annual Meeting Rosenheim, Germany,USDA Forest Service, Forest Products Laboratory, Madison, WI 53705, USA.