INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
07–382/SAB/2008/10–3–325–328
http://www.fspublishers.org

Full Length Article

Growth of *Trichosporon cutaneum R 57* in the Presence of Toxic Concentration of Cadmium and Copper

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ABSTRACT

The filamentous yeast *Trichosporon cutaneum R57* strain was cultivated in batch mode on a liquid medium in the presence of increased concentrations of cadmium and copper ions (from 1 to 112 mg L^{-1}). The experiments elucidated the ability of the investigated strain to grow and to accumulate cadmium and copper ions. The obtained results have shown low to middle bioaccumulation efficiency for Cd (from 6.8% to 11.4% removal efficiency at various external concentrations of Cd) and moderate to high level for Cu (from 23.5% to 49.9% removal efficiency at various external Cu concentrations).

Key Words: *Trichosporon cutaneum R57*; Growth; Bioaccumulation; Cadmium; Copper

INTRODUCTION

The removal of heavy metals from effluents and wastewaters by microbe-based technologies is very challenging alternative for metal recovery and environmental protection. There are several methods for remediation of polluted environment, which can be separated on the physical, chemical and biological approaches. Among the group of microorganisms used for bioremediation leading place occupied yeasts (Trevors *et al*., 1986; Vasudevan *et al.*, 2003). One of the reasons for their higher bioaccumulation ability to heavy metals is their higher growth rate at low pH of the medium. This is because most of the heavy metals ions are soluble with considerable rate at low pH (Gadd, 1990; Blackwell *et al.*, 1995). The Cu (II) and Cd (II) ions are known as main pollutants of wastewaters and soils. One of the major tasks in the field of bioremediation research with the microorganisms is discovery of species with high heavy metal accumulation ability. It is proved, that the use of microorganisms are more suitable than currently used chemical methods of metal removing from waste waters. One reason for this is that the heavy metals ions in industrial waste waters are usually in low concentrations (Wang *et al*., 1997; Castro-Silva *et al*., 2003; Kahraman *et al*., 2005).

Our previous study has shown high ability of the yeast *Trichosporon cutaneum R57* strain to grow in the media supplemented with high content of phenols (Ivanova & Yotova, 1993) revealed that the capacity of strain to sustain toxic concentrations of heavy metals in the medium often refer to its ability to accumulate harmful ions in the cells (Blackwell *et al.*, 1995). It is well known fact that there is close correlation between growth rate and ion accumulation capacity. However, the physiological phenomenon, correlation and the dynamic changes between the strain growth rate and its bioaccumulation capacity as a cell response to the toxic Cu (II) and Cd (II) ions concentrations are still unknown. Hence, the objectives of this study were to describe the growth response and bioaccumulation capacity of laboratory cultivated cells of the strain R57 of filamentous yeast *T. cutaneum* to added to the medium various inhibitory concentrations of Cu and Cd.

MATERIALS AND METHODS

Trichosporon cutaneum R57 strain registered by Ivanova and Alexieva (1996) and maintained in the culture collection of Bulgarian National Bank of Industrial Microorganisms and Cell Cultures less than 2414 number was used for the experiments. The strain was maintained by periodic culturing on agar medium at 28°C every 48 h with the following composition (g L^{-1})- glucose 20, bacterial peptone 20, yeast extract 10, agar 20 (Ivanova & Alexieva, 1996). After the incubation procedure, the colonies were picked up and suspended into a mineral salt medium with a glucose concentration of 20 g L^{-1} at 28°C for 24 h in 250 mL Erlenmeyer flasks filled up with 100 mL liquid medium. The cultivation process was carried out in a medium containing the following reagents (g L^{-1}): KCl – 0.3; $MgSO_4.7H_2O$ – 0.15; Ca $(H_2PO_4)_2$ – 0.042; tiamin. HCl – 0.001; biotin – 0.001; NH_4OH – 4 mL; H_3PO_4 (1:10) – 3.7 mL; CH_3COOH (1:10) – 18.5 mL; 0.1 N NaOH – 4 mL (Ivanova & Alexieva, 1996). The sterile glucose solution (10 g L^{-1}) as a sole carbon and energy source was added to

To cite this paper: Georgieva, N., 2008. Growth of *Trichosporon cutaneum R 57* in the presence of toxic concentration of cadmium and copper. *Int. J. Agri. Biol.*, 10: 325–8

the buffered growth medium.

The cultures used as a control were prepared by loop-inoculating 100 mL liquid medium and incubating for 18 h on a rotary shaker (180 rpm at 30°C). For experimental cultures, 100 mL of medium was inoculated with 5 mL of the control culture (with initial biomass concentration of approximately 0.1 mg dry wt mL^{-1}) and was incubated on the rotary shaker for 30 h. The strain was cultivated in the presence of various cadmium and copper ions concentrations. The cadmium ions were supplied in the form of $CdSO_4$ in the range of concentrations: control – 0 mg Cd L^{-1}, treatments – 22 mg Cd L^{-1} (0.2 mm $CdSO_4$), 89.6 mg Cd L^{-1} (0.8 mm $CdSO_4$), 112.4 mg Cd L^{-1} (1 mm $CdSO_4$). Similarly the copper concentrations in the treatments were in the form of $CuSO_4.5H_2O$, as follows: control – 0 mg Cu L^{-1}, treatments– 1.27 mg Cu L^{-1} (0.02 mm $CuSO_4.5H_2O$), 3.17 mg Cu L^{-1} (0.05 mm $CuSO_4.5H_2O$), 5.08 mg Cu L^{-1} (0.08 mm $CuSO_4.5H_2O$). After autoclaving, the obtained sterile solutions of $CdSO_4$ and $CuSO_4$ were added into the growth medium prior to inoculation of the culture.

Microbial growth was monitored by measuring the optical density of the cultural medium every two hours at 610 nm using a spectrophotometer Spekol 11 (Germany). Determinations of cadmium and copper ions uptake by *Tr. cutaneum R57* were done using an atomic absorption spectrophotometer Perkin-Elmer (England).

RESULTS

The growth analysis of *T. cutaneum R57* has shown that the copper inhibitory concentration in the medium was 1.27 mg Cu L^{-1}, while the lethal one was 5.08 mg Cu L^{-1}. The inhibitory effect of copper ions on the cells growth was firstly associated with the increase of growth lag-period and consequently, with decrease of both, the exponential and stationary growth phases (Fig. 1). At the inhibitory concentration value of copper ion in the medium, the growing cells have accumulated as much as 0.867 mg Cu DW biomass L^{-1} following 24 h of growth. This was about 68.3% of copper ion removing from the medium. When copper ion lethal concentration (5.08 mg Cu L^{-1}) was added into the medium, the growing biomass has accumulated 1.197 mg Cu DW biomass L^{-1}, which has represented of only 23.5% of copper ion removing (Table I).

However, when the inhibitory concentration of copper ion in the medium was decreased to the half of the lethal one (3.17 mg Cu L^{-1}), during the stationary phase of growth, the growing cells have accumulated much more copper ions – 1.58 mg Cu DW cells L^{-1}, which is calculated as 49.9% of Cu removing from the cultural medium.

In another set of experiments the cadmium ion inhibitory concentration on the R57 strain was determined such as 22.4 mg Cd L^{-1}, while the lethal one was about 112 mg Cd L^{-1}. Similarly, the copper ions stress effect on the strain growth has shown that the toxic levels of cadmium have prolonged the time of lag-period and have decreased the exponential and stationary growth phases (Fig. 2). At the level of Cd ion inhibitory concentration about 22.4 mg Cd L^{-1} the cells have contained 1.93 mg Cd g DW cell and have accumulated during that time as much as 1.53 mg Cd DW cells L^{-1}. This has represented 6.8% of Cd removal from the medium. At the lethal Cd ion concentration after 24 h of strain growth, the cells have removed as much as 12.83 mg Cd DW cells L^{-1}, which represent 11.4% of Cd removal efficiency (Table II).

DISCUSSION

Comparing the uptake and accumulation rate of two toxic ions by the strain, it can be concluded that the R57 strain can be more efficient for the bioremediation of Cu ions from the medium than for the Cd one. However, the removal efficiency of the strain for Cu ions was higher than the one for Cd ions under equal conditions of growth. The difference of the R57 bioremediation behavior in relation to the studied toxic ions can be explained by the difference in their growth tolerance. The maximal removal efficiency of Cd ions by the strain was close to the lethal external concentration level of this ion, while this value for the Cu ions was lower (approximately equal to 50% of the Cu ion lethal inhibitory concentration).

The effects of heavy metals on the yeast cells growth mainly depends on the processes that contribute to the mechanisms of metabolic or passive uptake of toxic ions into the cells. Each of these mechanisms has a major impact on the vitality of the cells (Gadd, 1990; Volesky *et al*., 1993). The processes associated with the toxic effects of heavy metals on cell growth can be classified to the water and osmotic relations disturbance of the cells such as decreased cell membrane stability or changed ions relations of the cells (Breierova *et al.*, 2002; Vasudevan *et al.*, 2003). The level of heavy metal tolerance of the microorganisms relates closely to the ability of the certain strain to sustain the toxic ions by absorbing them to metabolically less active compartments such as cell walls or vacuoles or by evolving an effective mechanism for ion extrusion outside of the cells. The lost of metabolic and, consequently, growth activity is often associated with deterioration of cell ion relations and loss of nutrients. This process often is due to degradation of the membrane system of the cells that control uptake or efflux of ions and other metabolites of the cells. This process is well documented in the literature like loss vitality of the cells, which is better observed during the time of stationary phase of growth (Volesky *et al.*, 1993; Blackwell *et al.*, 1995). The loss of significant amounts of useful metabolites by the cells subjected to the heavy metal stress is often connected with the disturbance of important parts of the cell metabolism. These can be decrease of energy utilization and synthesis of energy rich compounds such as ATP or NAD/P/H. Moreover, the taken by the cells harmful ion can

Table I. Bioaccumulation and removing efficiency of the strain R57 grown in the medium contained toxic levels of copper ions

Initial concentration of Cu in the cultural medium (mg Cu L^{-1})	*Cu uptake by the R57 strain during the stationary growth phase (mg Cu g^{-1} DW cells)*	*Cu accumulation by the R57 strain during the stationary growth phase (mg Cu DW cells L^{-1})*	*Cu removing efficiency by the R57 strain from the cultural medium (%)*
1.27	0.416±0.009*	0.867±0.007	68.3 %
3.17	0.496±0.012	1.585±0.20	49.9 %
5.08	0.746±0.014	1.197±0.042	23.5%

* - Data are means of 3 replicates ±SD

Table II. Bioaccumulation and removal efficiency of the R57 strain grown in the medium containing toxic levels of cadmium ions

Initial concentration of Cd in the cultural medium (mg Cd L^{-1})	*Cd uptake by the R57 strain during the stationary growth phase (mg Cd g^{-1} DW cells)*	*Cd accumulation by the R57 strain during the stationary growth phase (mg Cd DWcells L^{-1})*	*Cd removal efficiency by the R57 strain from the cultural medium (%)*
22.4	1.93±0.14*	1.531±0.15	6.8 %
89.6	35.11±1.10	7.794±0.42	8.7 %
112	81.22±2.42	12.832±1.10	11.4 %

* - Data are means of 3 replicates ±SD

Fig. 1. Growth curves of *Trichosporon cutaneum R57* in Cu-supplemented medium. Every point from the curves represents mean of 5 replicates ±SD

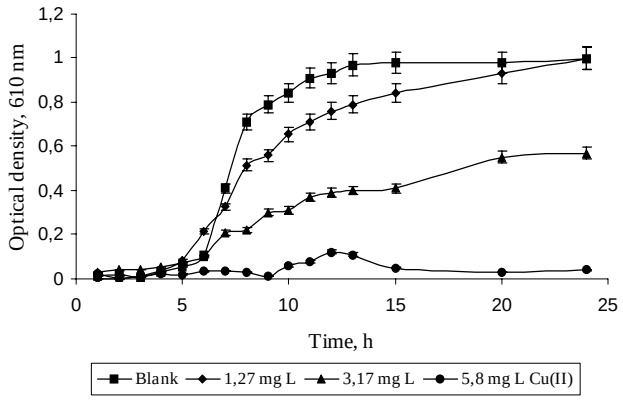
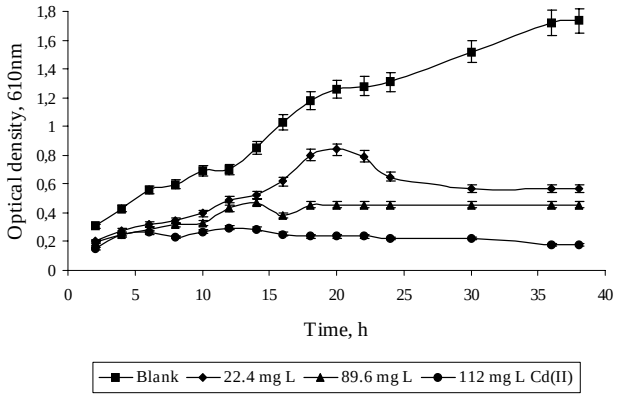


Fig. 2. Growth curves of *Trichosporon cutaneum R57* in Cd-supplemented medium. Every point from the curves represents mean of 5 replicates ±SD



disturb the structure and function of different low and high molecular compounds that play important role in the cell metabolism such as carbohydrates, proteins, nucleic acids and lipids. The efficiency of any strain to sustain by accumulation of higher levels of heavy metals inside the cells is connected with its ability to deliver these ions into the vacuoles or to keep them out of metabolically active sites of the cells. This can be a process of binding them to some metabolically inactive compartments such as the cell wall (Trevors *et al*., 1986; Wang *et al.*, 1997; Wang *et al*., 2000).

CONCLUSION

A considerable ability of the *T. cutaneum R57* strain to accumulate Cu and Cd ions from the cultural medium led to conclude that this preliminary data could be useful for the further research on the population level which is important for real industrial applications. These data on the bioaccumulation ability of studied R57 strain to remove toxic Cu and Cd ions from the cultural medium allowed quantifying the level of Cu and Cd ions on which the strain grew sufficiently well. Nevertheless, more research is imperative in the mineral medium components and working conditions changes.

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(Received 23 October 2007; Accepted 26 February 2008)