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Biosorption of Cadmium (II) Ions by the Cadmium Tolerant Bacteria Isolated from the Chemical Exposed Soil of Fireworks Industry

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The biosorption of cadmium removal by the isolated bacteria was studied in a laboratory simulated environment. The bacterium was isolated from fireworks industry soil. The bacterium was screened under various biochemical tests and identified as *Bacillus* sp. Various parameters such as pH, temperature, metal tolerant assay, kinetics assays were carried out to optimize and evaluate the cadmium ions removal. The isolated bacterium *Bacillus* sp removed 90% cadmium (II) ions in the simulated environment.

Key Words: Biosorption; cadmium; *Bacillus* sp; Firework industry.

The heavy metal cadmium is well known that is very harmful. Cadmium is a non-biodegradable and has the capacity to accumulate in living organisms, causes significant problems to both the environment and public health[1]. There are various physicochemical strategies types of techniques are available, such as filtration, chemical precipitation, electrochemical treatment, oxidation/ reduction, ion exchange, membrane technology, reverse osmosis, and evaporation recovery, have been developed to remove heavy metals, including cadmium, from polluted water. However, most strategies appear to be expensive, inefficient, and labor-intensive, or the treatment process lacks selectivity[1,2]. Biosorption using the biomass of microorganisms is an effective and economic technology for the removal and recovery of cadmium and other heavy metal ions from wastewater streams. Different types of biomass have been investigated for the biosorption properties of cadmium and other heavy metal ions[3].

In the current work, sorption of cadmium (II) ions from simulated laboratory aqueous solutions on to *Bacillus* sp which was isolated from firework industry chemical exposed soil was studied. Various parameters such as pH, temperature, metal tolerant assay, kinetics assays were carried out to optimize and evaluate the cadmium ions removal Further the protein variations between the metal trained and untrained *Bacillus* sp have been studied.

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MATERIALS AND METHODS

Collection of sample

Soil samples were collected from fire work industry, Sivakasi, Virudhunagar District, Tamilnadu India and stored at -20°C.

Isolation of cadmium tolerant bacterial species

Cadmium tolerant bacterial strain was isolated from the soil samples using bacterial medium [Luria Bertani Broth]. The medium was amended with different concentration of cadmium [10-100mg L^{-1}]. Serial dilution was carried out to decrease the microbial load in the sample and standard spread plate method was performed. The plates were than incubated at room temperature [30-35°C] for 48 h. After 48 h incubation larger identical colonies from each plate were isolated.

Heavy metal assay and biomass quantification

Cadmium concentration was determined by Pyronine G method with cadmium chloride as standard at 470 nm spectrophotometrically. The bacterial biomass was quantified at 595 nm [4].

Optimization of pH for cadmium removal

The bacterial isolate was inoculated into a series of 2100ml conical flasks containing cadmium chloride [100mg L^{-1}]. The pH was varied [2, 4, 6, 8 and 10] and kept in orbital shaker [120rpm] for 24 h incubation. The cadmium removal and bacterial biomass was measured spectrophoto metrically.

Optimization of temperature for cadmium removal

The bacterial isolate was inoculated into a series of 100ml conical flasks containing cadmium chloride [100mg L^{-1}]. The flasks were incubated at different temperatures [25, 30, 35, 40 and 45°C] and kept in orbital shaker [120rpm] for 24 h incubation. The cadmium removal and bacterial biomass was measured spectrophotometrically.

Measurement of the kinetics of broth cellular growth and cadmium removal

The bacterial isolate was inoculated into a 100ml conical flask containing cadmium chloride [100mg L^{-1}]. The flask was kept in orbital shaker [120 rpm] at optimum pH and temperature for 26 h. During the incubation period, heavy metal concentration and biomass were monitored for every two hours interval until heavy metal removal attains a saturation level.

Cadmium tolerance assay

To elucidate the tolerance of the isolate towards cadmium, optimal culture conditions were used with varying initial cadmium concentrations. To a freshly prepared growth medium, cadmium was amended [10-100 mg L^{-1}]. After 24 h incubation, the biomass was measured. The extent of tolerance was compared and the normalized biomass was

Table 1. Enumeration of Cadmium resistant bacterial species at different concentrations

Concentration of cadmium] [mg/L]	No. of Colonies	Colony forming unit [CFU/ml
10	78	78X 10^4
20	72	72 X 10^4
30	56	56 X 10^4
40	30	30 X 10^4
100	20	20 X 10^4
60	15	15 X 10^4
70	12	12 X 10^4
80	7	7 X 10^4
90	8	8 X 10^4
100	6	6 X 10^4

Table 2. Morphological, physiological and biochemical characteristics of the isolated bacterial species

Morphological/ Physiological/ Biochemical characteristics	Isolated bacterial strain
Gram's staining	+
Cell shape	Rod
Indole production test	-
Methyl red test	-
Voges- Proskauer test	+
Citrate test	+
Triple sugar iron test	K/A
Gas production	-
Urease test	+
H_2S production	-
Motility test	-
Catalase test	-
Oxidase test	-
ONPG test	-
Nitrate test	+
Gelatin hydrolysis test	+
Starch hydrolysis test	+
Strain name	*Bacillus* sp

calculated, i.e., biomass at each heavy metal concentration per biomass using a control. All the experiments were carried out in triplicates.

Protein expression assays in cadmium trained and untrained bacteria

Proteomes from the bacterial isolate was extracted and purified using microbial lysis method[5]. Cell samples was taken from mid log phase of cellular growth to measure the kinetics of the cellular growth and heavy metal removal. The isolated protein was quantified by Bradford method and the pattern of proteomic expression was analyzed by 10% SDS- PAGE using Laemmli's method[6].

RESULTS AND DISCUSSION

Screening of cadmium tolerant bacterial species

The number of bacterial colonies decreased in the plates with increase in cadmium concentration Table 1. This shows the toxic nature and the tolerant nature of bacteria. The isolated bacterial species showed maximum cadmium tolerance up to the concentration of 100 mg L^{-1}

Identification of cadmium tolerant bacterial species

Identification of the isolated bacterial species was done by various biochemical tests and by using Bergeys Manual of systemic bacteriology Table 2.

Optimal pH for heavy metal removal by isolated bacterial species

Removal of cadmium increased [90%] with increased in pH up to 6 and further the cadmium removal decreased [Fig 1a] at alkaline conditions. Most of the microbial surfaces are negatively charged due to the ionization of the functional groups, thereby contributing to metal binding. The pH of the biosorption medium affects the solubility of the metal ions and the ionization state of the functional groups. Bacterial surfaces have a negative charge in the pH range of 2-6 [5]. The optimum pH of bacterial biomass was seen at temperature 35 °C which is shown in Fig 1b. The proton concentration is high at lower pH [<2] and heavy metal biosorption decreases due to the positive charge density on metal binding sites, i.e. hydrogen ions compete effectively with metal ions in binding to the sites. The negative charge density on the cell surface increases with increasing pH due to deprotonation of the metal binding sites. The metal ions then compete more effectively for available binding sites, which increase biosorption[7]. Decrease in biosorption at higher pH [>6] is due to the formation of soluble hydroxylated complexes of the metal ions and their competition with hydroxyl ions for active sites. Beyond pH 8.0, precipitations of the ions as hydroxides occur[8].

Optimal temperature for heavy metal removal by isolated bacterial species

Removal of cadmium by the isolated *Bacillus sp* appears to be temperature dependent. Maximum removal of cadmium [95%] was observed at 35°C [Fig 2a]. The optimum growth of bacterial biomass was seen at temperature 35 °C which is shown in Fig 2b. The range of optimal temperature values [30-35°C] were comparable to the range of room temperature that was used when isolating the microorganisms, suggesting that the selection of these isolates might have been influenced not only with the heavy metal but also with the temperature used in the isolation procedure. The temperature of the adsorption medium could be important for energy dependent mechanisms in metal removal by microorganisms. Temperature is known to affect the stability of the cell wall, its configuration and can also cause ionization of chemical moieties. These factors may simultaneously affect the binding sites on isolated fungal and bacterial species causing reduction in heavy metal removal. Energy-independent mechanisms are less likely to be affected by temperature since the processes responsible for removal are largely physiochemical in nature[9]. Mostly adsorption is an exothermic process [8], whereas, some examples of endothermic adsorption have also been reported[10].

In case of exothermic biosorption processes, an increase in temperature has been found to reduce the biosorption capacity of the biomass[11,12]. This is attributed to the increase in temperature may increase metal desorption tendency from the interface to the solution[13]. At that temperature 35°C, the bacterial growth was also higher. During endothermic biosorption processes, the extent of adsorption processes increases with increasing temperature. This effect may be due to the fact that at higher temperatures, an increase in active sites occurs due to bond

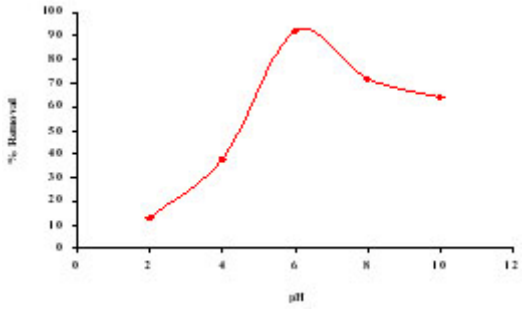

Fig 1a. Cadmium removal by *Bacillus* sp. in response to various pH. Temperature: 35°C, incubation time: 24h, concentration of cadmium:100 mgL^{-1}

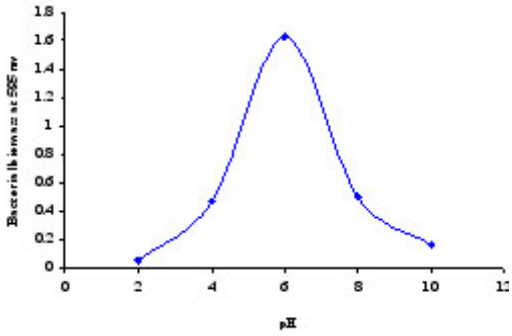

Fig 1b. Cellular growth by *Bacillus* sp. in response to various pH. Temperature: 35°C, incubation time: 24h, concentration of cadmium:100 mgL^{-1}

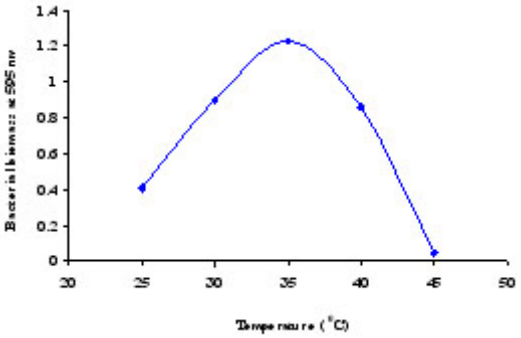

Fig 2a. Cadmium removal by *Bacillus* sp. in response to various temperatures. Concentration of cadmium: 100 mgL^{-1}, pH: 4, incubation time: 24h

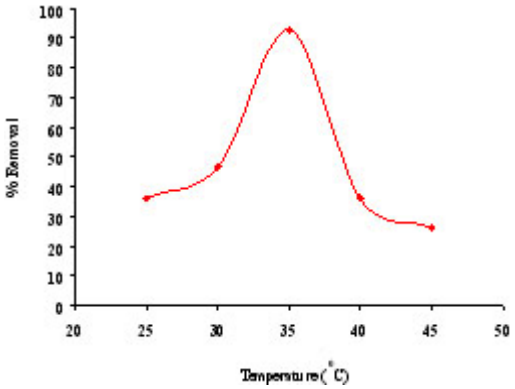

Fig 2b. Cellular growth of *Bacillus* sp. in response to various temperatures. Concentration of cadmium:100 mgL^{-1}, pH: 4, incubation time: 24h

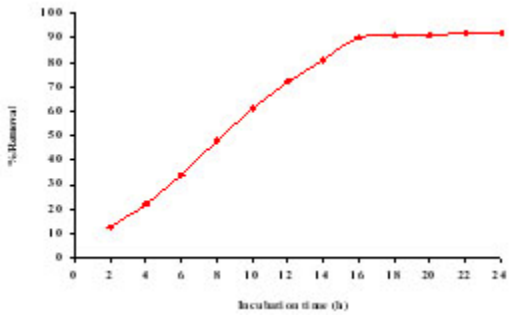

Fig 3a. Cadmium removal by *Bacillus* sp. Concentration of cadmium:100 mgL^{-1}, pH: 4, temperature: 35°C

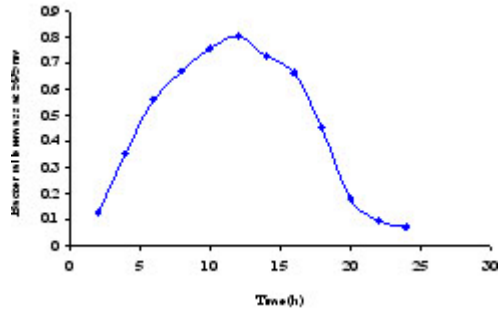

Fig 3b. Kinetics of cellular growth by *Bacillus* sp. Concentration of cadmium:100 mgL^{-1}, pH: 4, temperature: 35°C

rupture[14]. However, physical damage to the biosorbent can be expected at higher temperature

Measurement of the kinetics of broth cellular growth and heavy metal removal

Cadmium removal by *Bacillus* sp increased up to 24 hours of incubation. After that, sorption reached equilibrium. The maximum removal capacity of cadmium in aqueous solution

by *Bacillus* sp was found to be 90 % Fig 3a. The time-course data for heavy metal removal and cellular growth were observed for each isolate under its optimal pH and temperature conditions Fig 3b. For cadmium removal by bacterial isolates, specific metal bioaccumulation [accumulative biosorption [removal] of each heavy metal per accumulative biomass] increased when cells were in stationary phases. When these isolates are applied in removing heavy metal from industrial wastewater, information regarding the effect of growth phase will be important in designing bioreactor for wastewater treatment plants. The growth rate during the lag phase was very low because the isolated bacterial isolates was adapting with the environment. After this stage, the isolates grew in logarithmic form using the nutrients. In the third stage, the number of living and dead cells is fixed. In the initial stages the removal efficiencies of metal by biosorbents increased rapidly due to the abundant availability of active binding sites on the biomass, and with gradual occupancy of these sites, the sorption became less efficient in the later stages[15].

Heavy metal tolerance assay

The initial concentration of the cadmium in the solution remarkably influenced the equilibrium uptake. It was noticed that initial concentration increased the sorption of cadmium as is generally expected due to equilibrium process Fig 4. This increase in uptake capacity of the biosorbents with the increase in initial metal concentration is due to higher availability of metal ions for the sorption. Moreover, higher initial concentration provides increased driving force to overcome all mass transfer resistance of metal ions between the aqueous and solid phase resulting in higher probability of collision between metal ions and sorbents. This also results in higher metal uptake[16]. Tolerance implies a large change in sensitivity between sets of organisms to a particular toxicant. Tolerance can be adaptive, constitutive, or induced. Adaptive tolerance is where the organism colonizing a contaminated site is less insensitive than the same species colonizing uncontaminated sites and where this change in sensitivity is caused by the selection of genes that confer enhanced insensitivity. Induced tolerance, for which there is less evidence, is where particular enzymes that cause decreased sensitivity are induced on exposure to metal ions[17].

Protein expression assays in heavy metal trained and untrained bacteria

Heavy metals are increasingly found in microbial habitats due to natural and industrial processes, microbes have evolved several mechanisms to tolerate the presence of heavy metals [by either efflux, complexation, or reduction of metal ions] or to use them as terminal electron acceptors in anaerobic respiration[18]. When

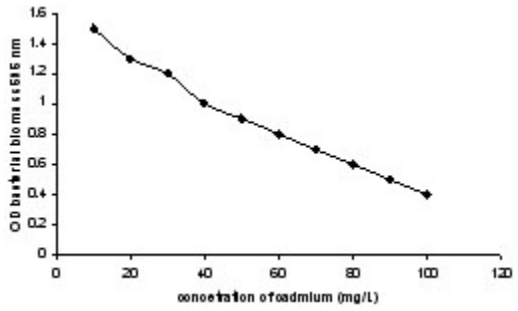

Fig 4. Normalized biomass [measured at 24h incubation time] in response to varying initial concentrations of cadmium concentration range: 10-100 mgL^{-1}, temperature: 35°C, pH: 4

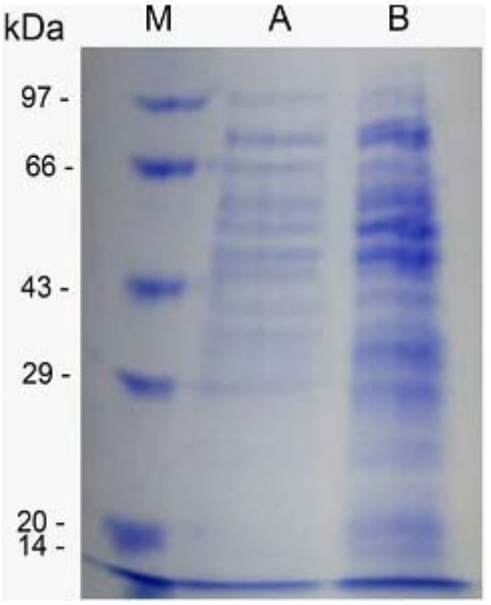

Fig 5. SDS- PAGE protein expression patterns in cadmium trained [resistant] and untrained; Lane M – Marker; Lane 1 and 2 – Cadmium untrained *Bacillus* sp*;* Lane 3 and 4 – Cadmium trained *Bacillus* sp

compared with controls [without heavy metals], *Bacillus* sp. expressed considerable amount of polypeptide [protein] which is separated by 10% SDS-PAGE. The intensity of band at 88, 43,47, 56, 61 kDa were high when compared with control. In *Bacillus* species in the presence of cadmium the organism express more proteins and a significant differential expression of polypeptide was seen on low molecular weight protein such as 22, 24 and 26 kDa is involved in response to heavy metals and probably pervasively exists in heavy metal resistant bacteria, which remains to be further examined Fig 5. This was probably attributed to a higher degree of functional diversity among bacteria. Similar results was reported by Thacker *et al*., [19] that protein with molecular weight 30 kDa induced in presence of chromium and this may possibly be associated with resistance of chromate. Similarly nickel resistant strain of *Pseudomonas fragi*[20] showed the induction of protein s with molecular weight 48 and 18 kDa proteins play a vital role in metal resistance mechanism. Heavy metals are essential micronutrients required for a variety of processes in the cell metabolism, but they are toxic above optimal cellular concentrations. All organisms must therefore critically balance the cellular concentrations of these potentially toxic elements, as they do for non-essential heavy metals. The understanding of the molecular bases of heavy metal in microorganisms will open new technological perspectives in bioremediation, and attention has been focused on the influence of metal ions on gene expression and protein synthesis in different organisms [21]. Several intracellular polypeptides have been identified following metal induction, and play specific roles in metal tolerance. Metal-binding polypeptides such as cysteine-rich metallothioneins and phytochelatins are induced in the presence of heavy metals and reduce the concentration of free metal ions in the cell[22].

CONCLUSION

This study elucidated the soil isolated cadmium tolerant bacteria isolated from fire work industry was potential in removing Cadmium (II) ions in a simulated environment under an optimized conditions. The observed effect(s) of pH on bioaccumulation was attributable mainly to organism-specific physiology, as indicated by the observed positive correlation between biomass and heavy metal removal. Furthermore, the kinetic and tolerance experiments provided information for SRT design and the lethal tolerance limits, which are important in designing CSCM (Chiral selective cation-exchange membrane) bioreactors for removing heavy metals of high concentrations.

REFERENCES

1. Chen, G.Q., Zeng, G.M., Tang, L., Du, C.Y., Jiang, X.Y, Huang, G.H., Liu, H.L., Shen, G.L. Cadmium removal from simulated wastewater to biomass byproduct of Lentinus edodes. *Bioresour. Technol*., 2008; **99**: 7034–7040.
2. Tang, L., Zeng, G.M., Shen, G.L., Li, Y.P., Zhang, Y., Huang, D.L., Rapid detection of picloram in agricultural ûeld samples using a disposable immunomembrane-based electrochemical sensor. *Environ. Sci. Technol*. 2008; **42**: 1207–1212.
3. Yin, P., Yu, Q., Jin, B., Ling, Z., Biosorption removal of cadmium from aqueous solution by using pretreated fungal biomass cultured from starch wastewater. *Water. Res*. 1999; **33**: 1960-1963.
4. Rajeshkumar. R., Shankar C., Dexilin M., Sridevidhanarani T., Thamaraiselvi K. Phosphate removal by isolated bacteria from municipal wastewater effluent. *Environ. Pollu. Cont. Jr.* 2010; **13**: 17-22.
5. Rajeshkumar. R., Shankar C., Thamaraiselvi K. Evaluation of isolated fungal strain from e-waste recycling facility for effective sorption of toxic heavy metal Pb (II) ions and fungal protein molecular characterization- a mycoremediation approach. et al. *Asian J. Exp. Biol. Sci.*2011; **2**: 342-347
6. Laemmli, U.K. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature* **227**: 680-685. *Nature.*1970; **227**:680 -685.
7. Kapoor, A., Viraraghavan, T., Cullimore, D.R. Removal of heavy metals using the fungus *Aspergillus niger*. Bioresour. Technol. 70: 95-104.et al. *Bioresour. Technol*. 1999; **70**: 95-104.
8. Martins, B.L., Cruz, C.C.V., Luna, A.S., Henriques, C.A. Sorption and desorption of Pb^{2+} ions by dead *Sargassum sp*. biomass. *Biochem. Eng. J*. 2006; **27**: 310-314.
9. Gulay, B. Sema, A.M. Yakup, Biosorption of heavy metal ions onimmobilized white-rot fungus Trametes versicolor, *J. Hazard. Mater. B* 2003; **101**:285–300.

10. Davis, T.A., Volesky, B., Mucci, A. A review of the biochemistry of heavy metal biosorption by brown algae. *Wat. Res*. 2003; **37**: 4311-4330.
11. Mamrei, N., Boudries, N., Addour, L., Belhocine, D., Lounici, H., Grib, H., Pauss, A. Batch zinc biosorption by a bacterial non living *Streptomyces rimosus* biomass. *Wat. Res.* 1999; **33**. 1347-1354.
12. Suhasini, I.P., Sriram, G., Asolekar, S.R., Sureshkumar, G.K. Biosorptive removal and recovery of cobalt from aqueous systems. *Process. Biochem*. 1999; **34**: 239-247.
13. Sari, A., Tuzen, M., Uluozlu, O.D., Soylak, M. Biosorption of Pb (II) and Ni (II) from aqueous solution by lichen *(Cladonia furcata)* biomass. *Biochem. Eng. J*. 2007; **37**: 151-158.
14. Hawari, A.H., Mulligan, C.N. (2006). Heavy metals uptake mechanisms in a fixed bed column by calcium treated anaerobic biomass. *Process. Biochem*. 2006; **41**: 187–198.
15. Shankar, C., Sridevi, D., Park, J., Dexilin, M., Thamaraiselvi, K. Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. *J. Hazard. Mater.* 2007; **146**: 270-277.
16. Tewari, N., Vasudevan, P., and Guha, B.K., (2005). Study on Biosorption of Cr (VI) by *Mucorhiemalis*. *Biochem. Eng. J*. 2005; **23**: 185–192.
17. Meharg, A. The mechanistic basis of interactions between mycorrhizal associations and toxic metal cations. *Mycological Res.*, 2003; **107**(11): 1253–1265.
18. Anne. S. and Elizabeth. A. 2003. Implications of microbial heavy metal tolerance in the environment. *Reviews in Undergraduate Research,* 2, 1-6p.
19. Thacker, U., Parikh, R., Shouche, Y., and Madamwar, D. Reduction of chromate by cell-free extract of *Brucella sp*. isolated from Cr(VI) contaminated sites. *Biores. Technol*., 2007; **98**: 1541–1547.
20. Patel, J. S., Patel, P. C., and Kalia, K. Isolation and characterization of nickel uptake by nickel resistant bacterial isolate (NiRBI). *Biomed Environ. Sci*. 2006; **19**: 297–301.
21. Mejare, M. and Bulow, L. Metal-binding proteins and peptides in bioremediation and phytoremediation of heavy metals. *Trends Biotechnol.*, 2001; **19(2)**: 67-73.
22. Nedkovska, M., and Atanassov, A. Metallothionein genes and expression for heavy metal resistance. *Biotechnol. Biotechnology Equipment* 1998; **2**: 11-16.