

Comparative studies on Synthesis of Protease and Bioconversion of Cr^{+6} to Cr^{+3} by Bioleaching technique using Isolated Microbial consortia in Lab scale Batch experiment

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Abstract—Presence of heavy metal like hexavalent chromium and high biological and chemical oxygen demands (BOD and COD) due to presence of animal flesh, skin etc. in the tannery effluent cause threat to the environment. *Bacillus* sp. (JUCHE2), *Micrococcus* sp (JUCHE3) and *Micrococcus* sp (JUCHE4) isolated from tannery waste, were used in a comparative study on synthesis of protease and bioconversion of Cr^{+6} to Cr^{+3} by bioleaching technique. The isolated microbial consortia could tolerate high metal concentration and hostile environmental conditions with respect to temperature and pH. It was observed that among three types of bacterial growth media, namely, Nutrient broth, Soyabean Casein Digest Medium and Luria broth, bacterial growth was maximum at Casein Digest Medium and laboratory scale batch experiments were performed in Soyabean Casein Digest medium at 100 ppm chromium concentration at aerobic conditions. Growth characteristics such as maximum specific growth rate (μ_{max}), Monod Constant (ks), synthesis of protease, bioconversion of Cr^{+6} to Cr^{+3} by bioleaching technique, utilization of substrates, namely, carbohydrate and protein were measured at different time intervals in bacterial growth media. For *Bacillus* sp. (JUCHE2) optimum values of pH, temperature and agitation speed were found to be 7.0, 37°C and 100 rpm, respectively and those for both *Micrococcus* sp (JUCHE3) and *Micrococcus* sp (JUCHE4) were observed to be 6.0, 30°C and 100 rpm, respectively. It was observed that among three types of microbial consortia growth of *Bacillus* sp. (JUCHE2) was very high compared to other microbial consortia and followed Monod type model. Protease synthesis was strongly associated with microbial growth whereas bioconversion of heavy metals was not associated with microbial growth. Maximum protease activities were notified after 30 hour incubation of microbial culture and protease was characterized with respect to optimum pH, temperature and enzyme kinetics. Reduction of BOD and COD in form of reduction of proteins by synthesized proteolytic enzymes and bioconversion of Cr^{+6} to Cr^{+3} by bioleaching technique utilizing the isolated strains could be highly effective for the treatment of tannery waste.

Index Terms—Isolated microorganisms, Growth characteristics, Bioleaching, Protease synthesis, Enzyme characteristics

I. INTRODUCTION

DUE to stricter environmental legislations tannery waste, containing high amount animal flesh, skin, mainly responsible for high biological oxygen demand (BOD) and high concentration of chromium responsible for chemical oxygen demand (COD) are nowadays considered a great challenge. Improper disposal of contaminant effluent leads to environmental pollution, particularly soil contamination, and poses a serious threat to groundwater [1]. Many of the constituents

of tannery effluent are carcinogenic and immunotoxicants [2]. In India, approximately 1,50,000 tonnes of offals comprising raw hide trimmings, limed animal fleshings, green animal fleshings, hide splits and chrome shavings are available which are either utilized or under utilized, thus posing a solid waste disposal problem in tanneries [3]. Bioremediation is currently receiving considerable attention as a remediation option for sites contaminated with hazardous inorganic and organic compounds [4]. Among their advantages with respect to other widely used techniques, are simplicity, the possibility of being coupled with other physical or chemical treatment methods, cost-effectiveness and the capability of complete destruction of the pollutants [5]. It is a managed treatment process that uses microorganisms to degrade and transform organic chemicals in contaminated soil, aquifer material sludge, and residues. Bioremediation also reduces the toxicity and migration potential of hazardous constituents in the material being treated. Conventional treatment technologies can reduce Cr^{+6} to Cr^{+3} . Cr^{+3} form the chromate complex which behaves as an anion and can not form an insoluble hydroxide. The advantages of employing mixed microbial cultures instead of bioadsorbent for bioremediation have been widely demonstrated. It could be attributed to the effects of synergistic interactions among different members of the microbial association. It is possible that one type microbial species removes the toxic metabolites (that otherwise hinder microbial activity) of the species preceding it. It is also possible that the second type species are able to degrade compounds that the first are able to only partially [6]. Further research should be directed towards understanding the roles of individual microbial member in influencing the effectiveness of a microbial association. Bacterial metabolism is a complex process that involves a series of electron transfer pathways that ultimately results in the transfer of an electron to a metal atom resulting in the reduction of that metal atom to a lower valence state. The mechanisms of Cr^{+6} reductions are technologically and biologically important because they convert a toxic, mobile element, Cr^{+6} into a less toxic, immobile form, Cr^{+3} . During the reduction of Cr^{+3} , massive insoluble chromium-rich precipitates formed on the outer surface of the cells. Reduction of Cr^{+3} results through a coupled biotic-abiotic reaction pathway in which $Fe_{(aq)}^{+2}$ or H_2S produced during microbial respiration catalyses

the reduction of Cr^{+3} [7]. Following the above findings, many studies of hexavalent chromium transformation have employed mixed or single microbial (bacterial-fungal) cultures in efforts to maximize biodegradation. Obtained by microbial fermentation, the proteases are meant for use in the leather industry for dehairing, bating and soaking processes. The most important criteria for their selection of enzymatic routes are their specificity, pH activity range as well as pH and thermal stability. If an enzyme is to act uniformly, it must be able to diffuse into the hide and this is obviously achieved with skins rather than with hides and latter case, an accumulation of enzyme at the surface of the grain occurs [8]. The enzymes or enzymatic formulations need not be pure but must be cheap compared to that of commercial chemicals used in leather industry for reduction of BOD. Suitable biotechnological process for the treatment of solid wastes from the tannery industry had been practiced from a long prior. Although many work already successfully completed both of chromium reduction by microbial bioremediation and microbial protease synthesis using tannery effluent as a proteinaceous substrate but no work had been reported simultaneous Cr^{+6} to Cr^{+3} bioconversion and protease synthesis by microbial route for degradation of proteinaceous substances in terms of reduction of COD and BOD respectively in Indian scenario [9][10]. In the alarming stage of zero effluent discharge the objective of present investigation is synthesis of protease and bioconversion of Cr^{+6} to Cr^{+3} by bioleaching technique by different types microbial consortia isolated from tannery waste because of these particular microbes already survive in hostile condition in respect of high chromium concentration and protein rich tannery effluent.

II. MATERIALS AND METHODS

A. Materials

A.1 Chemicals

All chemicals used in the experiment both for microbiological and chemical assays namely Agar agar, Glucose, Tryptone, Beef Extract, Yeast Extract, Di-potassium phosphate, Sodium chloride, Soybean peptone, Potassium dichromate, Tris-Phosphate Buffer, 3,5-dinitrosalicylic acid (DNS), N-t-BOC-L-Glutamic Acid -Phenyl Ester Substrate Solution, (N-t-BOC-GAPE), Bovin serum albumin (BSA), Biochemical Test Kit (KB001-10KT), Folin Ciocalteu solution, potassium sodium tartarate, Sodium carbonate, Potassium di-Chromate, Lead nitrate, Arsenic nitrate, Cadmium nitrate, Mercuric chloride, Copper sulphate, S-diphenylcarbazide, Sodium hydroxide, Hydrochloric acid and acetone (AR) were procured from either E. Merck (Mumbai, India) or HIMEDIA (Mumbai, India).

A.2 Analytical Instruments

The deionized water used in all experiments was obtained from Arium 611DI, ultrapure water system (Sartorius AG, Göttingen, Germany). REMI made centrifuge, local company S.C. Deb Pvt. Ltd. made indigenous autoclave, BOD incubator shaker, laminar air flow chamber and micro-filtration unit

were used for centrifugation, sterilization, incubation and microbiological transfer accordingly during the experiments. Spectrophotometer (Hitachi, Japan) and atomic absorption spectrophotometer (Perkin Elmer) were used during experiment for measuring concentration in different aspects.

B. Methods

B.1 Isolation of Chromium reducing Bacterial Strain

Cr^{+6} reducing and potential of protease synthesis bacterial strains were isolated from Tannery waste water, collected from Chingrighata, Kolkata, India. Briefly 100 ml of sterile plastic containers were used for collecting the tannery waste water, enriched by chromium followed by centrifugation at 12000 rpm for 20 minutes and supernatants were considered for isolation of bacteria. The chromium reducing bacteria were isolated by conventional repetitive adaptations method at 100 ppm chromium containing Luria broth. Conventional serial dilution method and four quadrants repetitive striking was followed for isolating single colonies of chromium reducing bacteria. The genres of isolated microorganisms have been identified Bacillus sp. (JUCHE2), Micrococcus sp (JUCHE3) and Micrococcus sp (JUCHE4) through biochemical and sugar fermentation techniques.

B.2 Maintenance of Microbial culture

Stock cultures were maintained at $-80^{\circ}C$ on Microbank vials (Pro-Lab Diagnostics). Prior to assay, strains were transferred into respective different bacterial growth medium i.e. Nutrient Broth, Soybean Casein Digest media, Luria Broth and incubated at $37^{\circ}C$ for 24 hours. Each culture was subcultured successively three times into respective different bacterial growth medium. For routine daily use, agar slant cultures were prepared and liquid seed culture of respective different bacterial growth medium were used for batch studies.

B.3 Preparation of Bacterial growth media

Three types of bacterial growth media namely Nutrient broth composed by 5 gram Tryptone, 1.5 gram Yeast extract, 1.5 gram Beef extract, and 5 gram Sodium chloride in 1000 ml Distilled water, Soybean Casein Digest medium composed by 15 gram Tryptone, 3 gram Soybean peptone, 2.5 gram Di-potassium phosphate, 2.5 gram Glucose and 5 gram Sodium chloride in 1000 ml Distilled water and Luria medium composed by 10 gram Tryptone, 5 gram Yest extract and 5 gram Sodium chloride in 1000 ml Distilled water were used for microbial growth. pH of all medias 7 were adjust by 10 N Sodium hydroxide and 10 N Hydrogen chloride. Respective agar media of described broths were prepared using 2% agar for solid growth media. Initially the inoculums of each bacterium was inoculated from solid agar slant in 30 ml sterile (15 Psi, 1200C at 20 min.) each liquid growth medium. Each growth medium was supplemented with appropriate amount of Potassium dichromate (100 mg.dm^{-3}), was dissolved in sterile water followed by micro-filtration.

B.4 Batch Studies

Adaptation of each consortium to the new environment was carried out by incubating at 37°C in a rotary BOD shaker at 170 rpm and keeping for several days. Enriched cultures were obtained by repeated inoculation of proceeding bacterial culture in fresh each bacterial growth medium containing (100 mg.dm^{-3}) Potassium dichromate. To determine the growth kinetics of the microorganisms with respect to time in culture medium, batch experiments were conducted in Erlenmeyer flasks with constant shaking 170 rpm. For each run the working hold up volume was maintained at 30 ml containing 10% of inoculums. A constant temperature was maintained during incubation for each run. Samples were withdrawal at an interval of 2 hours with a total time span of 60 hours for measuring biomass concentration, substrate utilization, reduction of Cr^{+6} and synthesis of protease. Separate batch experiments were conducted with different bacterial growth media for respective microorganisms.

B.5 Identification of Minimum inhibitory Concentration (MIC)

Different concentrations of heavy metals such as Chromium, Lead, Arsenic, Cadmium, Mercury and Copper were prepared by serial dilution method in Soyabean Casein Digest medium and working volume was considered 10 ml in 20 ml Test tube. 10% inoculums were added additionally for each dilution and those were incubated for 24 hours. Bacterial growths were identified by spectrophotometrically at 600 nm with respect to blank solution.

B.6 Determination of bio mass concentration

Concentration of bacterial mass in the reaction broth of batch type under steady state was determined both by dry weight method. In this method 20 ml broth, enriched with bacterial strains, was centrifuged at 10,000 rpm for 15 minutes and the separated (bacterial) mass was washed with phosphate buffer solution. The washed mass was transferred to a pre-weighed aluminum cup and was dried at 80°C for 24 hours. The exact weight of the bacterial mass was determined by subtracting the weight of dry cup from that of the cup containing dry bacterial mass.

B.7 Hexavalent chromium analysis

The sample under investigation was centrifuged at 10,000 rpm for 15 minutes and the supernatant was collected to measure the residual Cr^{+6} concentrations. The analysis of Cr^{+6} concentrations was carried out spectrophotometrically using Cr^{+6} specific colorimetric reagents S-diphenylcarbazide. Initially 0.025 gram S-diphenylcarbazide was dissolved in 9.67 ml acetone (AR) to prepare the solution. The test solution was prepared by combining 200l sample under investigation, 400l 20mM MOPS-NaOH buffer, 33 μl 3M H_2SO_4 , 40 μl S-diphenylcarbazide solution and 327 μl distilled water. Optical density of the above solution was measured at 540 nm using a spectrophotometer. A standard calibration curve of Cr^{+6} concentrations against optical density was prepared in the similar way except that in this case 20 μl solution of known

concentration of $\text{K}_2\text{Cr}_2\text{O}_7$ was used in place of supernatant. Using this standard curve the Cr^{+6} concentrations was determined during experiments.

B.8 Studies on Protease synthesis potentiality of Microorganism

Protease synthesis potentialities of microorganism were identified by agar wheel diffusion method using casein as a substrate. Briefly 50 ml of sterile milk prepared by microfiltration was mixed with 50 ml of 2X molten agar and wheels were subjected by extracellular fluid synthesized by respective microorganism. Protease activities were measured after 48 hours incubation at 37°C of those plates. Clear region formation comparing with blank solution in the wheel signifies protease synthesis potentiality of respective microorganisms. But for large production of protease Erlenmeyer flux were withdrawn at different times intervals followed by centrifugation at 10000 rpm for 15 minutes at 4°C and supernatant were considered to study the protease activities using N-t-BOC-L-Glutamic Acid -Phenyl Ester as a substrate.

B.9 Glucose Estimation

Supernatant of harvested broth considered for Glucose estimation by 3,5-dinitrosalicylic acid (DNS) assay method for identifying substrate glucose utilization of microbial consortia. Briefly 60 gram potassium sodium tartarate was dissolved in 100 ml distilled water along with 40 ml of 2(N) sodium hydroxide solution in a 200 ml volumetric flux and 2 gram DNS reagent was added followed by volume make up for 200 ml. Approximately diluted supernatant of harvested broth were add with DNS solution and heated at 100°C for 20 minutes followed by optical density measurement at 510 nm with respect to blank sample.

B.10 Protein Estimation

Supernatant of harvested broth considered for protein estimation by Lowry assay method for identifying substrate protein utilization of microbial consortia. Briefly 50 ml of 2 gram of Sodium carbonate in 100 ml. of freshly prepared 0.1 N Sodium hydroxide solution mixed with 1 ml of 0.5 g.L^{-1} of Copper sulphate dissolved in 1% potassium sodium tartarate solution. Approximately diluted supernatant of harvested broth and diluted Folin Ciocalteau solution were add and took into dark condition for 20 minutes followed by optical density measurement at 750 nm with respect to blank sample.

III. RESULTS AND DISSCUSION

Three types of high metal tolerant bacteria were isolated from tannery waste and relative Colony Formation Unit (cfu) count were 4×10^5 , 3×10^5 and 4×10^5 for white bacterial colony, whitish orange bacterial colony and orange bacterial colonies respectively. According to the biochemical test and sugar fermentation test it was identified that white colony bacteria was Bacillus sp. (JUCHE2), orange white colony bacteria was Micrococcus sp (JUCHE3) and orange colony bacteria was Micrococcus sp (JUCHE4). Isolated three types

TABLE I
KINETIC PARAMETERS FOR DIFFERENT TYPES OF BACTERIAL GROWTH MEDIA FOR RESPECTIVE ISOLATED MICORBIAL CONSORTIA

Kinetic Parameter	Bacillus sp. (JUCHE2)	Micrococcus Sp. (JUCHE3)	Micrococcus Sp. (JUCHE4)
Maximum Specific growth rate (μ_{max}) Soyabean Casein Digest Medium	$0.005h^{-1}$	$0.048h^{-1}$	$0.050h^{-1}$
Monod Constant(K_s) for Soyabean Casein Digest Medium	$15mg.dm^{-3}$	$8mg.dm^{-3}$	$8mg.dm^{-3}$
Maximum Specific Growth rate (μ_{max}) for Lunia broth	$0.050h^{-1}$	$0.042h^{-1}$	$0.045h^{-1}$
Monod Constant (K_s) for Soyabean for Lunia broth	$12mg.dm^{-3}$	$6mg.dm^{-3}$	$8mg.dm^{-3}$
Maximum Specific Growth rate (μ_{max}) for Nutrient broth	$0.045h^{-1}$	$0.035h^{-1}$	$0.038h^{-1}$
Monod Constant (K_s) for Lunia broth Nutrient broth	$10mg.dm^{-3}$	$4mg.dm^{-3}$	$6mg.dm^{-3}$

of bacteria were used in comparative studies on synthesis of protease and bioconversion of Cr^{+6} to Cr^{+3} by bioleaching technique. The isolated microbial consortia could tolerate high metal concentration and hostile environmental conditions with respect to temperature and pH. Results of the investigations showed that the microbial consortium Bacillus sp. (JUCHE2) could sustain an environment containing 1000 mg.dm⁻³ lead, 1500 arsenic/ copper, 0.5 mg.dm⁻³ mercury, 100mg.dm⁻³ cadmium, 2000mg.dm⁻³ chromium, Micrococcus sp (JUCHE3) could sustain an environment containing 1500mg.dm⁻³ lead/ arsenic, 1000mg.dm⁻³ copper, 1 mg.dm⁻³ mercury, 50mg.dm⁻³ cadmium and 1500mg.dm⁻³ chromium and Micrococcus sp (JUCHE4) could sustain an environment containing 1000mg.dm⁻³ lead/ arsenic, 1500mg.dm⁻³ copper, 1mg.dm⁻³ mercury, 100mg.dm⁻³ cadmium and 2000mg.dm⁻³ chromium. From table 1 it was observed that among three types of bacterial growth media namely Nutrient broth, Soyabean Casein Digest Medium and Lunia broth, bacterial growth were maximum at Casein Digest Medium and laboratory scale batch experiments were performed in Soyabean Casein Digest medium at 100(mg.dm⁻³) chromium concentrations at aerobic conditions. Table 1 described those kinetic parameters namely (Maximum Specific growth rate (μ_{max}) and Monod Constant (Ks)) for different types of bacterial growth media for respective isolated microbial consortia. It was also observed that among three types of microbial consortia Bacillus sp. (JUCHE2) had high specific growth rate followed by Micrococcus sp (JUCHE4) and Micrococcus sp (JUCHE3) respectively. Growth characteristics, synthesis of protease bioconversion of Cr+6 to Cr+3 by Bioleaching technique and substrates mainly carbohydrate and protein utilizations were measured at different time intervals in bacterial growth media.

For Bacillus sp. (JUCHE2) optimum values of pH, temperature and agitation speed were found to be 7.0, 37°C and 100 rpm, respectively those for Micrococcus sp (JUCHE3) and Micrococcus sp (JUCHE4) were 6.0, 30°C and 100 rpm, respectively. Although Bacillus sp. (JUCHE2) could withstand

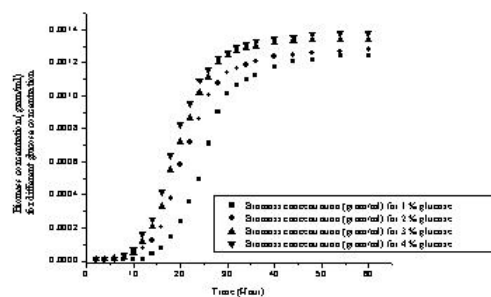


Fig. 1. Biomass concentration (gram/ml) for different concentration of glucose addition in Soyabean Casein Digest medium at 100 (mg.dm⁻³) chromium concentrations at aerobic conditions.

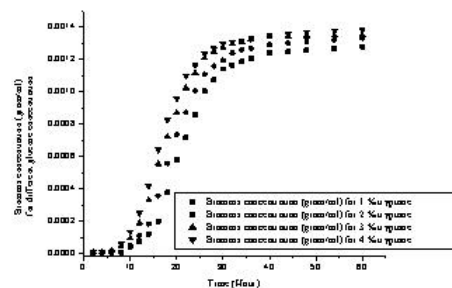


Fig. 2. Biomass concentration (gram/ml) for different concentration of tryptone addition in Soyabean Casein Digest medium at 100 (mg.dm⁻³) chromium concentrations at aerobic conditions.

wide range of temperatures, spanning from 25°C to 60°C and could withstand pH ranging from 4.5 to 12 where as Micrococcus sp (JUCHE3) and Micrococcus sp (JUCHE4) could withstand at temperatures, spanning from 25°C to 40°C and withstand pH ranging from 5 to 10.

FIG 1 and Fig 2 described that biomass concentration (gram/ml) for different concentration of glucose and tryptone addition respectively in Soyabean Casein Digest medium at 100 (mg.dm⁻³) chromium concentrations at aerobic conditions. The values of growth kinetic parameters, such as maximum specific growth rate (max), saturation constant (Ks) were determined for Bacillus sp. in presence of different glucose concentration and tryptone in Soyabean Casein Digest Medium.

From FIG 3 it was observed that in presence of 1% glucose concentration in Soyabean Casein Digest medium glucose utilization was very low by Bacillus sp. (JUCHE2) where as more than 1% glucose (2%-4%), glucose utilization by Bacillus sp. (JUCHE2) were almost same.

From FIG 4 it was observed that although 1% initial glucose in Soyabean Casein Digest medium but microbial cell growth was successfully occurred. However glucose utilization was very low by Bacillus sp. (JUCHE2) but presence of suitable amount of protein sources microbial growth was quite satisfactory. From FIG 4 it was observed that glucose uptake is very low but protein utilization was very high by Bacillus sp. (JUCHE2) and more than 1% tryptone (2%-4%), protein utilization by Bacillus sp. (JUCHE2) were almost same. The

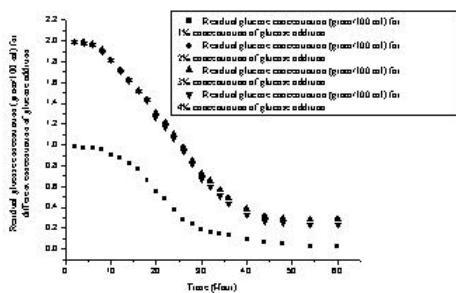


Fig. 3. Residual glucose concentration (gram/100 ml) for different concentration of glucose addition in Soyabean Casein Digest medium at 100 ($mg.dm^{-3}$) chromium concentrations at aerobic conditions.

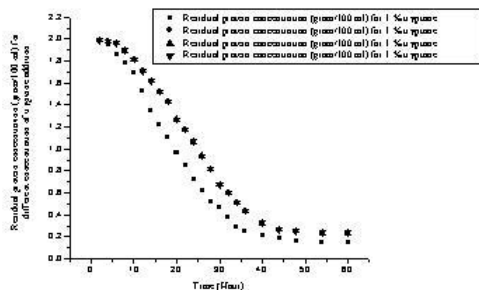


Fig. 4. Residual protein concentration (gram/100 ml) for different concentration of tryptone addition in Soyabean Casein Digest medium at 100 ($mg.dm^{-3}$) chromium concentrations at aerobic conditions.

same types of results were notified in both of *Micrococcus* sp (JUCHE3) and *Micrococcus* sp (JUCHE4). (Data were not showed)

From FIG 5 bioconversion of Cr^{+6} to Cr^{+3} by bioleaching techniques were notified with addition of different amount of glucose in Soyabean Casein Digest Medium. It was notified that addition of glucose in Soyabean Casein Digest Medium had a positive influence of bioconversion of chromium. From FIG 1 and FIG 2, it were notified that microbial growth follows

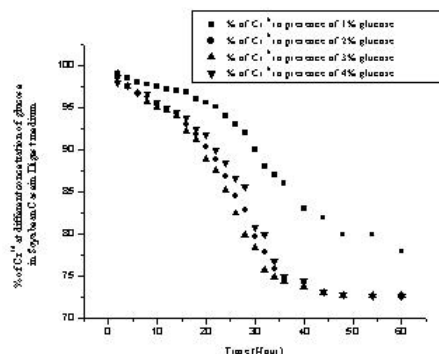


Fig. 5. % of Cr^{+6} (gram/100 ml) for different concentration of glucose addition in Soyabean Casein Digest medium at 100 ($mg.dm^{-3}$) chromium concentrations at aerobic conditions.

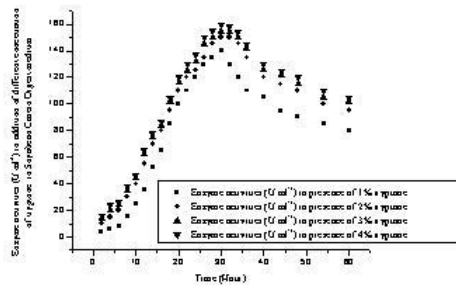


Fig. 6. Synthesis of protease for different concentration of tryptone addition in Soyabean Casein Digest medium at 100 ($mg.dm^{-3}$) chromium concentrations at aerobic conditions.

Monod model and it was notified that bioleaching were slightly diminished for tryptone addition comparing with glucose addition in Soyabean Casein Digest medium. Addition of 2% glucose in Soyabean Casein Digest Medium, bioconversion of chromium after 36 hours were 75%, 60%, 50% for *Bacillus* sp. (JUCHE2) *Micrococcus* sp (JUCHE4) and *Micrococcus* sp (JUCHE3) respectively when initial concentration of Cr^{+6} was 100 ppm where as with addition of 2% tryptone in Soyabean Casein Digest Medium, bioconversion of chromium after 36 hours were 65%, 50%, 45% for *Bacillus* sp. (JUCHE2) *Micrococcus* sp (JUCHE4) and *Micrococcus* sp (JUCHE3) respectively when initial concentration of Cr^{+6} was 100 ppm.

Protease synthesis potentialities were identified for isolated microorganisms by clear region observation in milk agar plate. From FIG 6 it was notified that protease synthesis by isolated microorganisms strongly associated with all types of microbial growth. From comparative studies it was notified that additions of tryptone rather than glucose in Soyabean Casein Digest medium had a positive influence on protease synthesis. Maximum protease activities were found after 24 hour incubation for *Bacillus* sp. (JUCHE2). It was observed that among three types of microbial consortia *Bacillus* sp. (JUCHE2) had high potentiality for protease synthesis followed by *Micrococcus* sp (JUCHE4) and *Micrococcus* sp (JUCHE3). Mximum protease activities were found $100U.ml^{-1}$, $85U.ml^{-1}$ and $60U.ml^{-1}$ for *Bacillus* sp. (JUCHE2), *Micrococcus* sp (JUCHE4) and *Micrococcus* sp (JUCHE3) respectively and beyond 30 hour incubation the protease activities were low for *Bacillus* sp. (JUCHE2) in case of different amount of glucose addition where as addition of different amount of tryptone in Soyabean Casein Digest medium maximum protease activities were found $150U.ml^{-1}$, $100U.ml^{-1}$ and $85U.ml^{-1}$ for *Bacillus* sp. (JUCHE2), *Micrococcus* sp (JUCHE4) and *Micrococcus* sp (JUCHE3) respectively and beyond 24 hour incubation the protease activities were low for *Bacillus* sp. (JUCHE2). Extracellular Protease was characterized in respect to optimum pH, temperature and enzyme kinetics were studied using N-t-BOC-L-Glutamic Acid -Phenyl Ester as a substrate. Enzymatic reaction kinetics were identified for respective isolated microbial consortia. Maximum reaction velocity (V^{max}) $1.721 \mu mol/ min mg$ protein, K_m 3.5 Mm respectively for *Bacillus* sp. (JUCHE2), (V^{max}) $1.55 \mu mol/ min mg$ protein, Km 3.0

Mm respectively for *Micrococcus* sp (JUCHE4) and (V^{max}) 1.65 $\mu\text{mol}/\text{min mg protein}$, K_m 4.0 Mm respectively for *Micrococcus* sp (JUCHE3). Above findings were also approved for 1:1 dilution of Soyabean Casein Digest medium by tannery effluent in vitro condition where as direct use of tannery effluents, values of kinetic parameter of microbial growth, bioconversion and protease synthesis by microorganisms were pronouncedly diminished. Reduction of BOD and COD by proposed technology had a great impact on environmental concern. (Data not showed)

IV. CONCLUSION

Isolated microbial consortia had potential in both of bioconversion of Cr^{+6} - Cr^{+3} by bioleaching techniques and protease synthesis simultaneously which had a high impact in case of tannery waste treatment. Among different types of bacterial growth media Soyabean Casein Digest medium containing 2% glucose and 2% tryptone were suggested for using bacterial growth medium. *Bacillus* sp. (JUCHE2) had high maximum specific growth rates, bioconversion and protease synthesis compareing with other isolated microorganisims. Addition of glucose had positive influence on bioleaching techniques where as addition of tryptone had positive influence on protease synthesis. In case of tannery effluent treatment 1:1 dilution of Soyabean Casein Digest medium by tannery effluent were suggested for bacterial growth medium as well as bioremediation.

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