

Microbiological Decolorization of Crystal Violet Dye and Amylase Production by Iraqi Isolate *Geobacillus thermoleovorans* Strain Ir1 (JQ912239)

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ABSTRACT

Alpha amylases hold a variety of capabilities into distinctive industries. Immobilization regarding alpha -amylase evolved via a pressure regarding *Geobacillus thermoleovorans* Ir1 isolated beyond hydrocarbons filthy soils about Iraq used to be studied. A partially clean enzyme with 189 U/mL undertaking used to be old because immobilization instruction using sodium alginate and agar, the immobilized enzyme was very effective in increasing the enzyme activity to be (10.7, 14.8 U/mL) respectively for the sodium alginate and agar. This immobilized enzyme performs stand-old commercially like a replacement on free enzyme because that has proven larger operational give then higher enzymatic activity. The *G. thermoleovorans* strain Ir1 shows high decolorization (99%) of crystal violet toxic dyes in the concentration 0.25 mg/mL for three days while (98%) decolorization in the concentration 0.5 mg/mL for ten days.

Keywords: Apha amylase, Crystal violet, Decolorization, *Geobacillus thermoleovorans*, Immobilization.

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INTRODUCTION

Geobacillus thermoleovarans is widely chronic because of thermostable α amylase manufacturing in conformity with meet technical needs, with the growing population, the revolution in industries is taking part to get all the basic needs that have a direct influence on our environment.¹ Proper management of discharged wastewater coming from various industries is a significant concern worldwide. However, the scenario is even worse for a country like, Iraq. The immobilized enzyme for the *Geobacillus* is the enzyme attached to inert non-soluble material. This method immobilizes the enzyme used to defend and stabilize the enzyme, so enhancing their houses and their repetitive using both in bunch yet non-stop anger Immobilization on the amylase enzyme prevents their deactivation through many bodily then chemical denaturing marketers, thereby bettering their operational stability.² Amylase enzyme is a dead vital enzyme the principal use over that enzyme hydrolysis regarding the size after propagating glucose syrup, prosperous amylase grind yet the formation over the dextrin for the duration of baking the meals industries.³ The immobilization regarding the amylase enzyme bears specific advantages: It can lie reused involved strategies lie cleft constantly with better controls, effortless severance

concerning the products, smile dealing with over substances yet high-quality discount into technique cost. Sodium alginate torse then agar is broadly old into the enzyme immobilization. The gel construction occurs at moderate conditions and no danger concerning human's bodily entrapment of α amylase among sodium alginate chaplet or agar has shown following be enormously easy, raped or sure technique.⁴ So, the makes use of over the enzyme into a soluble and broad shape should keep considered as thrifless because the enzyme can't stay healthsome at the stop regarding the reaction hence the modern thought about the enzyme technology is as involved together with immobilization of the enzymes regarding insoluble polymers membranes then particles work as like incorporates then support because of the enzyme.⁵ Therefore, biological agents are gaining more importance for bioremediation purposes to their economic scheme and potentially produced less sludge that reduces pollution risk.⁶ In the last few years, *Bacillus* species are gaining more awareness because they have massive potential in degrading and mineralizing various complex chemicals, including environmentally unsafe synthetic commercial dyes.⁷ Crystal violet (CV), a triarylmethane dye, is used for various purposes, including textile and printing industries and manufacturing ball-point pens, inkjet printers,

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and coloring diverse products like leather and detergent. These dyes are considered a biohazard substance because of their carcinogenic and mutagenic effects along with their recalcitrant property.⁸ To reduce the toxicity for the environment, we tried to detect whether newly strain of *Geobacillus* can decolorize and biodegrade CV dye.

MATERIAL AND METHODS

Dyes and Chemicals

Chemicals and solvents used in all experiment were analytical grade, The textile dyes, were obtained from Himedia, India.

The tested Microorganism

G. thermoleovorans pressure Ir1 (JQ912239) was used, that permanency is a young thermophilic bacteria successful according to using one-of-a-kind aromatics compounds. It was isolated between previous learning from the oil-contaminated floor within Iraq.⁹

Culture of *G. thermoleovorans* Strain Ir1 (JQ912239)

G. thermoleovorans strain Ir1 (JQ912239) was preserved within a silica gel. To revive the culture, 0.1 mg of bacterium isolate was added into 500 mL of LB medium provided with 0.1 mL MnCl₂ in a conical flask, the culture was incubated in a shaker incubator at 65°C and 150 rpm for one to two days. After the incubation period, a loopful culture was streaked on LB broth and incubated at 65°C for one day. A single colony was picked up with a sterile loop, transferred into LB broth flasks, and incubated at the same conditions. The bacterium was stained with Gram +ve stain, and the purity was checked by microscopic examination.

Study the Alpha Amylase Activity

The amylase endeavor was decent the use of agar hypocripsy technique.¹⁰ Stability, 5 mL of 24 hours old subculture soup containing soluble starch, yeast extract, peptone, MgSO₄·7H₂O, NaCl yet CaCl₂ (Himedia, India) was transferred following 45 mL concerning the same fruitless middling or was incubated because 55°C. The lifestyle was centrifuged at 10,000 rpm because x minutes at 0°C (Refrigerated Centrifuge, Sigma, Germany). The supernatant was ancient as a pointless enzyme.

Partial Purification on the Enzyme -Ammonium sulfate precipitation

Alpha-amylase was once incompletely correct through capacity on ammonium sulfate fractionation accompanied via dialysis then gel filtration chromatography. The substance enzyme was once delivered of pursuance, including 90% ammonium sulfate saturation at 4°C in a comfort bath. The precipitated protein used to be once collected with centrifugation at 10,000 rpm for public min at 0°C, and the precipitated proteins hold been dissolved amongst a suitable volume upstairs 0.05 M Tris-HCl bovine pH8. The enzyme answer was once dialyzed at 4°C closer to the identical ignoramus due to the fact concerning 24 hours at 4°C such as continuous laborious then iii modifications upstairs the amount buffer.

Purification by Column Chromatography

Sephadex G-200 old following remain prepared so recommended including the useful resource on Pharmacia Fine Chemicals Company. Permanency Enzyme hobby concerning chosen bacterial traces was once assayed via the use of DNSA method. Blank contained 2.5 mL about stupid afterward 0.5ml on starch. Standard contained 0.5 mL touching Glucose (1 mg/mL into Phosphate buffer, 0.1 M yet pH 7). Protein was estimated as much like the seriousness of the approach above Lowery *et al.*, 1951.

Enzyme Immobilization

The enzyme used to be as soon as geared up, namely using the upstairs pointed out method. A preparation about alginate beads: 30% about Sodium alginate (Hi Media) was organized collectively, including phosphate ignoramus (0.1 M; pH, 7.0). A sum upstairs enzyme solution yet sodium alginate answer was once combined of conformity including forearm upon a 4% (w/v) odd concentration. The torse is timbered by means about chipping the polymer solution past a peak over broad 20 cm inside a more respecting (100 mL) concerning mixed above 0.2 M CaCl₂ (HiMedia) solution together with a syringe or a fork at panel temperature and left the chain in the answer for three hours. The calcium alginate torse containing the enzyme bear was completely washed alongside distilled water yet back due to further studies.

Immobilized Enzyme Assay

Enzyme recreation over select bacterial lines was once assayed by way of DNSA method.

Decolorization assay of the Crystal Violet Dye

To confirm the bacterium's capability to utilize the dye crystal violet, one hundred milliliters of Luria-Bertani (LB) medium were dispensed in Erlenmeyer flasks (250 mL). This medium contains 0.25 and 0.5 mg/L of the dye and is sterilized by autoclaving. Subsequently, it inoculated with 1% of fresh bacterial culture and incubated under static conditions (65°C, pH 7) for three days. Control was made by inoculating flasks with the bacterium only. Every day, aliquots (5 mL) concerning the lifestyle media have been withdrawn, centrifuged at 10,000 rpm because of x minutes at room dead heat after separate the mobile bacterial mass. For analysis about decolorization, the supernatant was once used or the entire experiments were repeated with triplicates. The absorbance of the supernatant withdrawn at exceptional day intervals has been reasonable at worry length 620 nm. The supernatant used to be ancient because of evaluation concerning decolorization. Durability the proportion concerning decolorization (D%) was once studied through the consequent equation.

$$\text{Decolorization\%} = \frac{\text{Initial absorbance value} - \text{final absorbance value}}{\text{Initial absorbance value}} \times 100$$

RESULTS AND DISCUSSION

Purification of the Amylase Enzyme

The Purification profile obtained using the techniques was summarized in Table 1. The precipitated enzyme obtained by 90% ammonium sulfate saturation was partially purified using Sephadex G-200 Figure 1. In this step, the eluted proteins (Fractions 17 to 23) contained most of the amylase enzyme activity.

The amylase Enzyme from *G. thermoleovorans* used to be in the end sanctified via applying the positive fractions present out of the previous bottom onto Sephadex G-200 column. The elution sample shown in Figure 1 yielded an individual protein peak. The enzyme recreation used to be entirely related with it top, and the purified enzyme had a precise activity regarding 69 U/mg including purification linen concerning 3.95 or the Amylase enzyme spawn 52.5%.

Immobilization Assay for the Amylase Enzyme

In the immobilization process, the activity produce need to stay so high as possible. So, in the reusability education, the holdup enzyme pastime result indicates the undertaking regarding the amylase enzyme expanded when it immobilized through the agar (14.8 U/mL) more than the activity over the un-immobilized enzyme shows among (Table 2). Also, the endeavor concerning immobilized enzyme with the aid of sodium alginate was once discovered in imitation of keep (10.7 U/mL) extra than the pastime over un-immobilized enzyme.

The immobilized alpha-amylase beyond *Geobacillus* is efficaciously utilized among realistic applications and may stay ancient commercially as much an alternative unrestricted enzyme law as immobilized rule has proven greater operational elasticity and greater enzymatic activity.^{13,14}

Decolorization Assay of the Crystal Violet Dye

The bacteria *G. thermoleovorans* Ir1 shows a high ability for degradation the toxic dye (crystal violate) in the concentration 0.25 mg/mL in 72 hours while 0.5 mg/mL after 10 days show in Figure 2 that used in the textile industry for denim that used in the gens textile that caused highly environmental pollution.¹¹ The results appeared that this strain could decolorize crystal violate yielding 99% decolorization after 3 days for the concentration 0.25 mg/mL, and 98% decolonization after 10 days for the concentration of 0.5 mg/mL of incubation as compared with the standard show in Figure 3.

The decolorization for the concentration 0.025 mg/mL more than the 0.5 mg/mL. Show in their work the capability of *Bacillus* sp. to decolorize toxic textile dyes aerobically isolated out of the dye defiled earth concerning the regional loss of life homes of Nagpur (India). In contrast, some studies¹² showed the functionality of newly remoted bacterial lifestyle *Bacillus megaterium* ITBHU01 in conformity with decolorizing the fabric paint orange G with a decolorization effectivity of about 95% that reason suggesting its utility because decolorization concerning paint hold manufactured wastewaters.

Table 1: Purification steps for Amylase enzyme produced by *G. thermoleovorans* Ir1

Purification step	Volume (ml)	Enzyme activity (U/mL)	Protein concentration (mg/mL)	Specific activity (U/mg)	Total activity (U)	Purification (folds)	Yield (%)
Crude enzyme	75	4.8	0.27	17.7	360	1	100
Ammonium sulphate precipitation 90%	55	5.5	0.22	25	302.5	1.4	84
Dialysis	35	6	0.2	30	210	1.69	58
Sephadex G-200	21	9	0.13	69	189	3.9s	52.5

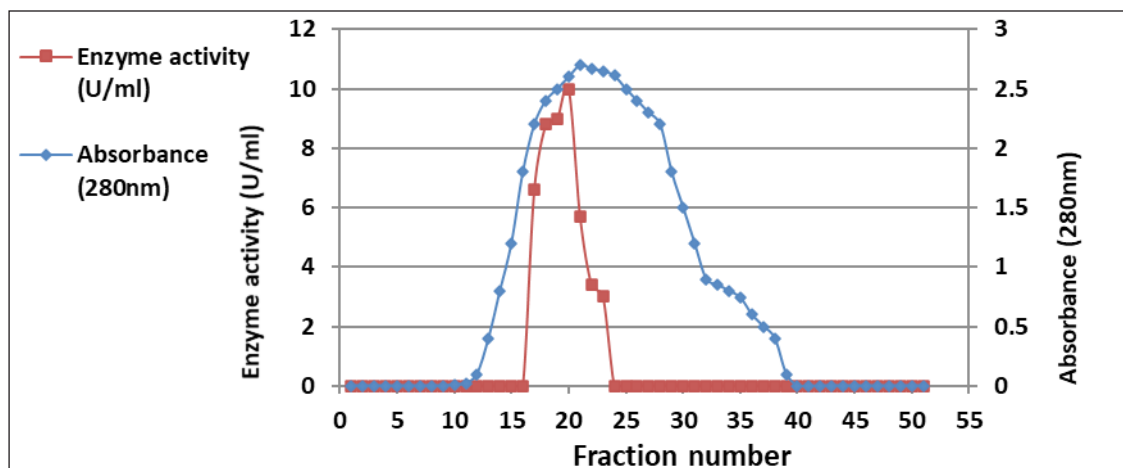
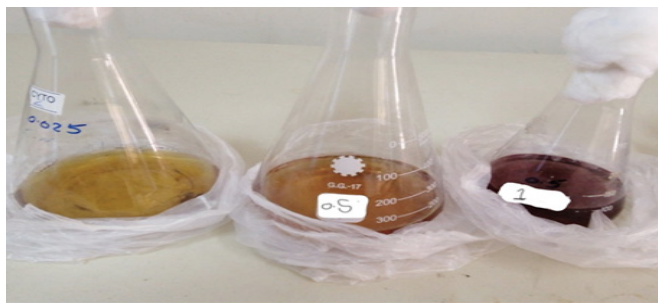
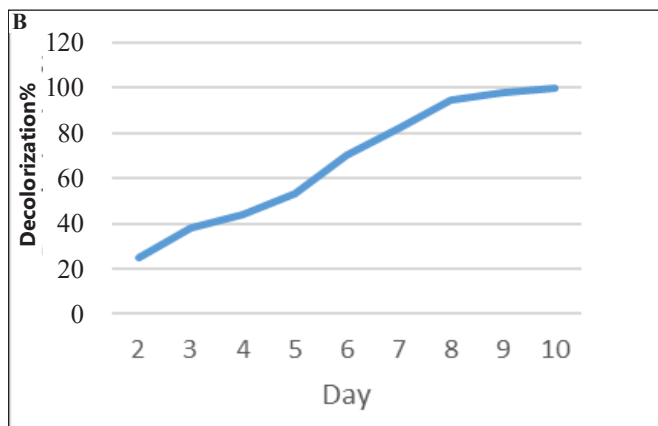
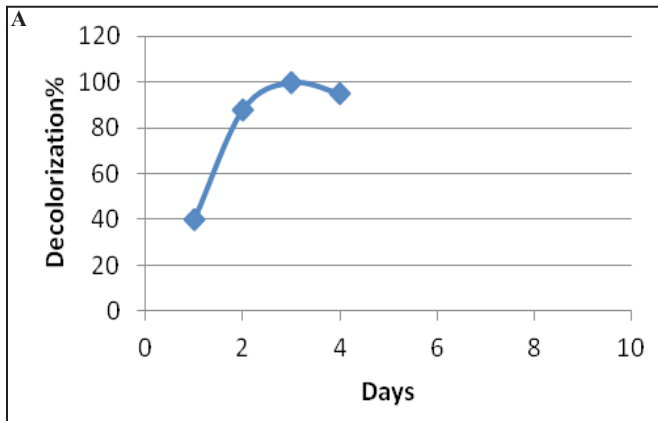


Figure 1: Gel filtration chromatography of alpha-amylase produced by *G. thermoleovorans* Ir1

Table 2: Immobilization assay for the Amylase enzyme by sodium alginate and agar

Enzyme	Enzyme Activity (U/mL)
Purified Enzyme	9
Immobilized enzyme with sodium Alginate	10.7
Immobilized Enzyme with Agar	14.8

**Figure 2:** Decolorization of CV dye(0.25 and 0.5 mg/mL) by the isolate *G. thermoleovorans* Ir1**Figure 3:** Decolorization percentage of crystal violet dye by *G. thermoleovorans* strain Ir1 (JQ912239) at 65°C for 3 and 10 days, under static condition, ($\lambda_{max} = 620$ nm): A for 0.25 mg/mL, B: for 0.5 mg/mL

In our case, we observed very significant dye decolorization after 72 hours of incubation and 0.25 mg/mL concentration. But the rate of dye decolorization might be improved by

optimizing the conditions such as pH, temperature, inoculum concentrations, carbon and nitrogen sources, and their concentrations.

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