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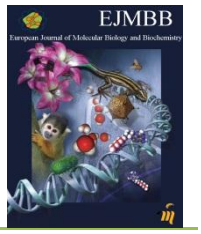

GROWTH RESPONSE OF *AZOTOBACTER CHROCCOCUM* SPP. AGAINST $ZnFe_2O_4$: A VITRO ANALYSIS

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ABSTRACT

The Zinc iron systems were prepared by sol–gel auto combustion methods—varying the synthesis conditions (precursor solutions, chemical compositions, catalysts, temperature and time of aging and heat treatment). The precursors of sol–gel procedures were Zinc nitrate, iron nitrate and glycine was applied as catalysts. The processing features and the micro structural characteristics of $ZnFe_2O_4$ phases formed during self-combustion reactions of the gels have been investigated by TG-DTA, XRD and TEM. The oxide has been annulated at 800^0C temperature and resulted in $ZnFe_2O_4$ nano-particles with crystallites size in the range of 50-60 nm. The prepared Zinc iron systems was treated against the *Azotobacter chroccocum* by using sole carbon source as a control, the *Azotobacter chroccocum* important and playing key major role for improving crop productivity, it supply the atmospheric nitrogen by nitrogen fixation process. *Azotobacter chroccocum* was showing the highest growth response to Zinc iron ($ZnFe_2O_4$) ceramics and had excellent bioactivity at low concentration, performing positive correlation but at high concentration of Zinc iron, it decrease their bioactivity, performing negative correlation. Hence the Zinc iron act as best bioactivator for the activity of *Azotobacter chroccocum* to accelerate the growth.

INTRODUCTION

No. of Azotobacter species are found in environment, which fix the atmospheric nitrogen, generally *Azotobacter chroccocum* is the one spp. of Azotobacter is generally present in soil and is one major species carried out the nitrogen fixation in every type of soil at each and every climate, it provide the sole nitrogen source to the plant species. The nitrogen is a major and key element required for the plant growth. Due to wide application of *Azotobacter chroccocum* species, it was used for same research.

These *Azotobacter chroccocum* are free living microorganisms present in soil where they can synthesize the nitrogenase enzyme which favour to convert atmospheric nitrogen towards ammonia is known as nitrogen fixation process. These bacterial cells have one specific gene located in the bacterial cell. Such gene is known as Nif gene is responsible for the production of nitrogenase enzyme. The enzyme is nothing but the combination of Azoferrodoxin and Molibdoferrodoxin. These both are playing the major role in nitrogen fixation process. The bacteria create the association with the plant species with providing the nitrogen source without any arrest form hence such type of association is known as non symbiotic association. The provided nitrogen does promote the growth yield with product capitulate. No. of different factors are responsible for promoting the growth yield viz. nutritional source, cofactor, activators, Opt pH and Temperature etc. in the term of growth response, the bacterial cells are given the wide variation range to different chemical and physical agent. In said research the growth response was detected against prepared $ZnFe_2O_4$. The tremendous work had done on *Azotobacter chroccocum* for their maximum activity but most work are

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depend upon Biological material. In this research $ZnFe_2O_4$ is prominent material used against *Azotobacter chroccocum* and act as Biological active agent refer as chemical catalyst.

A wide range of ceramic materials is used for biomedical applications: glasses, ceramics (polycrystalline, sintered, hot pressed, sol–gel ceramics) and composites.$CaO{\cdot}SiO_2$ was found to be bioactive in a simulated body fluid environment. Especially, the chain silicate minerals (e.g. wollastonite ($ZnFe_2O_4$) and diopside ($CaMgSi_2O_6$)) have been synthetically prepared for use as bioactive ceramic materials [1–3]. Calcium silicate ($ZnFe_2O_4$) ceramics have excellent bioactivity and degradability/resolvability [4–7], and thereby could be applied in hard tissue repair or as 3D scaffolds for tissue engineering. Siriphannon et al [8] and De Aza et al [9] has reported the preparation of dense calcium silicate ceramics by pressing $ZnFe_2O_4$ powders and conventional sintering at different temperatures. As numberof applications are discovered therefore the aim of this paper was expected to activate the growth of azotobactor by $ZnFe_2O_4$.

MATERIAL AND METHODS

Isolation of *Azotobacter chroccocum*

For the isolation of *Azotobacter chroccocum* the selective media nitrogen free mannitol agar media was used for the isolation purpose, the soil sample was allow to enrich in same broth at room temperature for 4 days. The slurry of bacterial growth from the surface of broth was stricken on Nitrogen Free Mannitol Agar Media plate, plate allow incubating at room temperature for 24 hrs. The slippery growth with brown pigmentation indicates the presence of *Azotobacter chroccocum*

Preparation of Suspension

In aseptic condition the scratched growth then washed in Sterilized distilled water and immersed in electrolytic saline, measure and maintains the turbidity of bacterial growth in saline with the help of calorimeter in the term of optical density. The prepared suspension was subjected for the growth response.

Powder preparation

In this study, $ZnFe_2O_4$ powder was successfully synthesized by solution combustion route using the starting regents as $Zn(NO_3)_26H_2O$ (7.43g), $Fe(NO_3)_26H_2O$ (7.27g) and glycine (6.05g) as a fuel. Glycine possesses a high heat of combustion. It is an organic fuel providing a platform for redox reactions during the course of combustion. Initially zinc nitrates, iron nitrates and glycine are taken in the 1:1:4 stoichiometric amount and homogenous paste was made. The paste formed was evaporated on hot plate at about 70 to 80^0C to get thick gel. This kept on a hot plate for auto combustion and heated at 170 to 180^0C. To obtain nanocrystalline $ZnFe_2O_4$ powder. The powder was sintered at 800^0C for 4 hrs. Which resulted into a white colour shining powder.

Materials Characterization

The prepared $ZnFe_2O_4$ samples were characterized using TG/DTA thermal analyzer (SDT Q600 V 20.9 Build 20), X-ray diffract meter (PW-3710) using Cu-K_α radiation, scanning electron microscope (JEOL JED 2300) coupled with an energy dispersive spectrometer (JEOL 6360 LA), and transmission electron microscope operating at 200 kV.

Tablet Preparation

The prepared powder of nonocrystaline $ZnFe_2O_4$ was pressed to make the tablet. The same tablet was used for the growth response at their different concentration.

Growth Response

At different concentration of $ZnFe_2O_4$ the prepared suspension of *Azotobacter chroccocum* was tested for their growth response. The results were noted after 24hr, 48hr, 72hr, and 96 hr. The control was kept by using the sole carbon source.

RESULT AND DISCUSSION

Microanalysis and TG-DTA

Figure 1 TG-DTA analysis was performed at a heating rate of 10 K min^{-1} to investigate the thermal properties of $ZnFe_2O_4$. The TG spectrum and its 1^{st} derivative in shown Fig.1 the thermal decomposition process for the dried gel oxidation and decomposition processes. The precursor measured a total weight loss of 76.26% with 5 distinct temperature intervals. The first three intervals are entwined from 36^0C to 350^0C with broad endothermic peaks and a weight loss of 16%. These are attributed to the evaporation of residual water and burning of residual organic materials. The second from 400^0C to 600^0C with a rapid weight loss of 40% and a broad exothermic peak around 620^0C, this is attributed to decomposition of the organic compounds. The last interval from 600^0C corresponds to a weight loss of 15% followed by a long gradual decreasing rate these as shown in equation (1,2), indicating that the synthesized powder was almost stable from the 800^0C.

XRD Analysis

The XRD patterns of the calcined $ZnFe_2O_4$ are shown in Figure 2. The structure possesses the hexagonal may be attributed to the different preparation method which may yield different structural defects. The crystalline size was determined from full width of half maximum (FWHM) of the most intense peak obtained by scanning X-ray diffraction pattern. The grain size was calculated by using following Scherrer's formula.

$$d = 0.9\lambda/ \beta\cos\theta$$

Where, d is the crystalline size, λ is the X-ray wavelength of the Cu K_α source (λ=1.54056 A^0), β is the FWHM of the most predominant peak at 100 % intensity, θ is the Braggs

angle at which peak is recorded. The grain size was found to be 55nm [10-11].

TEM Analysis

The TEM specimen was prepared by placing microdrops of colloid solution on a carbon film supported by a copper grid. To get better understanding of the morphology the TEM image of the mixed precursor calcined at 800 ^{0}C in air for 4 h is shown in Fig. 3 (a,b.c). It indicates the presence of $ZnFe_2O_4$ nanoparticles with size 50-90 nm which form beed type of oriental aggregation throughout the region, on the contrary the image shows distinct nanoparticles of nearly spherical structure which are correlated well with the XRD results. The selected area electron diffraction (SAED) pattern Fig. 3 (d) shows the spot type pattern which is indicative of the presence of single crystallite particles. No evidence was found for more than one pattern, suggesting the single phase native of the material.

Growth response of *Azotobacter chroccocum* to $ZnFe_2O_4$

Growth response of *Azotobacter chroccocum* to $ZnFe_2O_4$ at 0.5% represented in table no. 1 It was shown that growth response of *Azotobacter chroccocum* via Sole C source as a control were 0.8, 1.4, 2.8 and 4.1 after 24hr, 48hr, 72hr, and 96 hrs. respectively. It was found that growth response of *Azotobacter chroccocum* via Carbon being with $ZnFe_2O_4$ were 0.9, 2.1, 3.9 and 5.3 after 24hr, 48hr, 72hr, and 96 hr respectively. It was shown that as compare with sole carbon sources net growth response of *Azotobacter chroccocum* were 0.1, 0.7, 1.1 and 1.2 after 24hr, 48hr, 72hr, and 96 hr respectively

Growth response of *Azotobacter chroccocum* was increases in the presence of 0.5% $ZnFe_2O_4$ standardized with sole carbon source was represented in Fig. 4

Fig. 5 Signifying that calculated net growth response of *Azotobacter chroccocum* to $ZnFe_2O_4$ was in increasing manner, consider and belongs to positive correlation.

The Prescribed research was wrap up to establish that 0.5% $ZnFe_2O_4$ tablet act as enhancer which enhances growing *Azotobacter chroccocum* species at each tested time intervals.

Growth response of *Azotobacter chroccocum* to $ZnFe_2O_4$ at 1.0% represented in table no. 2 It was shown that growth response of *Azotobacter chroccocum* via Sole C source as a control were 0.8, 1.4, 2.8 and 4.1 after 24hr, 48hr, 72hr, and 96 hrs. respectively. It was found that growth response of *Azotobacter chroccocum* via Carbon being with $ZnFe_2O_4$ were 0.8, 1.4, 2.7and 3.4 after 24hr, 48hr, 72hr, and 96 hr respectively. It was shown that as compare with Sole Carbon sources net growth response of *Azotobacter chroccocum* were 0.0, 0.0, -0.1 and -1.7 after 24hr, 48hr, 72hr, and 96 hr respectively

Growth response of *Azotobacter chroccocum* was stable and decreases in the presence of 1.0 % $ZnFe_2O_4$ standardized with sole carbon source was represented in Figure 6

Figure 7 Signifying that calculated net growth response of *Azotobacter chroccocum* to $ZnFe_2O_4$ was in decreasing manner remain stable for some extend beyond that fall down which consider and belong attributed to negative correlation.

The Prescribed research was wrapping up to establish that 1.0% $ZnFe_2O_4$ tabletnor act as activator neither inhibitor up to 48 hrs. When extend time period beyond 48 hrs. 1.0% $ZnFe_2O_4$ tablet which inhibit growing *Azotobacter chroccocum* species at each tested subsequently time intervals.

Table 1. Growth response of *Azotobacter chroccocum* to 0.5% $ZnFe_2O_4$ tablet

Sr. No.	Time in hr	Growth at Sole C source (control) O.D	Growth at C and ZnFe2O4 O.D	Net growth response O.D
1	24	0.8	0.9	0.1
2	48	1.4	2.1	0.7
3	72	2.8	3.9	1.1
4	96	4.1	5.3	1.2

Where 'C' is sole Carbon source

Table 2. Growth response of *Azotobacter chroccocum* to 1.0 % $ZnFe_2O_4$ tablet

Sr. No.	Time in hr	Growth at Sole C source (control) O.D	Growth at C and ZnFe2O4 O.D	Net growth response O.D
1	24	0.8	0.8	0.0
2	48	1.4	1.4	0.0
3	72	2.8	2.7	-0.1
4	96	4.1	3.4	-1.7

Where 'C' is sole Carbon source

Figure 1. TG-DTA curve of mixed precursor $ZnFe_2O_4$

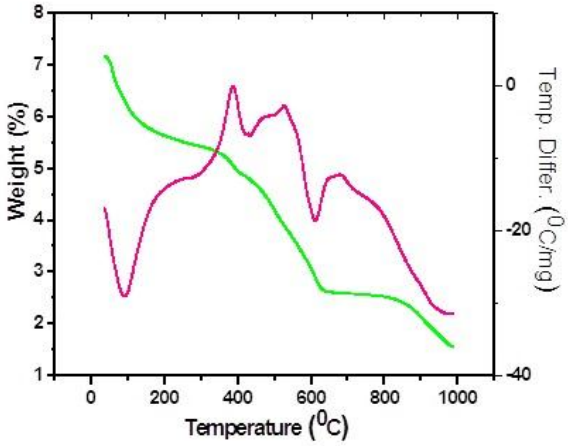


Figure 2. XRD pattern of calcined mixed precursor $ZnFe_2O_4$ at 800^{0}C in air for 4h.

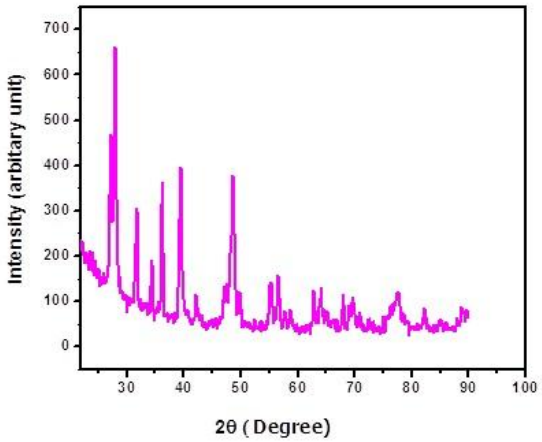


Figure 3. TEM (a,b,c) images of mixed precursor $ZnFe_2O_4$ (b) SAED pattern

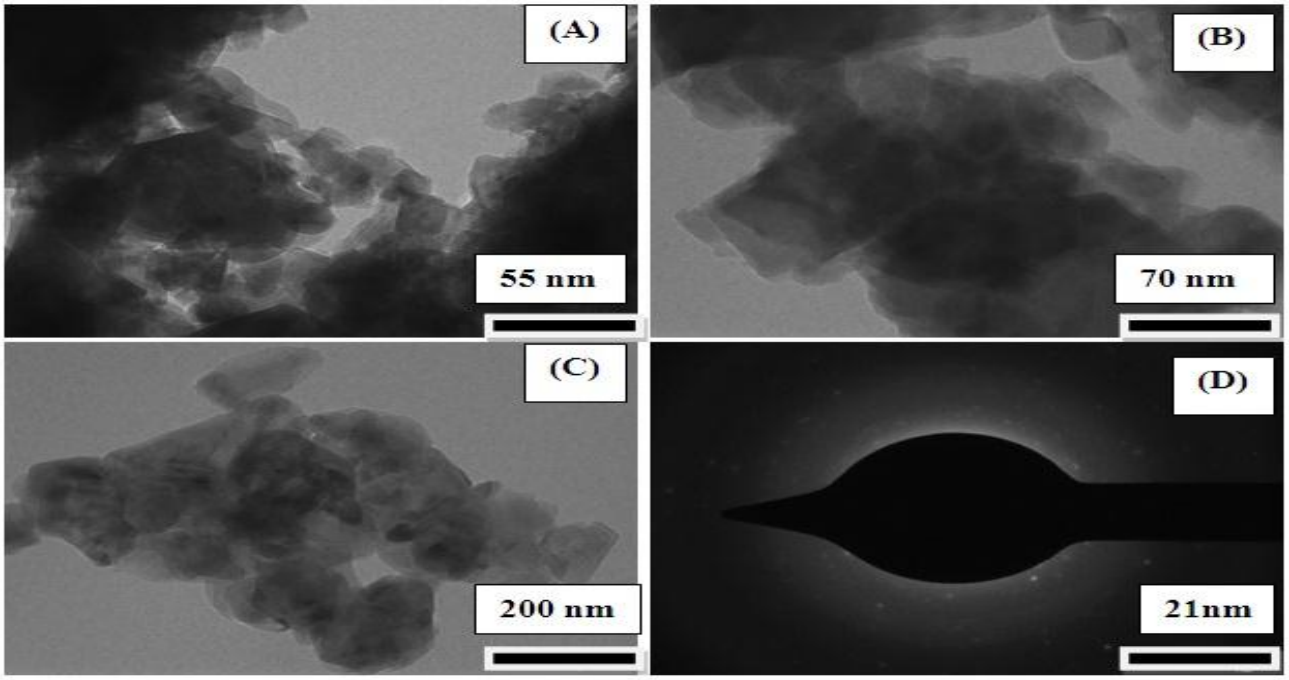


Figure 4. Growth response of *Azotobacter chroccocum* to 0.5% $ZnFe_2O_4$ tablet

Figure 5. Net growth response at 0.5% $ZnFe_2O_4$

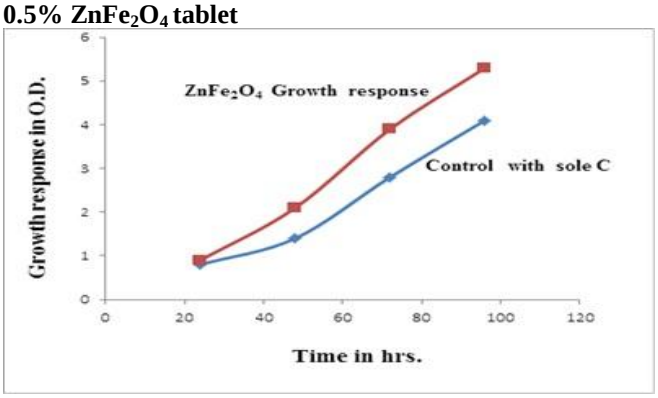


Figure 6. Net growth response at 1.0 % $ZnFe_2O_4$

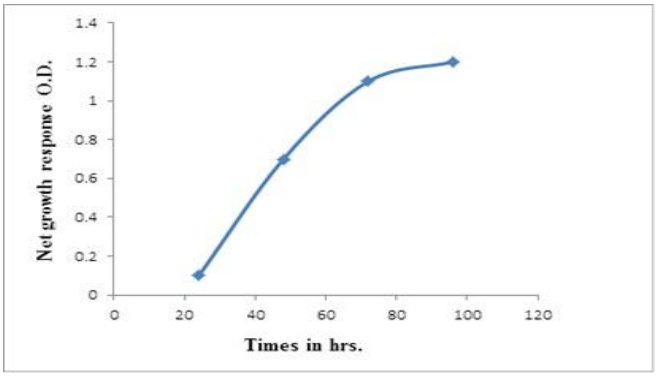
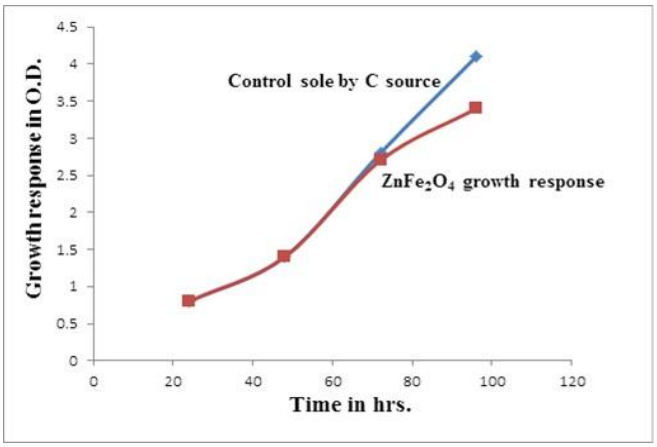
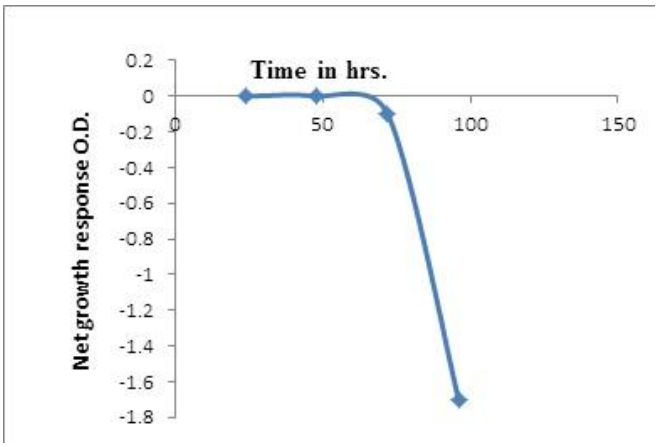


Figure 7. Negative net growth response at 1.0% $ZnFe_2O_4$





CONCLUSION

Nanocrystalline $ZnFe_2O_4$ has synthesized by self-combustion route. This synthesis route may be used for the synthesis of other metal oxide. The phage formation of the $ZnFe_2O_4$ is investigated by TG-DTA, XRD, TEM techniques. average diameter 50-60 nm. $ZnFe_2O_4$ was enhancing the growth response of *Azotobacter chroccocum* at low $ZnFe_2O_4$ concentration (0.5 %). At high concentration of ZnFe2O4 growth of *Azotobacter chroccocum* was inhibited From above result it was concluded that at high concentration of $ZnFe_2O_4$ growth of *Azotobacter chroccocum* was inhibited whereas at low concentration of $ZnFe_2O_4$ maximize the same growth response. At low concentration it act as activator and at high concentration it act as inhibitor. At Low concentration (0.5% $ZnFe_2O_4$), $ZnFe_2O_4$ will use as growth enhancer for growing *Azotobacter chroccocum* which make sure to improve the crop yield.

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