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RESEARCH ARTICLE

STRESS RESPONSE ANALYSIS ON LIPID PRODUCTION BY MICROALGAE ISOLATED FROM ESTUARIES BY UV RADIATION.

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Abstract

Rapid depletion of fossil fuels with increasing energy consumption and global warming have resulted in a move towards alternative energy sources with less emissions of greenhouse gas. Oil rich microalgae might be alternative sources of lipids for biodiesel production. Microalgae offer a high potential for lipid storage as well as high growth rates. Ultraviolet (UV) irradiation was applied to various microalgae for lipid induction. In this study various microalgae such as *Scenedesmus* sp, *Pseudokrichneriella* sp, *Nannochloropsis* sp and *Chlorella* sp was studied for the microalgal lipid production. Two strains such as *Scenedesmus* sp, and *Chlorella* sp shows increased dry cell weight and lipid content of 45.7µg/ml and 49.4µg/ml respectively which led to a general increase of biomass and total lipid content. The highest lipid content was observed in *Chlorella* sp of about 49.4 µg/ml. All these results indicate that UV mutation is an efficient method to improve probability for using microalgae as the potential raw material for biodiesel production. This study highlights that UV mutation for microalgae can be a viable approach to improve biomass and lipid productivity in microalgae. This process resulted in a significant increase of both biomass and lipid productivity of microalgae. Such strains could subsequently be used as commercial oleaginous algae and serve as an alternative to conventional petrol.

Introduction:-

The breathe taking issue on fossil fuel consumption and the deleterious threat to mankind is due to its unavoidable intake and generation of greenhouse gases like carbondioxide, nitrogen oxides, methane, sulfur dioxide and volatile organic compounds (Gavrilescu & Chisti, 2005). The answer to the question why is there a need for biofuels is highly challenging, as it implies on energy security, economic development and mitigation of climate change. There is a foretelling that around 2035 the energy consumption in developing countries is going to reach 84 percent, which would be alarming. The main resource and key source of industrialization is fossil fuel resources like coal and

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petroleum. There lies a high source of responsibility among the government, researchers and international organization to gear up the available resources to source for the masses.

Biodiesel is a nontoxic, most renewable and biodegradable, monoalkyl ester of long chain fatty acids. Biofuel was obtained from food crops, then non-food crops such as wood, organic waste, food crop waste and biomass waste, it is termed as first generation and second generation respectively. The third generation emphasized on the improvements in the biomass production i.e algal crops were used. The search for alternate fuel has made microalgae more demanding. Microalgal oil doesn't contribute to the net rise in the level of carbondioxide in the atmosphere, but it leads to minimize the intensity of the greenhouse effect (Deng et al., 2009).

Microalgae are sunlight driven cellular factories that are easily cultured and they require less space for cultivation. They can convert carbon dioxide to possible biofuels, valuable bioactive compounds such as proteins, carbhohydrates, lipids and pigments. Algal lipid is a great opportunity as it serves as the feedstock for the future's sustainable biodiesel production (Liu et al., 2008). Microalgae provides a range of renewable biofuels which are produced by anaerobic digestion of algal biomass. These biofuels include methane produced anaerobically, biohydrogen produced photobiologically abd biodiesel (Melis, 2002)

Microalgae are categorized into groups based on color, which includes, Cyanobacteria; Chlorophyta; Rhodophyta; Glaucophyta; Euglenophyta; Haptophyta; Dinophyta; Chloarachniophyta; Cryptophyta and Stramenophiles (Graham, 2009).

Microalgae produce lipids i.e., neutral lipids, polar lipids, sterols, wax esters, hydrocarbons; phenyl derivatives i.e., tocopherols, carotenoids, terpenes quinines; and pyrrole derivatives such as chlorophylls. The lipid constituents differ in plants and microalgal oils. Microalgal oil shows a higher percentage of polysaturated fatty acids than found in plants. (Tran et al., 2010). Lipid are extracted in various methods. The most common among them are oil presses, liquid – liquid extraction (solvent extraction), supercritical fluid extraction and ultrasonic extraction. The best result producing technique for lipid extraction in microalgae is solvent extraction method, with solvents such as hexane, acetone, and chloroform. Oil is soluble in organic solvents than in water. (Bligh and Dyer et al., 1959).

Microalgae can modify lipid metabolism efficiently in response to changes in environmental conditions. Wide range of studies are being carried out on lipid induction in microalgae i.e, use of nutrient stress which includes nitrogen and/or phosphorus, light irradiation, pH, temperature, heavy metals and other chemicals (Xiong et al., 2008). Strain improvement is done by mutation, which falls into two main categories i.e., physical (eg., UV, X-ray and gamma rays) and chemical (eg. Ethyl methane sulfonate (EMS) and nitrosomethyl guanidine (NTG).

UV irradiance in microalgae is a promising focus with the impact of UV-A and UV-B radiation on algal growth, morphology, physiology and oxidative stress (Fouqueray et al., 2007). In bulky scale production UV radiation has been suggested. UV radiation induces cell mutagenesis and the selected mutants are suggested under strain improvement method to enhance lipid production. For mutation studies a little knowledge on the biochemical pathway is required to synthesis the product of desire. The main aim of this study was to investigate the stress response of the microalgae isolated from estuaries. The effect of the radiation and photo reactivation was studied on biomass, pigment content, carbohydrates, proteins, carotenoid and lipid.

Materials and Methods:-

Microalgae culture conditions:-

Water samples from north eastern estuaries of Tamilnadu were collected and microalgae were isolated and mass cultivated in the laboratory conditions in BG11 medium in 16:8 hours light flux at 28^0C incubation in a growth chamber. Pure cultures of *Nannochloropsis* sp, *Chlorella* sp, *Scenedesmus* sp, and *Pseudokrichneriella* sp were cultivated and studied.

Monitoring of the microalgal cultures:-

Two sets of cultures were implied in the UV stress analysis, they were exposed to UV radiation and photo reactivation was done to one set. The cultures were exposed to various timings under UV i.e., 20 mins, 40 mins, and 60 mins. One set was placed in dark for 2 hours and the others were incubated immediately in 16:8 hours light flux at 28^0C in a growth chamber. A periodic study of the biomass, chlorophyll, carotenoid, protein and lipid were done at 7 days interval.

Analysis of lipid production:-

Lipid extraction was done by Bligh and Dyer method. The cells were harvested by centrifugation, for 1g of algal biomass, 2ml of methanol and 1ml of chloroform was added and kept for 18 hours at 25^0C. The mixture was agitated and 1ml of chloroform was added and was shaken vigorously for 1min. After that, 1ml of distilled water was added and the mixture was placed in a vortex again for 2min. The layers were separated by centrifugation for 10min at 2000 rpm. The lower layer was separated and the procedure was repeated with the pellet. The two supernatants collected were allowed to sand for 2 hours. Lower organic layer with the lipids was transferred to a clean pre-weighed vial (W_1). Evaporation was carried out in hot air oven at 80^0C for 50min. The weight of the vial was again recorded (W_2). Lipid content was calculated by subtracting W_1 from W2 and expressed as % dry cell weight.

Result and Discussion:-

The microalgae such as *Scenedesmus* sp., *Pseudokrichneriella* sp., *Nannochloropsis* sp., and *Chlorella* sp., were isolated (Fig 1) and selected for the UV stress analysis. Dohler et al., (1997) have reported that Dunaliella grew to be negatively affected by short time exposure to UV radiation, whereas Salguero et al., (2005) reported that enhanced growth was observed after long time exposure to moderate UV radiation. The cultures were exposed to UV light in varied timings. The growth of all the four cultures with 40 min and 60 min in UV light grew up much faster during the exponential phase than in the control cultures (Fig 2 and 3). The total lipid content was determined by Bligh and Dyer method in both control and UV exposed cultures. The total lipid content of the cultures exposed with 60 min UV light was higher than in the control cultures (Fig 4 and 5). Along with the increase of UV irradiation time, the dry cell weight increased dramatically in this study. The cells would have bursted open to release the lipid bodies into the medium to maximize the lipid content. Forjan (2011) have reported that modulated use of UV radiation could lead to increased production of fatty acids in *Nannochloropsis* sp. UV has been successfully used to generate microalgal mutants and was selected as a mutagenic agent because it is practically safer than chemical mutagens such as EMS and MTG. A pretreatment with UV radiation leads to breaking of the cell, which has a positive effect on the lipid extraction efficiency. (Carlton et al., 1981).

Fig 1. Microscopic observation of the microalgal cultures a) *Scenedesmus* sp.,
b) *Pseudokrichneriella* sp., c) *Nannochloropsis* sp., d) *Chlorella* sp.

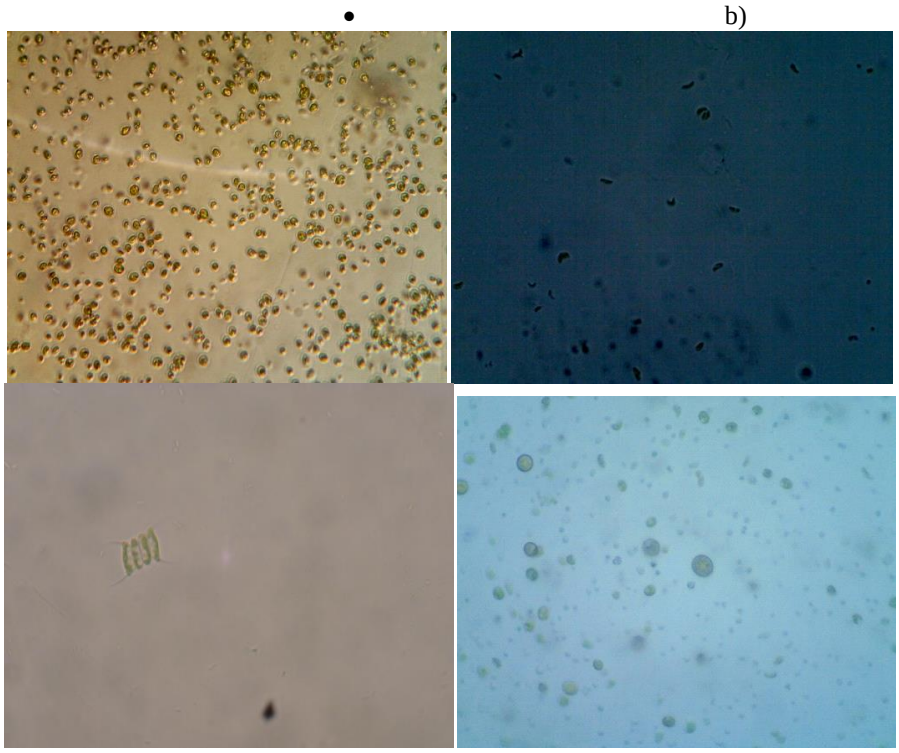
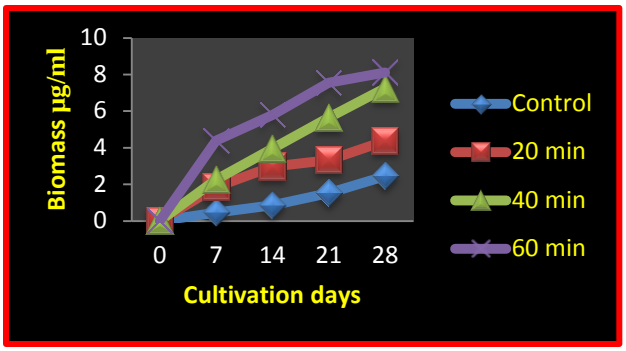
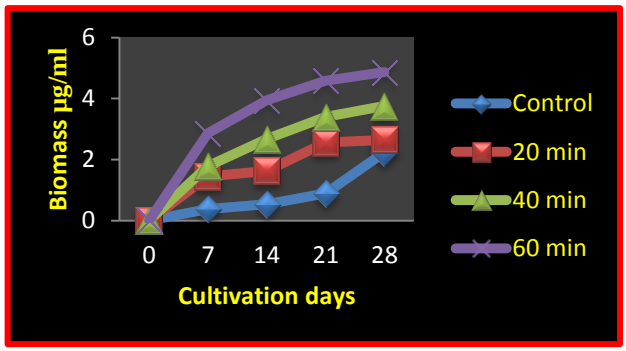


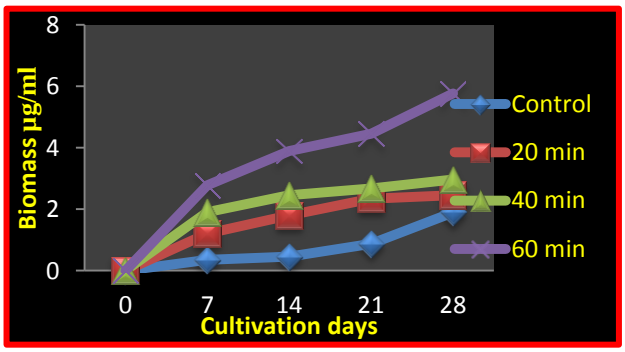
FIGURE NO. 2 ESTIMATION OF BIOMASS (µg/ml) BY DRY WEIGHT METHOD IN MICROALGAL SPECIES CULTIVATED IN BG11 MEDIUM INCUBATED IN 28^{0}C IN 16:8 LIGHT FLUX AFTER UV EXPOSURE AT VARIED TIMINGS



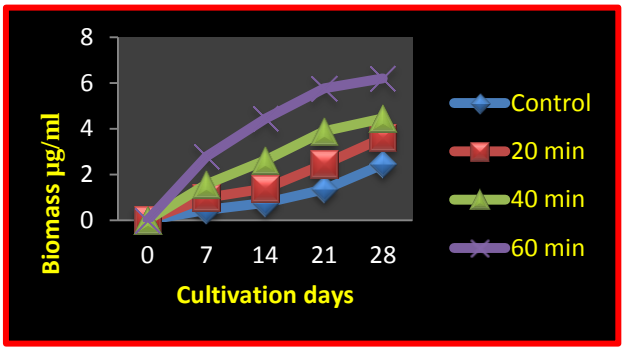
A. ***Scenedesmus*** **sp.**



B. Pseudokrichneriella **sp.**



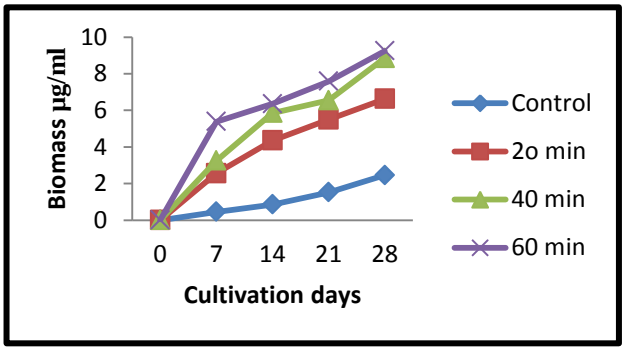
C. Nannochloropsis **sp.**



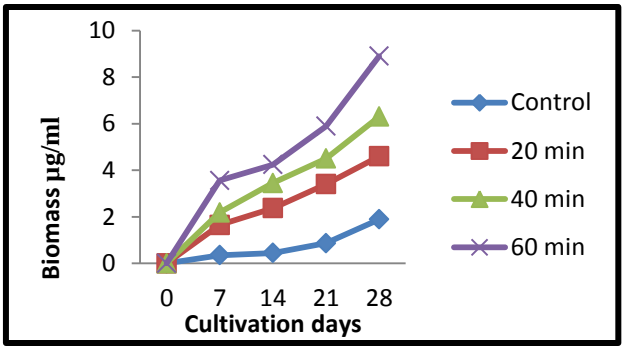
D. ***Chlorella*** **sp.**

FIGURE NO. 3 ESTIMATION OF BIOMASS (µg/ml) BY DRY WEIGHT METHOD IN MICROALGAL SPECIES CULTIVATED IN BG11 MEDIUM INCUBATED IN 28^{0}C IN 16:8 LIGHT FLUX AFTER PHOTOREACTIVATION OF THE UV EXPOSED CULTURES

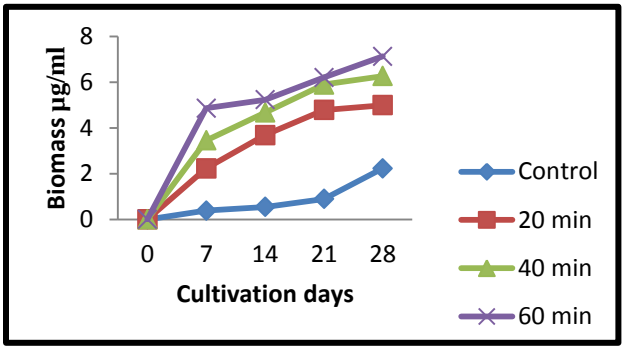
A. ***Scenedesmus***



B .Pseudokrichneriella



C. *Nannochloropsis* sp.



D. *Chlorella* sp.

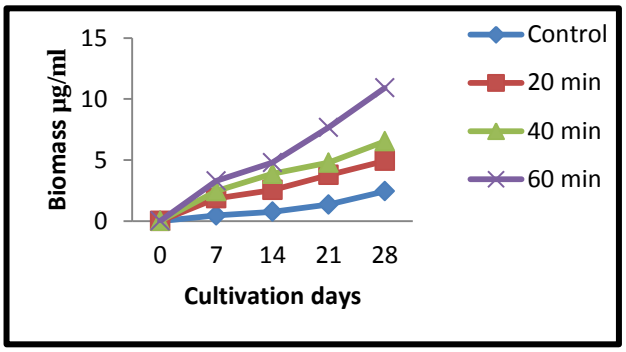
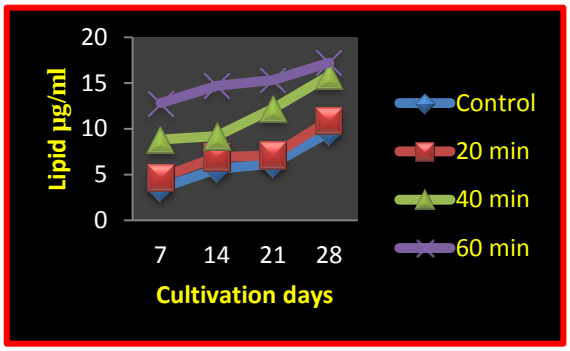
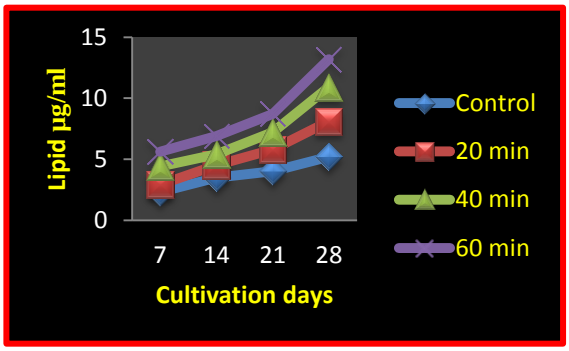


FIGURE NO. 4 ESTIMATION OF BIOMASS (µg/ml) BY DRY WEIGHT METHOD IN MICROALGAL SPECIES CULTIVATED IN BG11 MEDIUM INCUBATED IN 28^{0}C IN 16:8 LIGHT FLUX AFTER UV EXPOSURE AT VARIED TIMINGS

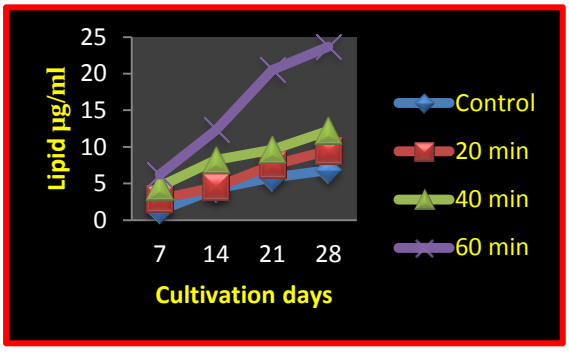


A. *Scenedesmus.*



B. *Pseudokrichneriella*

C. *Nannochloropsis* sp.



D. *Chlorella* sp.

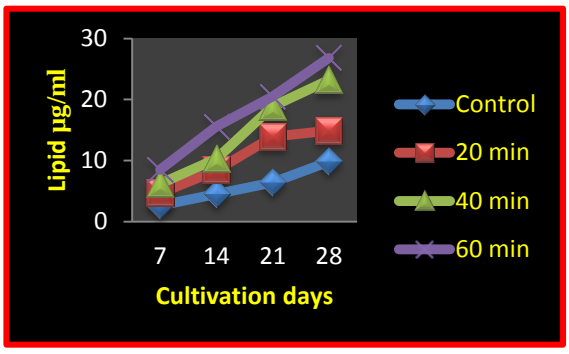
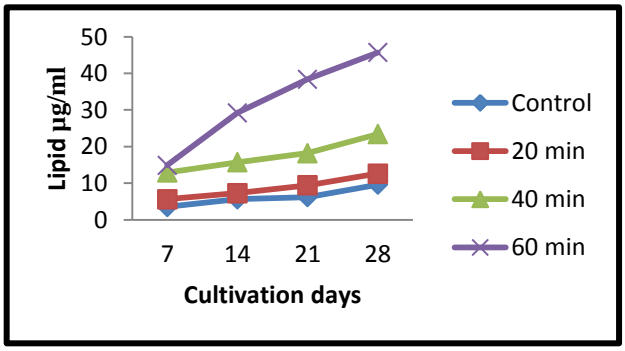
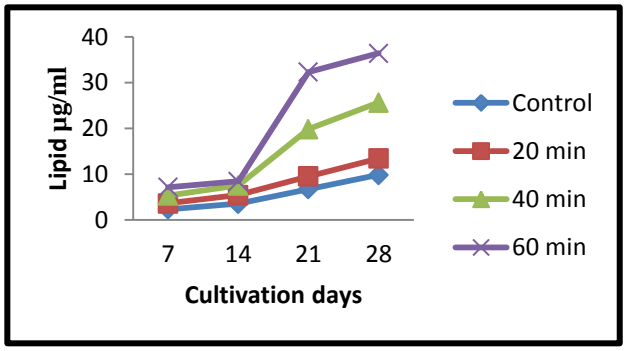


FIGURE NO. 5 ESTIMATION OF BIOMASS (µg/ml) BY DRY WEIGHT METHOD IN MICROALGAL SPECIES CULTIVATED IN BG11 MEDIUM INCUBATED IN 28^{0}C IN 16:8 LIGHT FLUX AFTER PHOTOREACTIVATION OF THE UV EXPOSED CULTURES

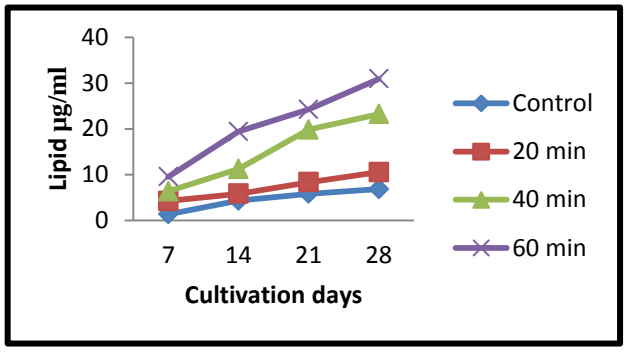


A. Scenedesmus

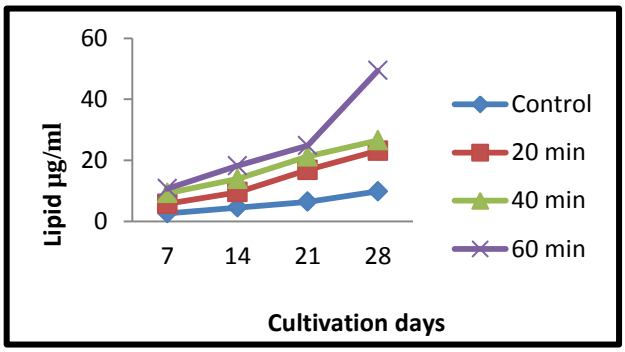


B. Pseudokrichneriella

***C. Nannochloropsis* sp.**



D. *Chlorella* sp.



Conclusion:-

Transportation fuel requirement in India is unique in the World. India is found to consume six to seven times more diesel fuel than gasoline. But the whole of the World use gasoline than diesel. Hence India has a more demanding emphasis in the search of alternate fuel. Microalgae have been attracting attention as a source of high lipid material to produce biofuel, because the biofuel they produce are biodegradable, renewable, nontoxic fuel and do not compete with food crops. This paves a way to produce microalgal lipid content at cost effective and environmentally friendly state.

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