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Studies on evaluation of Renewable Carbon sources on the Growth and Pigment production in yeast *Rhodotorula glutinis*

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Abstract

Wide applications of synthetic pigments in the food, cosmetics, textiles is increasing in recent era, but due to the harmful effect of synthetic dyes, a number of natural pigments are produced by plants, animals and microorganisms. As the production processes of natural pigment from the biological source make the high market price hence low-cost processes are required to replace synthetic pigments. The main objective of the present research work was to evaluate the renewable, cheap, easily available carbon sources as alternative substrates for production of carotenoid pigment by yeast *Rhodotorula glutinis*. Renewable carbon sources such as banana peel waste, corn-steep liquor collected from starch factory, dairy effluent collected from dairy industry and sugar industry effluent were tested as economically feasible cheap carbon sources for pigment production by yeast *R. glutinis*. The results obtained revealed that banana peel waste was found to be the best cheap, easily available substrate for pigment production in *R. glutinis* followed by banana peel, corn steep liquor and dairy industry effluent was also found to favours the growth of yeast. Utilization of banana peel, corn steep liquor, dairy and sugar industry effluents can be used as growth medium for carotenoid production by *Rhodotorula*, provides economic benefits and reduce production cost of pigment and help in reduction of environmental pollution.

Key words : carotenoid pigments, *Rhodotorula* yeast, banana peel waste, corn steep liquor, sugar industry and dairy industry effluent.

Colors are the first significant variable of food. Colors are added to foodstuffs to make food attractive, as well as to make the consumer buy the product[1]. Pigments can be classified, according to their origin, as natural or synthetic. Natural pigments are further categorized based on their specific origin (plant, mineral, microbial, or animal), color formation, chemical constitution, and application method. Examples of natural pigments include

carotenoids, melanins, chlorophylls, flavonoids, anthocyanins, betalains, and quinones. Synthetic pigments, on the other hand are hazardous as they are cytotoxic, carcinogenic, or teratogenic. In addition to that, multiple synthetic colorants depend on precursors that are petroleum-based, a non-renewable resource. With the advent of the hazardous effects of chemically synthesized pigment demand for natural colors is increasing. At present, the production of natural dyes is mainly based on the extraction from plant materials that makes the current market prices still high. To solve this problem, other biological sources, have been adopted to improve the yields of pigment production[2]. However, microorganisms are superior to plants because use as renewable sources for bio pigment production with massive production, controlled scaled up bioprocessing, easiness in genetic manipulation, fastly growing organisms, low, cost, effective growth in production media, simplicity in handling, no need for big land areas for growth like plants, etc.[3] Agricultural industries produced large amount of residues per year if these residues are released into the environment without proper treatment it may cause environmental pollution and harmful effects on human and animal health[4]. Hence researchers are seeking strategies to utilize this waste for microbial pigments production. Some of the recent reports on bacterial pigments using agro-industrial wastes highlighted the use of *Erwinia uredova*, *Planococcus* sp., and *Rhodopseudomonas faecalis*. These bacteria have demonstrated their potential in the production of carotenoid-type pigments using different agro-industrial wastes[5-7,9]. Thus the objective of the present study was to investigate the efficacy of natural substrates for carotenoid pigment production by yeast *Rhodotorula glutinis* having potential applications in pharmaceutical and medicines.

Microorganism :

Intense red carotenoid pigment producing yeast cultures *Rhodotorula glutinis* 3379 was procured from NCIM.

Preparation of fermentation media :

The natural substrates like banana peel, corn steep liquor, sugar industry effluent, dairy waste effluent were collected for the present study. The main objective of the present research work was the evaluation of the best suitable alternative substrates for the maximum yield of pigment production.

Utilization of banana peel :

Fresh banana peels were crushed into mortar and pestle. Crushed banana peel sample was then filtered by Whatmann filter paper and supernatant obtained after filtration was collected in flask. It was again centrifuged to remove any suspended particles of banana peel present in it. Then supernatant was heated at 80^{o}C and boiled for 5 min for conversion of cellulosic sugar content of banana peel into simple sugar like glucose.

Glucose conversion was checked by adding 1ml of Benedict's reagent with slight boiling. Development of brick red color indicated presence of glucose in the banana peel extract which can be used as carbon source for the growth of yeast.




Preparation of culture media using banana peel extract :

Banana peel extract agar plates were prepared by adding 3 gm agar-agar into 100 ml of supernatant containing banana peel extract. Simultaneously control media containing yeast extract, peptone and dextrose was prepared to observe the growth of yeast on both plates. Similarly banana peel extract broth medium was prepared in different combinations with YPD medium and inoculated with 24 hrs yeast culture for pigment production. like banana peel, corn steep liquor was subjected to pre-treatment. Again different concentrations of corn steep liquor supernatant was prepared like 25, 50, 75 and 100 ml in order to grow cultures by using residual sugar as a carbon source for the growth. All flasks were supplemented with respective amount of YPD broth to check the efficacy of natural substrates utilized by yeast for the growth, biomass production and pigment formation.

Utilization of dairy waste and sugar industry effluent :

Dairy industry effluent includes mainly whey as a major byproduct. After proper pretreatment whey as well as sugar industry effluents were used in combination for the inoculation of yeast strain and carotenoid production.

After growth of the cells in the respective media absorbance was measured at 620nm on UV-Vis Spectrophotometer (Shimadzu, UV mini 1240) against un-inoculated broth as a control. For the biomass measurement, broth was centrifuged pellet obtained was dried and dry cell mass was expressed as g/100ml and pigment was extracted in dimethyl sulphoxide, dried and expressed as $mg.g^{-1}$.

Prominent carotenoid pigment producing yeast culture used for the present work was *Rhodotorula glutinis* was procured from NCIM, NCL, Pune. Culture was sub-cultured in the laboratory on Sterile Yeast extract, Peptone, Dextrose agar media and in broth. The main objective of the present research work was the evaluation of the naturally occurring cheap substrates as a renewable carbon source for the growth and pigment production by yeast. The utilization of low-cost carbon sources was to reduce the production cost of pigment and to increase the yield of pigment.

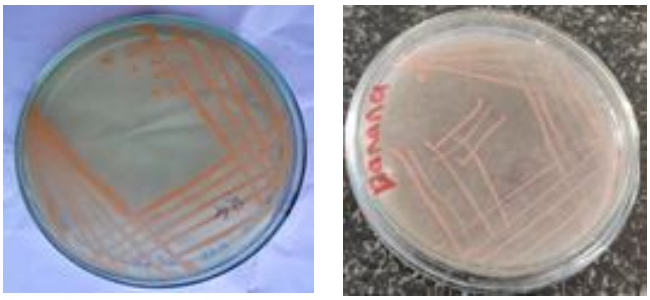

Fig. Growth of Yeast *Rhodotorula glutinis* on YPD medium A and Banana Peel containing medium B

Fruit peel waste is the unused or unconsumed parts of a fruit. Fruit peels contain macronutrients (carbohydrates and proteins) and nutrient sources to grow microorganisms. Present research was conducted for utilization of banana fruit peel for growth of yeast.

Table-1. Varying concentrations of banana peel with YPD medium

S.N.	Banana Peel extract In ml	YPD Broth In ml	Cell dry weight g/100ml	Carotenoid pigment $mg.g^{-1}$
1	25	75	1.05	0.89
2	50	50	1.11	0.96
3	75	25	0.45	0.25
4	100	---	0.23	0.15

Banana peel is rich source of nutrients as well as sugar. Hence an attempt was made to utilize banana peel as an easily available carbon source. Result obtained revealed that maximum carotenoid pigment production was observed medium of 50 % banana peel supernatant with YPD broth of 0.46 mg/g. But further increase in banana peel supernatant no more growth was observed. It was observed that an increasing amount of banana peel concentration results in precipitation of medium and lowers the growth of organism. *Rhodotorula glutinis* could efficiently utilize sugar and nutrients present in medium and could be used as cheap carbon source for pigment accumulation up to 50% concentration.

Production of pigment using corn steep liquor :

Corn steep liquor contains residual starch which can be utilized as carbon source for the growth and pigment production by organism. Corn steep liquor was also subjected to hydrolysis and in various combinations was used for growth of the organism. Results obtained in table 2 reveals that in 75ml of CSL maximum biomass was accumulated and pigment obtained after extraction was 0.58 mg. g^{-1}.

Table-2. Utilization of varying concentrations of corn steep liquor

S.N.	Corn Steep Liquor In ml	YPD Broth In ml	Cell dry weight g/100ml	Carotenoid pigment $mg.g^{-1}$
1	25	75	0.57	0.44
2	50	50	0.76	0.56
3	75	25	0.82	0.58
4	100	---	0.31	0.22

Use of Dairy industry effluent and Sugar industry effluent for growth of yeast :

Dairy industry effluent includes whey mainly which is rich source of lactose can be utilized by yeast cells for growth. Different concentrations of dairy industry effluent and sugar industry effluent which contain sucrose were prepared and inoculated with yeast cells. Results obtained revealed that both effluents were strongly supported the growth of yeast cells.

Table-3. Utilization of dairy industry effluent

S.N.	Dairy industry effluent ml	YPD Broth In ml	Cell dry weight g/100ml	Carotenoid pigment $mg.g^{-1}$
1	25	75	0.82	0.76
2	50	50	0.85	0.80
3	75	25	0.93	0.83
4	100	---	0.97	0.86

Table-4. Utilization of sugar industry effluent

S.N.	Sugar industry effluent In ml	YPD Broth In ml	Cell dry weight g/100ml	Carotenoid pigmentmg. g^{-1}
1	25	75	0.85	0.73
2	50	50	0.92	0.83
3	75	25	0.99	0.90
4	100	---	1.03	0.99

Maximum production observed in 100 ml of sugar industry effluent was 0.99mg. g^{-1} and in 100 ml of dairy industry effluent was 0.86 mg. g^{-1}. Both waste effluent samples were found to produce biomass and carotenoid pigment production. Dairy effluent and sugar industry effluents are agro-industrial waste products which have high nutritive value and can be utilized for pigment production by isolate. Natural pigments extracted from microorganism can be used as environment friendly, biodegradable colors in number of

different fields. Bio-pigments, are eco-friendly and proved additionally propitious as antitoxic, antitumor, antioxidant, anticancer, and antimicrobial agents. Cost-efficient production using agricultural residues for the production of microbial pigments can also be made more convenient to produce on large scale.

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