Faculty of Science

Improvement of Plant Oil Extraction by Highly Safe Microbial Enzyme Cocktails

Thesis

Submitted for Ph.D. Degree in Microbiology

By

Waill Ahmed Mohamed Abd-Elhafez El-Khateeb

B.Sc. Microbiology-Chemistry (1998)

M.Sc. Microbiology (2005)

In Microbiology

Faculty of Science
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Declaration

This Thesis has not previously been submitted for any other university. The references were being checked whenever possible, show the extent to which I have availed myself of the work of other authors.

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كليــة العلـوم

تطوير إستخلاص الزيوت النباتية بإستخدام الكوكتيلات الإنزيمية الميكروبية عالية الأمان

رسالة مقدمة من

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بكالوريوس علوم (١٩٩٨) ميكروبيولوجى / كيمياء

ماجستير العلوم (٢٠٠٥) الميكروبيولوجي

للحصول على درجة دكتوراه الفلسفه فى العلوم

(قسم الميكروبيولوجى)

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كليـة العلـوم

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٢٠١٣

AIM OF THE WORK

The enzymatic cocktails **(Multienzyme system complex, MESC**) especially those containing cellulases, pectinases and hemicellulases are currently known in the world market, due to their high potential biotechnological applications in many fields, which include their uses as very safe extracting agents of oils and starches from plant materials instead of the carcinogenic solvents and chemicals.

The increasing demands of the world market to these influential enzyme cocktails jusify therefore, the need for their production in low costs and through new clean technologies just as the controlled applied biotechnological methods.

The present study aimed at the production of an enzyme cocktail containing active cellulases, pectinases and hemicellulases suitable for jatropha oil extraction through the microbial utilization of some selected abundant agro-lignocellulusic wastes, such as jatropha seed cake and sugar beet pulp, as very cheap and available by-products containing the fundamental substrates inducing the microbial production of the required enzyme cocktail.

The study also extended to bench and pilot-scale application of the most promising precipitated enzyme cocktail fraction in jatropha oil extraction process applying the most proper conditions previously specified.

Objectives of this study:

1- Production of the enzymes used as eco-friendly helping agents in oil extraction from plant sources.

2- Studying the favorable condition for growing the fungal strains to produce cellulase, hemi-cellulase and pectinase enzymes.
3- Studying the enzymes properties.
4- Determination of the produced enzymes efficiency in oil extraction from the different plant sources

1. INTRODUCTION

1.1 Biodiesel and peculiar extraction of jatropha oil

Biodiesel had gained high importance in the recent years for its ability to replace fossil fuels, which are likely to run out within a century. The environmental issues concerned with the exhaust gases emission by the usage of fossil fuels, also encourage the usage of biodiesel which has proved to be eco-friendly more than fossil fuels. Biodiesel is known as a carbon neutral fuel, because the carbon present in the exhaust was originally fixed from the atmosphere. Biodiesel is a mixture of mono-alkyl esters obtained from vegetable oils like jatropha oil, soya bean oil, rape seed oil, palm oil, sunflower oil, corn oil, peanut oil, canola oil and cotton seed oil **(Peterson, 1986)**.

Biodiesel production has received considerable attention in the past as a biodegradable and nonpolluting fuel. The production of biodiesel by transesterification process employing alkali catalyst has been industrially accepted for its high conversion and reaction rates. Recently, enzymatic transesterification has attracted much attention for biodiesel production as it produces high purity product and enables easy separation from the byproduct, glycerol **(Ranganathan *et al.*, 2007)**.

1.2 Enzymatic cocktails and agricultural wastes

Enzymatic biodegradation of the agricultural wastes as lignocellulosic wastes requires multienzyme systems containing cellulases, pectinases and hemicellulases, which act synergistically upon these wastes. Production of the multienzyme systems adequate

for jatropha oil extraction preluding to biodiesel preparation, could be optimally gained through the microbial biodegradation of some abundant and selective lignocellulosic wastes as sugar beet pulp or jatropha seed cake **(Ali 2006)**

1.3 Agricultural wastes and environmental pollution

Agricultural wastes are excess of agricultural products that have not effectively utilized worldwide. Most waste management approaches are methods of concentration and or relocation of wastes, such as source separation, biological waste treatment, incineration or land disposal. Recycling, reprocessing and utilization of the wastes in a positive manner offer the possibility of returning the excesses to beneficial use as opposed to the traditional methods of waste disposal and relation. The keys to successful processes of this mature are a beneficial use, an adequate market and an economical, although not necessarily profit-making process. Many such processes would be satisfactory if they caused the overall cost of waste management to be less than other alternatives. Any additional steps in utilization should repay extra storage, processing and distribution costs that are incurred. A return greater than the extra cost of utilization is desirable in that it reduces the total cost of waste management, but such reduction may be sufficient to result in an overall profit of the producer **(Loehr, 1974)**.

Environmental pollution occurs by numerous factors due to all wastes continuously generated by the agricultural, industrial and municipal segments of the population. Particularly, the increasing amounts of lignocellulosic wastes produced annually overall the world represent

hard environmental pollution loads, as only very little amounts (less than 1%) of these wastes are used for some diminutive purposes such as animal feed, production of energy by burning, some industries, while the rest of these wastes are left in the field to rot **(Loehr, 1974)**.

When these materials accumulate, they present a disposal problem and rank as pollutants, simply by their volume and lack of profitable use **(Meller, 1969).**

1.4 Agricultural wastes in Egypt

In Egypt, vast and increasing amounts of agriculture wastes are produced annually, and except very small quantities used in animal feeding and soil conditioning, these huge amounts of agricultural wastes are almostly not utilized in any way and causing hard environmental impacts, especially after burning. The combined results of the investigation concerning feed from waste will serve as a sound basis for continued technological developments as well as applied research for different products from local wastes. It will help also to ensure that these wastes will be of real value as an outlet for the main products from which they have been derived (**Ministry of Agriculture and Land Reclamation (2010)**

1.5 Sugar beet pulp wastes

In Egypt, sugar beet is considered the second important source following sugar cane for sugar production and the average area cultivated by sugar beet increased. The beet sugar is exclusively produced by four companies' namely Delta sugar company (in Kafr-El-Sheikh), Dakahlia sugar company (in Belkas) El-Fayoum sugar

company (in El-Fayoum) and Abu-Kurkas sugar company (in Abu – Kurkas**), Ministry of Agriculture and Land Reclamation, 2010)**

Sugar beet pulp consists mainly of cellulose, hemicellulose, pectin, protein with little amounts of lipids and some minerals **(Silin, 1967; Silin and silina, 1977).**

The poor crystallinty of the beet cellulose and abundance of amorphous regions beside the easy of hemicellulose fraction for hydrolysis are excellent advantage for beet pulp to be more susceptible for biodegradation by microorganisms. **(Kjaergaard, 1984; Michel *et al*., 1988).**

It is worthy to mention that the high pectin content of beet pulp by-product (~30%) represents a real problem and causes bad digestibility in ruminants as this pectin is highly esterified so that it becomes strongly water binder. **(Rai and Mudgal, 1988; Tanaka *et al.,* 1993).**

In addition, the high content of methyl and acetyl groups within beet pulp pectin causes poor gelling ability **(Mc Cready, 1966)** and this hinders the use of this by-product as raw material for pectin production in low costs.

Accordingly, the above mentioned waste is a good candidate to be utilized for microbial enzyme cocktails production.

1.6 Jatropha seed cake and enzymatic oil extraction

Jatropha seed cake (JSC) is the residue remaining after jatropha seeds oil extraction by hexane. The dried cakes contain holocellulose, pectin, lignin, lipids. Due to the various toxins present in this cake, it is unsuitable as fodder **(Francis *et al.,* 2005)** but may be used as fertilizer

and microbially digested to biogas **(Lopez *et al.*, 1997, Azam *et al.*, 2005; Radhakrishna, 2007 and Tiwari *et al.*, 2007)**. As jatropha seed cake contains mainly cellulose, hemicellulose & pectin, this waste may be utilized for successful microbial enzyme cocktail of cellulases, hemicellulases and pectinases production.

Generally the accumulated amounts of agricultural wastes in Egypt are mostly not utilized in any other way and cause very hard environmental impacts, especially after burning. Accordingly, the very creative methods already proposed for the eco-friendly utilization of such agricultural wastes seemed to be the perfect solution of double benefit accomplishments, the first is the environmental safe by eco-friendly of harmful wastes and the second is the production of a very variable product in low costs, namely the active enzyme cocktail suitable for very effective, cheap and safe oil extraction. The most pronounced method is the biodegradation of these wastes either microbially or enzymatically affording many influential products (Enzymes, proteins, sugars, amino acids and others **(Filemon, 2010)**

2. REVIEW OF LITERATURE

2.1 Petroleum oil problem and fuel deficiency

Biodiesel is a substitute for, or an additive to, diesel fuel that is derived from petroleum oil. Biodiesel is obtained from oils and fats of plants, like jatropha, sunflower or canola. It is an alternative fuel that can be used in diesel engines and provides power similar to conventional diesel fuel. Biodiesel is a renewable domestically produced liquid fuel that can reduce the countries dependence on foreign oil imports and contributes to their economy. Biodiesel is a much cleaner fuel than conventional fossil-fuel petroleum diesel. Biodiesel can be used in any diesel engine without modification and it is better for the environment because it is made from renewable resources and has lower emissions compared to petroleum diesel. It is less toxic than table salt and biodegrades as fast as sugar, **(Filemon, 2010).**

2.2 Utilization of the agricultural wastes

Agricultural wastes are excesses of agricultural production that have not effectively utilized. Agricultural wastes originate from primary agricultural production, plant products such as straw, culls, leaves, press cakes, from intensive farm production (animal and poultry by-products as manure) and from livestock processing plants, by–products of slaughtered animals, tannery **(El Boushy, 1990)**.

Wastes are generated particularly by the agricultural, industrial and municipal segments of the population, nowadays, confrontation with the challenge of the processing and disposal of these byproducts as a result of modern industrialization is taking place. **(El Boushy, 1986)**.

Recycling, reprocessing and utilization of these wastes in a positive manner offers the possibility of returning the excesses to beneficial use as opposed to the traditional methods of waste disposal and relation. The problem of waste disposal and its utilization had been regularly postulated **(Birch *et al*., 1976; El Boushy, 1986; Inglett, 1973; Boda, 1990; El Boushy, 1990)**.

However, other methods of conversion of wastes are possible such as the conversion into feed by ensilation, dehydration, chemical treatment or fermentation to yield enzymes and protein biomass **(Rolfe, 1976)**.

At the present, the fundamental development for agricultural wastes utilization is the controlled biological degradation of the waste by microorganisms (bacteria, fungi and actinomycets), for the production of very valuable compounds as, proteins, polysaccharides, oligosaccharides, vitamins, hormones, enzymes and others as raw materials for high medical and industrial applicabilities.

2.2.1 Sugar beet pulp (SBP)

Sugar beet (*Beta vulgaris* L.) crop is the most important sugar sources overall the world. In Egypt, sugar beet is considered the second crop following sugar cane for sugar production. **(Egyptian Crops Council, 2002)**.

The processing of sugar beet roots results in the production of two more valuable feeds: sugar beet pulp (SBP) and molasses. The latter may be further processed by fermentation to alcohol to yield another potential feed, vinasse.