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Open Access Journal of Pharmaceutical Research
ISSN: 2574-7797

Fungal Enzymatic Cocktails Benefits and Applications

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Review Article

Volume 6 Issue 1

Received Date: December 21, 2021

Published Date: January 31, 2022

DOI: 10.23880/oajpr-16000258

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Abstract

The present review focused on production of enzyme cocktail (Cellulases, hemicellulases and pectinases) suitable for perfect biodiesel extraction process from different oil seeds especially *Jatropha curcus* L. by potent fungal producer utilizing some different abundant Egyptian lignocellulosic wastes. Enzyme cocktails is an eco-friendly process saving human from n-hexane toxicity and carcinogenicity. The enzymatic oil extraction instead of the organic solvent n- hexane is of great interest all over the world.

Keywords: Enzymatic Cocktails; Ecofriendly; Biodiesel; Agriculture wastes

Introduction

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 [1]. 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 [1].

Biodiesel production has received considerable attention in the past as a biodegradable and nonpolluting fuel. The production of biodiesel by trans esterification process employing alkali catalyst has been industrially accepted for its high conversion and reaction rates. Recently, enzymatic trans esterification has attracted much attention for biodiesel production as it produces high purity product and enables easy separation from the byproduct, glycerol [2].

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 [3]. 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 [4].

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 [5].

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 [6].

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 [6].

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 [7].

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 [1].

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). Sugar beet pulp consists mainly of cellulose, hemicellulose, pectin, protein with little amounts of lipids and some minerals. The poor crystallinity 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 [5].

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. In addition, the high content of methyl and acetyl groups within beet pulp pectin causes poor gelling ability, and this hinders the use of this by-product as raw material for pectin production in low costs. Beet pulp by-product waste is a good candidate to be utilized for microbial enzyme cocktails production [5].

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 [8]. 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 [9].

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. 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 [10].

At the present, the fundamental development for agricultural wastes utilization is the controlled biological degradation of the waste by microorganisms (bacteria, fungi and actinomycetes), 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 [11].

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 [1]. 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. Sugar beet pulp is a by-product from the beet sugar industry. SBP has been reported to contain large amounts of cellulose. Cellulose might be converted to useful materials, bio-based chemicals and energy. One possible approach to SBP lingo-cellulose utilization is to hydrolyze the materials into fermentable saccharides, which can then be converted into value added products or bioenergy, as in the to effectively convert lignocellulose into reducing sugars, commercial cellulose enzymes could be used [12].

The commercial enzymatic process however is considered non-economical because the cost of commercial cellulose enzymes remains very high. If cellulase could be produced directly by help of SBP and then be applied to degrade the SBP cellulose, the cost of cellulase could be significantly reduced [13]. Cellulolytic enzyme could be produced by a number of fungi, e.g. *Trichoderma viride* and *Trichoderma ressei*). SBP is almost used as feed for ruminants. The material is rich in polysaccharides, particularly pectin, arabinan, galactan and cellulose. It is a potentially rich source of pectin, as it contains approximately 25% of the dry matter weight anhydrogalacturonic acid [13].

Types of Plant Oils

There are two distinct types of plant oils: (a) fixed oils such as coconut and castor oils, which do not readily evaporate on exposure to air; and (b) essential oils such as citronella and cinnamon oils, which readily evaporate or volatilize on exposure. Fixed oils are usually extracted by crushing and pressure, by boiling, or by chemical solvents. On the other hand, essential oils are almost always extracted by distillation, many of them from flowers such as *ilang-ilang* oil and *sampaguita* oil. Some fixed oils that are liquid at relatively high temperature become solid in ambient and lower temperatures. These fixed oils from plants are the oils of interest as possible replacement for diesel fuel or as diesel fuel extenders, while essential oils are of interest as components in the production of perfumes and other cosmetics and pharmaceuticals. Soybean oil, coconut oil and palm oil are the most widely used plant oils, followed by rape seed oil, sunflower seed oil, peanut or groundnut oil, cotton seed oil, olive oil and jatropha oil. Jatropha oil can be directly used in older diesel engines, planes engines or new big motors running at constant speed [1].

Jatropha Curcas L. Plant

Jatropha curcas L. is a tropical plant widely distributed in arid areas. The seeds contain about 55% oil, which is mainly used for the production of soap, as a fuel and after transesterification as biodiesel. Various methods for recovering of oil from the seeds, including extraction with organic solvents and water, have been investigated. Compared to hexane extraction (98%), the oil extraction using water only yielded 38% of the total oil content of the seeds. Using several cell wall degrading enzymes during aqueous extraction a maximum yield of 86% was obtained. *Jatropha curcas* L. is a drought resistant tropical tree and the oil from its seeds has been found useful for medicinal and veterinary purposes, as insecticide, for soap production and as a fuel substitute [14].

The interest in using *Jatropha curcas* L. as a feedstock for the production of bio-diesel is rapidly growing. The oil produced by this crop can be easily converted to liquid bio-fuel, which meets the American and European standards. Additionally, the press cake can be used as a fertilizer and the organic waste products can be digested to produce biogas (CH_4) [15-17].

Jatropha curcas in Egypt

Jatropha curcas L it is generally cultivated in tropics and subtropics and likes heat, although it does well even in lower temperatures. Jatropha trees grow in wide ranges of different environments, climatic conditions and soil types. Jatropha trees grow almost anywhere even on gravelly, sandy and saline soils. Its water requirement is extremely low and it can stand long periods of drought by shedding most of its leaves to reduce transpiration loss. Jatropha seeds were imported from India and cultivated in the southern part of Egypt. It's cultivation in Egypt started in Luxor and Qena governorates, also in El-Gabal El-Asfar, Kalubia governorate. It is worth mentioning that the cultivation irrigated by treated sewage. It's cultivation in desert soil does not make any competition with other food crops and also it decreases the environmental pollution [1].

Oil Extraction from *Jatropha curcas* Seeds

Jatropha seeds contain considerable amounts of oil. The oil contained in the seeds has to be extracted. The main inputs for this process, besides the seeds, are the production and use of machines, infrastructure and energy. On the output side, the main products are the *Jatropha curca*s L. oil and the seed or kernel cake, which is an important by-product. For extraction of the *Jatropha curcas* L. oil two main methods have been identified (i) mechanical extraction (e.g. by pressing) and (ii) chemical extraction (e.g. by solvents or by enzymes) prior to oil extraction the *Jatropha curcas* seeds have to be dried. Seeds can be dried in the oven (105°C) or sun dried (3 weeks) [18,19].

Based on the extraction efficiencies discussed and the average oil content of the whole seed (34.4% on a mass basis) and the seed cake will contain 9-12% oil by weight. This content will of course influence the gross energy value of this cake as well. This cake contains various toxins and is therefore not usable as fodder. Also, the average crude protein content of the seed cake is 58.1% by weight in case of mechanical oil extraction from whole seeds, the oil content of the seed cake will be higher, due to the lower efficiency of the expellers [20]. However the raw kernel or seed cake can be valuable as organic nutrient source, as it contains more nutrients than both chicken and cattle manure. Jatropha seed cake is useful as fertilizer [20]. The cake can serve as feed for biogas production through anaerobic digestion before using it as a soil amendment as well also, obtained, 446 m^3 of biogas, containing 70% CH_4, per kg of dry seed press cake using pig manure as inoculum were reported [21].

Jatropha curcas L. Oil Importance and Application

Jatropha oil had various uses. Apart from its use as a liquid fuel, the oil has been used to produce soap and biocides (insecticide, molluscicide, fungicide and nematicide), The oil can be directly used in older diesel engines or new big motors running at constant speed (ex. pumps, generator). Also, the oil can be transesterified into ethyl esters that can be used in conventional diesel engines [22].

Jatropha curcas L. seed cake as example of agriculture west and enzymatic oil extraction

Jatropha seed cake 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 but may be used as fertilizer and microbially digested to biogas [1,16,17,20]. As jatropha seed cake contains mainly cellulose, hemicellulose and 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) [1,4].

The enzymatic cocktails (Multienzyme systems complex, MESC) especially those containing cellulases, pectinases and hemicellulases are currently required due to their high potential biotechnological applications in many fields, which include their use as a new eco-friendly technique in the biodegradation of agricultural wastes and as a very safe extracting agent of oils and starches from plant materials. The need for the production of these enzymatic cocktails is due to their low cost, high safety and the fact that it represents new clean technology. Elkhateeb, reported that production of the enzyme cocktail, containing active cellulases, pectinases and hemicellulases by some locally isolated fungi through microbial utilization of agro lignocelluloses wastes such as jatropha seed cake and sugar beet pulp as a very cheap and available by-product containing the fundamental substrates inducing the required enzymes cocktail production followed by the optimization of their production through the study of the effect of different factors and finally the use of the produced enzyme cocktail in an application for the optimum extraction of oil from plant materials [1].

Oil Extraction Processing

The industrial method of oil extraction takes place in many steps. Figure: 1; Represents the process of seed oil extraction to obtain edible and non-edible oil (Biodiesel oil).

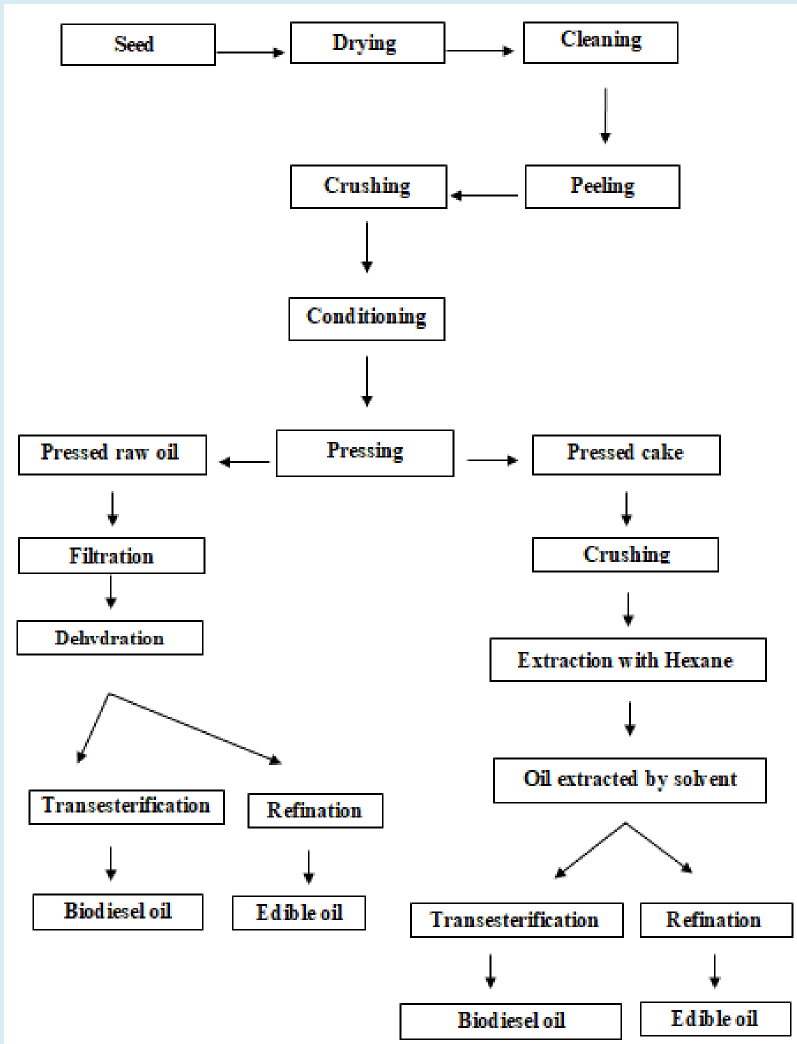

Figure 1: Seed oil extraction processing [1].

Vegetable Oils Extraction by Cocktail Enzymes

Enzyme based process for edible oil extraction is considered as an environmentally clean technology. The uses of enzymes for oil extraction have been reviewed by Elkhateeb [1]. The enzymatic process mainly hydrolyzes the structural polysaccharides, forming the cell wall of oil seeds or the proteins, which form the cell and lipid body membrane. This technology has been developed in bench scale to extract oil from many vegetable raw materials such as rapeseed, soybean, peanut, sesame, rice bran corn germ, palm, sun flower, avocado and coconut [23].

The use of hexane, generally employed for oilseed extraction is being questioned because of its toxicity and flammability involvement of high temperatures during hexane- extraction may result undesirable effect on the quality of extracted oil due to oxidative deterioration of polyunsaturated fatty acid and development of rancid and off flavors for this reason. There is considerable interest in seeking suitable alternatives to hexane for oil seed extraction [24].

Oil form peanuts are conventionally extracted by either mechanical pressing or solvent extraction. Mechanical pressing is a less efficient process, leading to low oil recovery (40- 60%). Solvent extraction although its recovery is in the 90- 98% range, has inherent disadvantages of poor quality of protein in oil cake (meal), and high investment, and energy requirements. The commercial hexane used as the most common solvent for oil extraction is listed among hazardous air pollutants associated with neurological and respiratory disorders on prolonged exposure. Hence, there is a need to explore alternative safe and efficient oil extraction processes that may also result in edible protein [25].

The pectinolytic enzymes alone account for about one- quarter of the world's food enzyme production by their degradative effect, pectinolytic enzymes became an indispensable part of fruit juice processing and oil process. They improve the extraction yield by degrading cell walls, enabling a more efficient liberation of various metabolites [26]. Aqueous enzymatic oil extraction is one such alternative eco-friendly process based on simultaneous isolation of oil and protein from oil seed by dispersing finely ground seed in water and separating the dispersion by centrifugation into oil, solid and aqueous phases. The presence of certain enzymes during extraction enhances oil recovery by breaking cell walls and oil bodies [26].

The wall of plant cells is composed of pectin and cellulose, which constitute the most abundant polymer's in nature. Many microorganisms are able to degrade these polymers in simple sugars by secreting enzymes such cellulases and pectinases. Pectinolytic enzymes are widely produced in fungi like *Trichoderma, Penicillium* and *Aspergillus* genera [26]. Oils from rape seed (canola), coconut germ, sun-flower seed, palm, kernel and olives are traditionally produced by extraction with organic solvents. The most commonly used solvent is hexane which is a potential carcinogen. Cell wall degrading enzymes, including pectinases, may be used to extract vegetable oil in an aqueous process by liquefying the structural cell wall components of the oil- containing crop [27].

Olivex, is an enzyme preparation derived from *Aspergillus aculeate* that contains Pectinolytic activity besides hemicellulases and cellulolytic activity has been shown to give good oil extraction and better stability when stored. The oil also shows increased content of polyphones and vitamin E, which stabilize the oil against rancidity. Treatments with a crude enzyme with both cellulase and a hemicellulase activities, only or in combination with a protease and a pectinases, all resulted in higher palm kernel oil yield than control treatments optimum yield occurred when a protease, a pectinases and the cellulase/ hemicellulase enzymes were combined, each at 1% concentration. Yield was influenced by the pH of the meal suspension and the meal/ water ratio during the enzyme treatment [1].

Aqueous enzymatic oil extraction is undoubtedly an emerging technology in the fats and oil industry since it offers many and vantages compared to conventional extraction for instance, it eliminates solvent consumption which reportedly may also lower investment costs and energy requirements. Also it enables simultaneous recovery of oil and protein from most oil seeds and the process yields oil of good quality complying with codex specifications. These enzymes formulations not only degrade the walls of oil bearing cells but also break the colloidal system in olive past thereby maximum oil and also improve the oil quality [28]. Cell wall degrading enzymes can be used to extract oil by solubilizing the structural cell wall components of the oil seed. This concept has already been commercialized for the production of olive oil and has also been investigated for other oil- bearing materials [29].

The plant cell wall degrading enzyme preparation has begun to be used in olive oil preparation. The enzymes are added during grinding of the olives, there by the oil is released easily in the subsequent separation techniques. Enzyme treatment hence causes an increase in yield of olive oil. The increase in the yield depends upon pH, temperature, and dosage of the enzyme used. The enzymes present in olive fruit are in general deactivated during the extraction process or crushing step, thus exogenous enzymes need to be added to the olive past during the mixing step to replace the deactivated enzymes. In terms of applications, it is becoming increasing popular for culinary, pharmaceutical, cosmetics, and medical purposes [30].

Sharma and Sharma, reported that combination (1:1) of pectinases and cellulose (0.05%, w/v) resulted in maximum oil recovery and minimum loss of oil in olive cake compared to individual enzymes pectinases, cellulases and hemicellulases (PCH) even at higher concentrations [31]. The obtained oil with enzymatic treatments had relatively higher natural antioxidants (total phenols), slightly higher oil clarity, and lower free fatty acids and peroxides. Enzymatic pretreatments significantly improved oil recovery and did not exert any adverse effect on the nutritional and pharmaceutical quality of the oil.

The olive fruit contains about 50% water, 20% oil, 20% carbohydrates (pectic, cellulosic and hemicellulosic substances) and 10% organic acids, pigments, phenolic compounds and minerals. The common methods of olive oil extraction include physical or mechanical processes, chemical procedures or a combination of both. During the conventional oil extraction processes, some of the oil not extracted and remains in the solid residue. Several methods have been proposed improving oil extraction procedures including enzymatic pretreatment. In order to effectively recover oil enclosed in the cell, the cell wall must be destroyed. This may be done by enzymes specific to the breakdown of the individual types of polysaccharides in the cell wall structure. The cell wall of plants consists mainly of pectic substances, cellulose, hemicellulose and lignin [32,33].

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