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## Review

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# An overview of the enzyme potential in bioenergy-producing biorefineries

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## ABSTRACT

Biorefineries are considered as an integrative thinking that focuses on the possibility of obtaining as many added-value products as technically and economically feasible. However, in practice most biorefineries comprise only enzymatic or chemical pretreatment followed by biofuel generation. The drop in oil prices may menace the development of this young industry, as has happened before in history. This has become a fundamental reason for which the biofuel industry should not consider only biofuels production, but enzyme and non-fuel based chemicals as well. Hence, this work aims at overviewing the most important enzymes involved in biotechnological processes and to describe their role in biorefineries. Bioethanol, biogas and biodiesel biorefineries are overviewed, along with the integrated and industrial types. Finally separation and purification processes in biorefineries are discussed.

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**Keywords:** biofuel; cell-surface display; inverse-cascading; integrative biorefinery; ionic liquids; organic wastes; non-fuel bio-based chemicals

# Perspectives on the utilization of waste fat from beef cattle and fowl for biodiesel production in Mexico

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## Abstract

The purpose of this research was to perform a preliminary inventory of the waste fat generated from beef cattle and fowl in Mexico that could be used for biodiesel production. Additionally, the CO<sub>2</sub> emissions reduction that could be achieved by using the potential biodiesel to replace an energy-equivalent amount of fossil diesel was assessed. Based on national reports for the year 2014, it was estimated that the non-edible fat from beef cattle and fowl annually generated in Mexico is sufficient to produce 216.0 kt of biodiesel, which would furnish 8379 TJ. This amount is equivalent to 1.5% of the energy annually consumed in Mexico as fossil diesel for road transport. The potential 216.0 kt of animal fat-based biodiesel that can be produced annually could replace 198.3 kt of fossil diesel and thus allow a reduction in the WTW (well to wheels) emissions of 592.3 kt CO<sub>2</sub>, which represents 1.5% of the WTW CO<sub>2</sub> emitted from the combustion of the fossil diesel used for road transportation in Mexico in the year 2014.

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**Keywords:** animal fat; inventory; biodiesel; CO<sub>2</sub> emissions; Mexico

# Volumetric oxygen transfer coefficient as a means of improving volumetric ethanol productivity and a criterion for scaling up ethanol production with *Escherichia coli*

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## Abstract

**BACKGROUND:** This study evaluated the influence of different micro-aerated conditions, including the aeration rate, the volumetric oxygen transfer coefficient ( $k_L a$ ) and the oxygen transfer rate (OTR), on improving ethanol productivity and scale-up of the fermentation step of the ethanologenic *Escherichia coli* strain MS04 in mineral medium supplemented with xylose, glucose, and sodium acetate.

**RESULTS:** Growth and ethanol production results using 0.75 L of fermenter showed that micro-aeration (0.1 vvm, 400 rpm) improved the volumetric ethanol productivity and sugar consumption rate compared with the anaerobic condition (0 vvm, 400 rpm) or higher aeration rates (>0.2 vvm) without reducing significantly the ethanol yield. The  $k_L a$  and the OTR were estimated and a  $k_L a$  value of  $7.2 \text{ h}^{-1}$  was used as a criterion to scale-up the fermentation process from 0.75 L to 9.16 L and 110 L. During scale-up, the volumetric ethanol productivity and ethanol yield of consumed sugars were maintained at similar levels to those obtained in the laboratory in the 0.75 L fermenter.

**CONCLUSIONS:** The controlled supply of low levels of oxygen promoted an increase in the concentration of biomass favoring the production and volumetric productivity of ethanol. The use of  $k_L a$  allowed the fermentation step to be scaled up with ethanologenic *E. coli* maintaining similar ethanol yields and productivities.

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**Keywords:** *Escherichia coli*; glucose; scale-up; volumetric ethanol productivity; volumetric oxygen transfer coefficient; xylose

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# Metabolic engineering and adaptive evolution of *Escherichia coli* KO11 for ethanol production through the Entner–Doudoroff and the pentose phosphate pathways

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## Abstract

**BACKGROUND:** Ethanologenic *Escherichia coli* KO11 was modified to channel carbon flux from glucose through the Entner–Doudoroff (ED-P) and pentose phosphate (PP-P) pathways by using a phosphoglucose isomerase (*pgi*) knockout in the glycolytic pathway. This strain grows very slowly under non-aerated conditions with minimal media supplemented with 4% glucose. To improve the capacity to grow, KO11  $\Delta$ *pgi* was evolved for 60 days; the resultant strain was named KO11 E35, which directs the carbon flux through the PP-P and ED-P to lactate and acetate production.

**RESULTS:** The activities of glucose-6-phosphate dehydrogenase and the ED-P enzymes increased 17-fold and 2-fold, respectively, in KO11 E35 in comparison with KO11. A homoethanologenic derivative was constructed from KO11 E35 by deleting the *pta*, *ack* and *ldh* genes, yielding the KO11 PPAL strain. This strain channels most of the carbon flux from pyruvate to ethanol and increased expression of heterologous pyruvate decarboxylase and alcohol dehydrogenase from *Zymomonas mobilis* allowed us to obtain specific ethanol production rates similar to those found in KO11, but with half the cell mass, i.e. larger ethanol/glucose and ethanol/biomass yields.

**CONCLUSIONS:** These results suggest that it is possible to obtain the same carbon flux using the PP-P and ED-P as when using the Embden–Meyerhof–Parnas pathway for glucose catabolism to ethanol.

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**Keywords:** ethanologenic *Escherichia coli*; glucose; ethanol; pentose phosphate pathway; Entner–Doudoroff pathway; adaptive evolution

## Review

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# Heterotrophic cultivation of microalgae: production of metabolites of commercial interest

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## Abstract

Several microalgal species are capable of growing heterotrophically, exhibiting considerable metabolic versatility and flexibility. As demonstrated in this review, heterotrophic conditions can enhance the biomass concentration by as much as 25-fold compared with phototrophic conditions. Currently, these types of cultivation are economically feasible only for high-value products, including polyunsaturated fatty acids (PUFAs), pigments, antioxidants, polysaccharides, food and aquaculture feed from carbon sources, such as glucose, acetate or glycerol. To make heterotrophic cultivation economically viable for high-volume, low-value commodities, such as biofuels, the use of unconventional carbon sources, such as food and agricultural wastes and wastewater, is recommended. Since microalgae are capable of modifying their metabolism according to varying culture conditions, it is possible to modify, control and therefore maximize the production of target compounds. This manuscript not only offers a review of the most relevant and recent findings in the use of heterotrophic microalgal cultivation for enhanced metabolite production but also provides recommendations for future research on this promising subject.

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**Keywords:** microalgae; heterotrophic growth; carbon sources; lipids; polyunsaturated fatty acids; pigments; biodiesel



# An economic model for estimating the viability of biodiesel production from *Jatropha curcas* L.

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## Abstract

**BACKGROUND:** At commercial level, the biodiesel production process is well established for many types of feedstock. However, economic feasibility depends on regional fluctuating data, making each case unique. A calculation model to analyze the economic feasibility of biodiesel production from *Jatropha curcas* was developed, along with an analysis of the energetic balance derived from this process.

**RESULTS:** Yucatán state has the cultivation surface to replace 10% of its fossil diesel fuel consumption with biodiesel. Two scenarios were studied: the use of biodiesel–fossil diesel blends B5 and B10. The net energy ratio (NER) of biodiesel production is 2.88, indicating that the system provides more energy than it consumes. The economic analysis indicates that biodiesel cost remains constant with production capacities of 10 000 m<sup>3</sup> year<sup>-1</sup> and higher. Field labor, pesticides, and fertilizers are the major costs at *J. curcas* plantations, representing 64.3, 16.3 and 11.5% of total biodiesel cost, respectively. The net present value (NPV) was always negative, proving that the biodiesel–jatropha chain is not economically viable.

**CONCLUSIONS:** Actual seed productivity (1 495 kg ha<sup>-1</sup> year<sup>-1</sup>) must be increased 2.17 times to attain economic viability. Agricultural practices need to be enhanced in order to lower labor cost and the use of fertilizers.

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Supporting information may be found in the online version of this article.

**Keywords:** renewable energy; biofuels; net energy balance; seed productivity; transesterification



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# A review of the potential of pretreated solids to improve gas biofuels production in the context of an OFMSW biorefinery

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## Abstract

The organic fraction of municipal solid waste (OFMSW), mainly composed of lignocellulosic polymers, is extremely complex. Therefore, it is necessary to apply pretreatments to remove the lignin content and decrease the cellulose crystallinity in order to use the OFMSW for gas biofuels production in the context of biorefineries from waste. This work focused on critically reviewing the conventional pretreatments applied to OFMSW, with the goal of improving the H<sub>2</sub> production, as well as other biofuels in modern biorefineries. There are a wide variety of pretreatments that have successfully been used, mainly alkaline, milling and dilute acid. In addition, some research has focused on the recovery and reutilization of the alkali, acid or solvents after the pretreatment, to be incorporated into new cycles of production, minimizing the environmental impacts. Moreover, it would be necessary to incorporate analytical tools, in order to determine the sustainability of the biorefinery project. It is concluded that waste pretreatments could significantly contribute to increased yields of biogas fuels in organic waste-based biorefineries. Therefore, establishing preliminary stages for conditioning biomass or wastes is essential to improve the degradation of wastes and bio-product generation.

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*Supporting information may be found in the online version of this article.*

**Keywords:** biohydrogen; biorefinery; cellulose; lignin; methane; OFMSW; organic wastes; pretreatment



# Control properties of hybrid distillation processes for the separation of biobutanol

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## Abstract

**BACKGROUND:** Butanol produced from fermentation has attracted the interest of research groups because its physicochemical properties show several enhancements over bioethanol. Recent studies have proposed alternative methods to separate and purify biobutanol from a fermentation broth. These alternatives offer energy and economic savings; in addition, a reduction in environmental impact is observed. However few studies have analyzed the control properties of the process which involves separation of an acetone–butanol–ethanol (ABE) mixture.

**RESULTS:** A controllability analysis using the singular value decomposition technique and a closed-loop dynamic analysis was performed on several hybrid distillation processes including conventional, thermally coupled, thermodynamically equivalent and intensified designs. The results indicated that under the closed-loop control policy, an intensified design which is integrated for only two distillation columns instead of three distillation columns, showed good dynamic properties. In addition, thermally coupled sequence A showed better control properties under open-loop analysis.

**CONCLUSIONS:** Using both SVD analysis and closed-loop tests the dynamics properties were obtained for several hybrid processes to separate an effluent produced by fermentation. It was possible to control all schemes under both methodologies and it was clear that when the base case became more complex with thermal coupling, section movement or elimination of a column section improved the control properties.

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**Keywords:** control properties; thermal couplings; ABE fermentation; biobutanol

