*Chapter 4*

# THE PAUPER AND THE PRINCE: GLYCEROL IN A VIEW FROM BIOFUELS AND BIOREFINERIES

*Alejandro J. Beccaria[1], Alberto A. Iglesias[1] and Raúl A. Comelli*[\*,2]*

[1]Instituto de Agrobiotecnología del Litoral – IAL (UNL-CONICET).
FBCB, Santa Fe, Argentina
[2]Instituto de Investigaciones en Católisis y Petroquímica – INCAPE
(FIQ-UNL, CONICET), Santa Fe, Argentina

## 1. INTRODUCTION

Glycerol (1,2,3-propanetriol, abbrev. Gro), a sweet-tasting alcohol, was discovered in the 18th century (1779) by the Swedish chemist C.W. Scheele, who isolated the poly-alcohol after heating olive oil and litharge. The chemical is also known as glycerin or glycerine. It is abundant in nature after being a component of many lipids and a main compatible solute produced by cells for osmoregulation, to manage water activity variations in the medium. Also, many microorganisms are able to use glycerin as a source for carbon and energy. Main physicochemical properties of Gro can be summarized by being a colorless, odorless, highly hygroscopic and viscous liquid having a boiling point at 290 ºC, a specific gravity of 1.26 and a molecular weight of 92.09 (Pagliaro and Rossi, 2008). It forms crystals at low temperatures that tend to melt at 17.9 ºC. All its characteristics make of Gro a compound of utility for inclusion in different industrial processes and/or for being a key constituent in several chemical preparations. Without trying to make a detailed and complete listing of its applications, chief uses of the polyol include:

– Gro is extensively used in the pharmaceutical and cosmetic industries taken advantage of its emollient, humectant and demulcent properties (Pagliaro and Rossi,

---

[\*] Correspondence to: Prof. Raúl A. Comelli, INCAPE, Instituto de Investigaciones en Católisis y Petroquímica,
(FIQ-UNL, CONICET), Santiago del Estero 2654, S3000AOJ – Santa Fe, Argentina, Telephone 54 0342
4571164 (int. 2739), Fax +54-342 4531068, Email: rcomelli@fiq.unl.edu.ar.

2008). Also, its capability as a solvent (alone or as stabilizer in combination with water and alcohols) for preparation of different formulations and elixirs carrying active ingredients for variable purposes going from tinctures to key agents benign for specific treatments (i.e. theophyllin and xanthine derivatives with clinical applications for respiratory diseases).

– Food industry has Gro as a key compound of many utilities: preservative moistening agent for baked goods, preservative for juices, anti-crystallization mediator in candies, and solvent of chemicals serving as carriers of color and flavors (Pagliaro and Rossi, 2008).

– Gro is used as a plasticizer of different polysaccharides, such as starches (Qiao et al., 2011) or chitosan (Epure et al., 2011), this application being decidedly relevant for the production of biodegradable polymers with improved properties as plastic-like compounds.

– Gro conversion into nitroglycerin serves in contrasting applications (Pagliaro and Rossi, 2008). Thus, the nitro-derivative is a main component in production of dynamites and explosives; although it also has a laudable use as an active component in medication for heart disease, since nitroglycerin has the capacity of generate nitric oxide (Agvald et al., 2002).

Although the many faceted uses and applications for Gro, its market value is in down-hill, principally because the rapid expansion of biodiesel production that generated a glut of the polyol (Johnson and Taconi, 2007). Biodiesel is produced from triacylglycerides contained in animal fats or vegetable oils through transesterification with low molecular weight alcohols (mainly methanol or ethanol). The process generates methyl-(or ethyl-) esters of fatty acids as the major biodiesel product and about 10% (v/v) of Gro as a by-product. This is not only converting the polyol in a low value chemical; but it is further creating an environmental problem because of the limited possibility for disposal, which is also quite expensive (da Silva et al., 2009). The situation is obligating to revisit current uses and the design of new processes for adding value to the propanetriol. For the latter, overall possibilities include the finding for new applications of the chemical as well as its conversion to other molecules serving in different industry systems, in a way improving competitiveness in the productive chain of the biodiesel business.

Paradoxical to the relatively pauper state of affairs for Gro, it can be visualized as a key compound for developing processes in the emerging field of biorefineries. Limited reserves of petroleum and the associated dramatic increases in its price, together with critical environmental concerns and climate changes provoked by the use of fossil fuels are creating obligatory demands for generate renewable fuels as well as for reconvert industrial processes. The necessity is to make them more ecology-friendly and compatible with a sustainable environment. This leads to the concept of biorefinery, which embraces different technologies allowing convert biomass into materials, chemicals and energy (Ohara, 2003; Kamm and Kamm, 2004, 2007; Schell et al., 2008; Amidon and Liu, 2009). In this framework, the positioning of Gro as a by-product in a process to obtain biofuel that needs to be revalorized is relevant and it represents a fair challenge for the development of biorefinery tools. In the present work we analyze current and potential ways to valorize glycerol. Globally, we review

chemical as well as biological approaches and we analyze possibilities for combine strategies to develop biorefinery-like solutions.

## 2. CHEMICAL APPROACHES TO VALORIZE GLYCEROL

Different alternatives for valorize Gro have been proposed from the chemical field. Figure 1 illustrates on the different compounds that can be obtained from the polyol by utilizing chemical strategies. It is important to consider that they are not the only ones. Then, Gro can be a versatile raw material and plays an important role as a key compound in the environment of the future biorefinery.
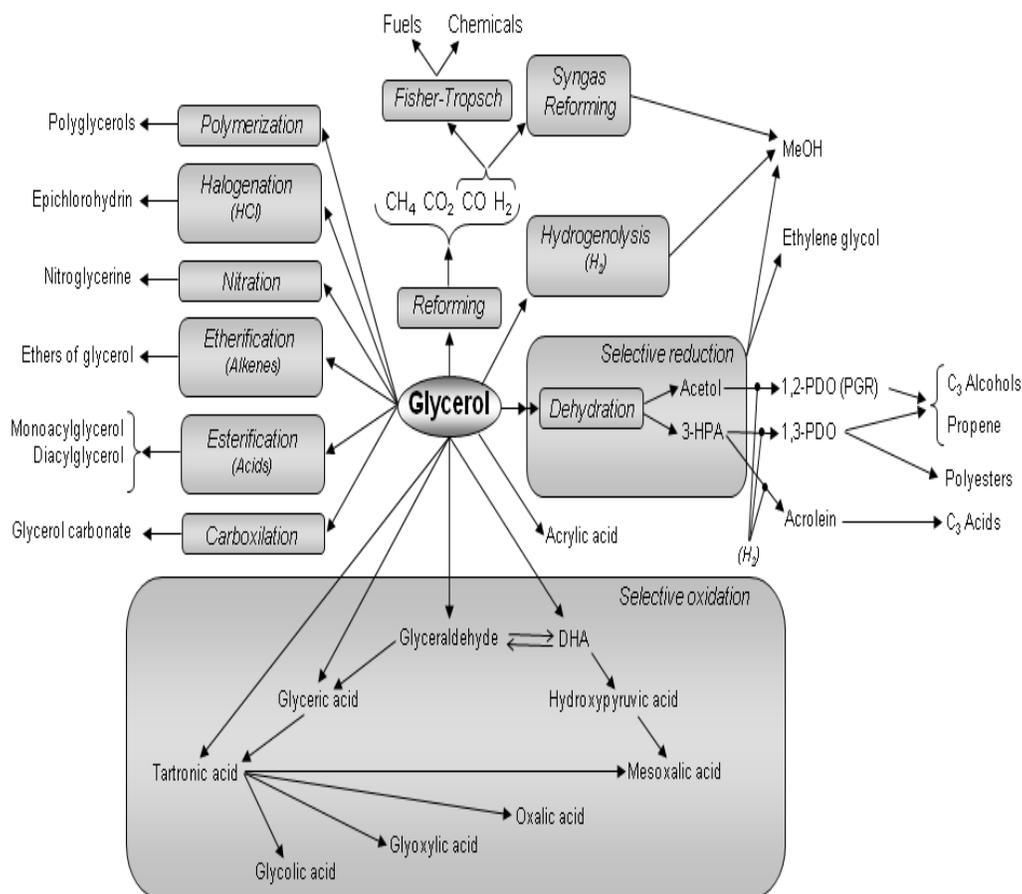


Figure 1. Glycerol valorization by catalytic ways.

## 2.1. Dehydration and Oxyhydration Reactions

Dehydration of Gro can directly produce important chemicals such as 3-hydroxy-propionaldehyde (3-HPA), monohydroxyacetone (acetol), and acrolein. 3-HPA is a precursor

for 1,3-propanediol (1,3-PDO), acrolein, and acrylic acid, and used for polymer production; it also exhibits antimicrobial activity towards a wide range of pathogens and food spoilage organisms, and it finds use both as a food preservative and as a therapeutic auxiliary in the pharmaceutical industry. Currently 3-HPA is produced by synthesis from petrochemicals; Degussa and Shell chemical processes produce it as an intermediate in the formation of 1,3-PDO. The Degussa catalytic process transforms propylene into acrolein, which is hydrated to 3-HPA. The Shell process transforms ethylene into ethylene oxide, which is converted to 3-HPA by a hydroformylation reaction with syngas at 150 bar pressure; ethylene is inexpensive and intermediate products are not toxic, but 3-HPA has to be recovered from the organic phase. More recently, dehydration of Gro to 3-HPA which is latter hydrogenated to 1,3-PDO was suggested by Miyazawa et al. (2006).

Dasari et al. (2005) proposed that Gro is dehydrated to acetol which is then hydrogenated to 1,2-propanediol (1,2-PDO). Metal supported catalysts, especially Ru/C, combined with a strong solid acid as an Amberlyst ion-exchange resin, were active in the hydrogenolysis of Gro to 1,2-PDO (Kusunoki et al., 2005); the ion-exchange resin was responsible to produce acetol, which is an intermediate product (Miyazawa et al., 2006).

Acrolein is an important intermediate for the chemical industry; it can be polymerized to acrylic resins and forms the basis of superabsorbent polymers, widely employed in the baby hygiene market. Acrolein is currently produced by the oxidation of propylene, however, the increase of propylene price making commercially attractive its production from Gro. A hybrid process coupling the oxidation of propylene with the dehydration of Gro is conduced by Arkema.

By considering a one-step oxydehydration of Gro in the presence of molecular oxygen and at atmospheric pressure, the commercially important acrylic acid can be produced; the dehydration reaction to acrolein is followed by the direct oxidation to acrylic acid. Thermal balance of the overall process is improved coupling both exothermic oxidation and endothermic dehydration reactions. The reactor contains two catalytic beds, a first one of tungstated zirconia, and a second one of a mixed W–Sr–V–Cu–Mo oxidation catalyst with acetic acid as binder. At 280 °C and feeding a 20wt% Gro aqueous solution, conversion was complete while acrylic acid reached 74% yield.

## 2.2. Selective Reduction Reactions

The selective reduction of Gro through the hydrogenolysis reaction allows to obtain compounds with added value, such as 1,2-PDO, ethylene glycol (EG), and 1,3-PDO. 1,2-PDO, also known as propyleneglycol, is a major commodity chemical traditionally derived from propylene oxide, with applications in medicines, cosmetics, food, liquid detergents, tobacco humectants, flavors and fragrances, personal care products, paints, and animal feedstuffs, being also used as functional fluids (antifreeze, deicing fluid, and heat transfer liquids), and a solvent for the production of unsaturated polyester resins (Corma Canos et al., 2007). The selective production of 1,2-PDO by hydrogenolysis in liquid phase has been reported using catalysts such as Raney Ni, Ru/C, Pt/C, Ni/C, and copper chromite, which reached the best performance. That hydrogenolysis reaction was evaluated on catalysts containing Ru, Cu, Pt, Ni, Co, Rh, and Re, following catalytic performance the order Cu ≈ Ni

$\approx$ Ru > Pt > Pd (Roy et al., 2010). Pt-Ru/C is more stable, being Au-Ru/C altered by the harsh operating conditions (Maris et al., 2007). Using Ru-containing catalysts, the type of support (TiO$_2$, SiO$_2$, NaY, $\gamma$-Al$_2$O$_3$, and activated carbon) can influence the metal particle size and the reaction pathways (Feng et al., 2008). Ma et al. (2008) studied the promoting effect of Re on Ru impregnated on SiO$_2$, ZrO$_2$, Al$_2$O$_3$, C, and ZSM-5. Vasiliadou et al. (2009) reported the effect of both support and Ru precursor, obtaining a linear relationship between the total acidity of catalyst and the activity in hydrogenolysis. Lahr and Shanks (2005) increasing sulphur loading on Ru/C during the hydrogenolysis at high pH, increased the selectivity to 1,2-PDO, without modifying the selectivity to EG. Balaraju et al. (2009, 2010) reported Nb$_2$O$_5$ and TPA/ZrO$_2$ (TPA: 12-phosphotungstic acid) having moderate acid sites as the most active co-catalysts combined with Ru/C, influencing the amount of acidity in the acid solid its catalytic activity; the effect of preparation conditions of Ru/TiO$_2$ on the behavior during the hydrogenolysis of Gro was also studied (Balaraju et al., 2010). Roy et al. (2010) used a mixture of Ru/Al$_2$O$_3$ and Pt/Al$_2$O$_3$ to produce 1,2-PDO from Gro without added external hydrogen. Unfortunately, Ru catalysts promote an excessive breakage of C-C bonds decreasing the selectivity to 1,2-PDO. Copper-containing catalysts have a poor activity in the rupture of C-C bonds and a high efficiency for the hydrogenating/dehydrogenating of C-O bonds, then they were proposed as an alternative material (Huang et al., 2009). Commercial copper chromite and samples prepared by impregnation and co-precipitation, have been reported as efficient catalysts to produce 1,2-PDO. Cu/ZnO (Wang and Liu, 2007), Cu/ZnO/Al$_2$O$_3$ (Meher et al., 2009), Cu/Al$_2$O$_3$ (Akiyama et al., 2009; Guo et al., 2009), Cu/SiO$_2$ (Huang et al., 2009), and Cu/MgO (Yuan et al., 2010) were suitable materials for hydrogenolysis, while Cu supported on zeolites such as HY, 13X, H-ZSM5, and H$\beta$ did not produce 1,2-PDO from Gro (Guo et al., 2009). Catalytic behavior of copper chromite without and with barium and Cu/H-FER (being FER: ferrierite zeolite), Cu/K-FER, and Ni/13X, was measured in the hydrogenolysis of Gro in gas phase at 200ºC and atmospheric pressure (Comelli, 2011). Copper chromite stabilized with barium was the most active catalyst, reaching 83.0% conversion while both copper chromite samples were the most efficient in selectivity to acetol and 1,2-PDO; sample without barium was the most selective to acetol (67.8%), while the one with barium improved the selectivity to 1,2-PDO up to 29.7%. Cu/H-FER was more active than Cu/K-FER, which was more selective to acetol and 1,2-PDO. Ni/13X was very active, 79.9% conversion, but few selective to acetol and 1,2-PDO; methane was the only product detected in the gas stream while EG was the major one in the condensed fraction.

1,3-PDO is used as a solvent for the production of adhesives, laminates, and paints, and in coolant formulations, although it can have the same applications as EG, 1,2-PDO, 1,3-butanediol, and 1,4-butanediol. Nevertheless, its use as monomer in polycondensation reactions concentrates attention to produce polyesters, polyethers, and polyurethanes; 1,3-PDO copolymerized with acid produces polytrimethylene-terephthalate (PTT) polymers, recognized for their excellent elastic properties, and marketed with the commercial name Corterra™ and Sorona®, by Shell Chemical Company and DuPont, respectively. Both companies produce the largest commercial amount of 1,3-PDO, DuPont hydrating acrolein to 3-HPA followed by hydrogenation to 1,3-PDO, while Shell produces it by hydroformylation of ethylene oxide followed by hydrogenation. The high pressure applied in both hydroformylation and hydrogenation steps together with the high temperature, the use of expensive catalysts, and the release of toxic intermediate compounds are problems in those

processes (Saxena et al., 2009). By considering 1,3-PDO, a selective hydroxylation involving three steps, acetalization, tosylation, and detosylation was reported as an alternative synthesis way (Wang et al., 2003). Kurosaka et al. (2008) studied the selective hydrogenolysis of Gro to 1,3-PDO on $Pt/WO_3/ZrO_2$, using 1,3-dimethyl-2-imidazolidinone (DMI) as a solvent, while Gong et al. (2009) studied the effect of both protic and aprotic solvents such as sulfolane, DMI, ethanol, and water, using the same catalyst. Other materials employed to produce propanediols by the hydrogenolysis of Gro are $Pt/WO_3/TiO_2/SiO_2$ (Gong et al., 2010), Pt/amorphous silica-alumina (Gandarias et al., 2010), and Pt on MgO, HLT (hydrotalcite), and $Al_2O_3$ (Yuan et al., 2009). $Pt/WO_3/ZrO_2$ and $Pt/WO_3/Zr(OH)_4$ allow forming 1,3-PDO at ratios higher or similar to 1,2-PDO; different ratios allow to consider the effect of catalyst preparation on the catalytic behavior which can be associated to the interaction of Pt and tungsten, the modifying of active sites, and/or the formation of new ones. It has been previously reported for this catalytic system (Vaudagna et al., 1997). The production of 1,3-PDO by hydrogenolysis of Gro on $Pt/WO_3-ZrO_2$ was previously reported at higher pressures, 5.5 and 8.0 MPa, higher weigh ratio of catalyst/Gro, and in the presence of organic solvents (Kurosaka et al., 2008).

In 2007, Davy Process Technology Ltd., a Johnson Matthey company in a joint venture with Ashland and Cargill announced the production of 1,2-PDO, starting from renewable resources in the context of sustainable chemical technology search; Gro is reacted with hydrogen on a heterogeneous copper catalyst under relatively moderate conditions (20 bar, 200 ºC), being conversion around 99%, adequate selectivity to the desired product, and by-products removed by distillation. Senergy Chemical, a consortium of propylene glycol consumers and marketers has also licensed a process for the first commercial facility. Renewable Alternatives was the first to focus on creating an antifreeze based on Gro mixed with propylene glycol. Products containing propylene glycol are slightly more expensive, but the new process will bring the price down and make it the predominant product. There is also a significant positive environmental advantage, since the ethylene glycol currently in almost universal use is toxic, whereas propylene glycol is not.

## 2.3. Selective Oxidation Reactions

Primary products of oxidation of Gro are glyceraldehyde, glyceric acid, tartronic acid, and dihydroxyacetone (DHA); tartronic acid can be oxidized to glycolic, glyoxylic, oxalic, and mesoxalic acids, while the latter one can be obtained by oxidation of DHA, being hydroxypyruvic acid an intermediate product. The main products have not been yet developed due to low selectivities and yields reached with the existing processes, which operate with low concentration solutions of Gro. Glyceric acid, is mostly produced by a fermentation process (Kenji et al., 1989; Teruyuki and Yoshinori, 1989). Hydroxypyruvic acid is obtained by oxidation of Gro or sugars using mineral acids and it is the precursor of serine amino acid. Tartronic acid is widely used as a precursor of major products such as oxalic acid (Fordham et al., 1995). In aqueous solution, DHA is found as monomer, which can gradually tautomerize to glyceraldehyde. The equilibrium between both compounds depends largely on pH; DHA is favored in an acid medium (greater stability at pH 3) while glyceraldehyde in an alkaline one (Yaylayan et al., 1999). DHA is a chemical used in the cosmetic industry to make artificial

tans, varying its concentration between 2 and 5%; people with sensitive skin should limit exposure to sun due to the consequences that may ensue. Due to its potential capacity of taining, DHA is also used for the treatment of vitiligo, an autoimmune disease that affects the melanocytes. DHA also finds an important application in the chemical industry as reagent of great versatility for the production of compounds like lactic acid, hydroxypyruvic acid, or 1,2-PDO (Hekmat et al., 2003; Bicker et al., 2005). Considering the oxidation of Gro in liquid phase using heterogeneous catalysts, both nature of metal and pH of medium affect the reaction selectivity. Catalytic oxidation of Gro using Pt supported on carbon (Pt/C) was more efficient than the conventional fermentation process, decreasing drastically the DHA selectivity in a basic medium (pH 8) and being less than 10% in an acid one (2-3 pH), increasing that selectivity up to 80% by incorporating bismuth on Pt/C (Kimura et al., 1993). In the same way, Abbadi and van Bekkum (1996) studying the oxidation of both Gro and DHA on Bi-Pt/C at 65°C, reached 95% conversion of Gro and 93% selectivity to glyceric acid at pH 5-6, being DHA, hydroxypyruvic acid, and oxalic acid the main products obtained under acidic conditions. The oxidation of primary or secondary hydroxyl group can be favored controlling reaction conditions (Garcia et al., 1995); Gro conversion was 90% with 70% glyceric acid and 8% DHA using Pd/C, while the DHA yield was 37% with 75% conversion on Bi-Pt/C at pH 2. Using gold catalysts supported on carbon, the selectivity to glyceric acid decreased markedly when gold nanoparticles with average diameter of 6 nm are well dispersed on the surface, while the selectivity increased to 92-95% with nanoparticles larger than 20 nm, indicating the importance of preparation method on performance in the oxidation reaction of Gro (Porta and Prati, 2004). Monometallic catalysts such as Pt/C, Pd/C, and Au/C are able to produce glyceric acid, while bimetallic ones as Au-Pd/C and Au-Pt/C produce tartronic acid and glyceraldehyde, respectively (Bianchi et al., 2005).

Supported catalysts were prepared by impregnation following the incipient-wetness techniques, being identified as Pt/C, Pt-Bi/C, Pt/K-FER and Pt-Bi/K-FER (Antuña and Comelli, 2010). Pt/C showed activity and oxidized Gro (34.9% conversion) but without producing DHA, agreeing with previous results indicating this catalyst as selective to glyceric acid; the addition of a second metal as Bi on Pt/C promoted the selective oxidation of Gro to DHA, being it associated to the changing on the environment of the active site (Kimura et al., 1993). Pt/K-FER having only Pt displayed activity and selectivity to DHA, allowing that zeolitic support the oxidation of the secondary hydroxyl group of Gro; Pt-Bi/K-FER reached the best performance in the selective oxidation to DHA with 48.3% Gro conversion and 90.5% selectivity to DHA.

## 2.4. Reforming Reactions

In the last few years, there has been a growing interest in environmentally clean renewable sources for hydrogen production. In this context, new technologies have been developed for Gro reforming. A complete review related to the processes capable to convert Gro into hydrogen was carried out by Adhikari et al. (2009). The processes include steam reforming, partial oxidation, autothermal reforming, aqueous-phase reforming, and supercritical water reforming; their main characteristics are:

- Steam reforming process: the substrate is reacted with steam in the presence of a catalyst to produce hydrogen, carbon dioxide, and carbon monoxide, being a highly endothermic process. Thermodynamically, it is favored by high temperatures, low pressures, and an excess of steam. This process is the most exhaustively studied to produce hydrogen from Gro.
- Partial oxidation process: the substrate is reacted with oxygen at sub-stoichiometric ratios. The oxidation reaction results in heat generation and high temperature. Reforming in the presence of air allows to balance the energy required for the process by oxidizing some of the substrate.
- Autothermal process: it combines the effect of partial oxidation and steam reforming by feeding together fuel, air, and water. This process is carried out in the presence of a catalyst. The steam reforming process absorbs the heat generated by the partial oxidation one; then, the main benefit is that, ideally, it should not require any energy for reaction to occur whereas the steam reforming is a highly endothermic process. Although the autothermal steam reforming process has advantages over the conventional steam reforming one, the amount of hydrogen produced from that process would be less on a thermodynamic basis.
- Aqueous-phase reforming process: it is a relatively new process and has opened a new pathway for hydrogen production from alcohols and sugars. It operates at relatively higher pressures and lower temperatures than the steam reforming process. The main advantage is that it is a liquid phase process and most biomass based liquids are difficult to vaporize; another advantage is the process also produces less amount of CO.
- Supercritical water reforming: it is performed under the critical temperature (374ºC) and pressure (22.1 MPa) of water. Hydrogen has been produced from Gro by this process using a $Ru/Al_2O_3$ catalyst.

By considering the steam-reforming of Gro, Adhikari et al. (2007a) performed a thermodynamic equilibrium study, resulting temperature higher than 900 K, atmospheric pressure, and 9:1 water:Gro molar ratio as the best conditions for producing hydrogen and minimizing the methane production. Wang et al. (2008) determined optima conditions for hydrogen production between 925 and 975 K and water:Gro ratios of 9-12 at atmospheric pressure, whereas higher temperatures and lower reactant ratios at 20-50 atm favored the production of synthesis gas. Hirai et al. (2005) reported the production of hydrogen using ruthenium catalyst, reaching $Ru/Y_2O_3$ the best performance; at 500°C, the hydrogen yield increased by increasing the ruthenium loading up to 3 wt.%, while a further increment to 5 wt.% did not affect the behavior. Zhang et al. (2007) studied the hydrogen production by reforming of ethanol and Gro over ceria-supported Ir, Co, and Ni catalysts, showing $Ir/CeO_2$ a quite promising catalytic performance with 100% Gro conversion and hydrogen selectivity higher than 85% at 400ºC. Adhikari et al. (2008) measured the hydrogen production using $Ni/CeO_2$, Ni/MgO, and $Ni/TiO_2$ catalysts; $Ni/CeO_2$ having the highest surface area and metal dispersion, reached the best performance in the steam-reforming process gaving the maximum hydrogen selectivity (74.7%) at 600ºC, 12:1 water:Gro molar ratio, and 0.5 mL/min feed flow rate, while the maximum hydrogen yield was obtained at 650ºC using MgO. Cui et al. (2009) evaluated the steam reforming of Gro on non-substituted and partially

Ce substituted $La_{1-x}Ce_xNiO_3$ mixed oxides, being $La_{0.3}Ce_{0.7}NiO_3$ highly active with conversions nearly to the equilibrium at 500 and 700ºC, and forming the smallest amount of carbonaceous deposits. The viability of steam reforming by combining Gro and water by-product streams of a biodiesel plant was studied on a platinum alumina catalyst; a high gas yield (almost 100%) with 70% selectivity (dry basis) can be reached at high temperatures (Slinn et al., 2008). Adhikari et al. (2007b) reported the behavior of fourteen catalysts prepared on ceramic foam monoliths (92% $Al_2O_3$ and 8% $SiO_2$) following the incipient wetness impregnating technique; $Ni/Al_2O_3$ and $Rh/CeO_2/Al_2O_3$ reached the best conversion and selectivity, respectively. Valliyappan et al. (2008) evaluated the steam gasification in the presence of a commercial $Ni/Al_2O_3$ catalyst in the range of steam to Gro weight ratio of 0:100–50:50 to produce either hydrogen or syngas. Pure Gro was completely converted to gas containing 92 mol% syngas at 50:50 weight ratio of steam to Gro. Monometallic (Ni or Pt) and bimetallic (Pt–Ni) catalysts and the effect of lanthana modified alumina support was evaluated during the Gro steam reforming; the lanthana addition on alumina improved catalytic activity of Ni catalysts, while the best selectivity to hydrogen was reached with the catalyst modified with the intermediate content of lanthana (Iriondo et al., 2009). Profeti et al. (2009) investigated the activity of $Ni/CeO_2–Al_2O_3$ catalysts modified with noble metals (Pt, Ir, Pd, and Ru); the formation of inactive nickel aluminate was prevented by the presence of $CeO_2$ dispersed on alumina while the highest catalytic performance for the Gro steam reforming was obtained with the NiPt catalyst, producing an effluent gaseous mixture with the highest $H_2$ yield and low amounts of CO. Sánchez et al. (2010) using a Ni-alumina catalyst reached 96.8% conversion at 600ºC increasing it to 99.4% at 700ºC.

Dou et al. (2009) evaluated a commercial Ni-based catalyst and a dolomite sorbent for the steam reforming reaction and in situ $CO_2$ removal, respectively. Hydrogen productivity was greatly increased with increasing temperature and the formation of methane by-product became negligible above 500ºC. Chiodo et al. (2010) investigated features of Rh and Ni supported catalysts in the steam reforming of Gro to produce syngas; at temperature higher than 720 K, Gro drastically decomposes before to reach the catalyst surface, and $Rh/Al_2O_3$ resulted more active and stable than Ni supported catalysts.

The hydrogen production from Gro by the aqueous phase reforming process using supported-Pt catalysts was studied (Luo et al., 2008). The reaction pathways showed that hydrogen generation is accompanied by side reactions to form alkane and liquid products. Lehnert and Claus (2008) studied the influence of Pt particle size and support type on the aqueous-phase reforming of Gro; independent of the metal precursors employed, similar Gro conversion (45%), and identical selectivity to hydrogen (85%) were obtained. The use of crude Gro as starting material was successful, although the rate of hydrogen production was lower than the one obtained feeding pure Gro, and decreased dramatically after 4 h-on-stream; it was related to impurities present in the crude Gro. Iriondo et al. (2008) compared the hydrogen production from Gro reforming in both liquid (aqueous phase reforming) and vapor (steam reforming) phase over alumina-supported nickel catalysts modified with Ce, Mg, Zr, and La; catalytic activity indicated different catalyst functionalities necessary to carry out aqueous-phase and vapor-phase reforming of Gro. For the aqueous phase reforming, the addition of Ce, La, and Zr to $Ni/Al_2O_3$ improved the Gro conversion respect to the unpromoted $Ni/Al_2O_3$; moreover, all samples showed important deactivation which was associated to oxidation of the active metallic Ni during reaction. In the Gro reforming in vapor phase, Ce, La, Mg, and Zr on Ni based catalysts promoted the hydrogen selectivity.

The operating conditions affect stability of Ni-supported catalysts, causing deactivation. Carbon deposition on the catalyst surface will result several undesirable reactions and products affecting the purity of the reforming products. Carbon occurrence may arise due to the decomposition of CO or $CH_4$ or the reaction of $CO_2$ or CO with $H_2$ (Adhikari et al., 2007b); carbon formation is thermodynamically inhibited at temperature higher than 900 K, atmospheric pressure, and 9:1 water:Gro molar ratio (Adhikari et al., 2007c). At water:Gro molar feed ratios lower than 3:1, the insufficient steam supply produced the methane decomposition forming solid carbon, which is an undesirable substance that decreases the hydrogen production and also causing catalyst deactivation (Rossi et al., 2009). The use of a $CO_2$ adsorbent can suppress the carbon-formation reaction and substantially reduce the lower limit of the water:Gro feed ratio (Chen et al., 2009). A minimal degradation of a platinum-alumina catalyst took place after several days of continuous operating under the optimum conditions for Gro reforming, only 0.4% of feed was deposited (Slinn et al., 2008). Hydrogen production on a supported-Pt catalyst is accompanied by side reactions which form carbonaceous entities on the surface causing catalyst activity drop (Luo et al., 2008). Nickel catalysts supported on commercial $Al_2O_3$ and $Al_2O_3$ modified by addition of $ZrO_2$ and $CeO_2$ were evaluated; $Ni/CeO_2/Al_2O_3$ was the most stable system; it was associated to the Ce effect in inhibition of secondary dehydration reactions forming unsaturated hydrocarbons that are coke precursors generating fast catalyst deactivation (Buffoni et al., 2009). Using $Ni/CeO_2–Al_2O_3$ catalysts modified with Pt, Ir, Pd, and Ru, the presence of noble metals stabilized the Ni sites in the reduced state along the reforming reaction, increasing conversion and decreasing coke formation (Profeti et al., 2009). Chiodo et al. (2010) investigated features of Rh and Ni supported catalysts in the steam reforming of Gro, independently of both impregnated metal and temperature, reaction is affected by coke formation mainly promoted by the presence of olefins formed by Gro thermal decomposition. Recently, characterization of deactivation processes of Ni impregnated on alumina during the hydrogen production from Gro and evaluation of regenerating conditions was made, indicating that selecting adequate conditions, activity can be restored (Sánchez and Comelli, 2012).

Finally, reforming of Gro can also produce synthesis gas which containing mainly carbon monoxide and hydrogen; syngas can be converted into methanol using conventional technology, and methanol used for methyl esterification of vegetable oils, then, 100% biomass-based bio-diesel fuel could be obtained. In the same way, BioMethanol Chemie Nederland BV uses this technology to produce biomethanol. Another commercial potential of the aqueous phase reforming technology is reinforced by coupling with the Fisher–Tropsch process, providing the characteristic product streams.

## 2.5. Miscelaneous Reactions: Chlorination, Etherification, Esterification, Nitration, and Polymerization

Direct hydrochlorination of Gro is an attractive way to produce 1,3-dichloro-2-propanol, an intermediate to obtain epichlorohydrin, which interests to produce ether polymers, including the commercially valuable epoxy resins. From 2007, Solvay started the production of epichlorohydrin from Gro obtained as by-product of biodiesel. Previously, Solvay produced both epichlorohydrin and Gro from propene, but the increment of propene prices

and the decrease of the Gro one, induced that company stop the production of synthetic Gro from epichlorohydrin, reversing the process, and converting the plant to produce epichlorohydrin from Gro. This process based on organic acid catalysts displays advantages over the propene route. Reaction to produce dichloropropanol takes place using anhydrous hydrochloric with 30% caprylic acid as catalyst at above 120 ºC, in order to minimized corrosion; then, epichlorohydrin is obtained by dechlorination with NaOH, recovering NaCl which is used to produce chlorine by electrolysis and recycling the fraction rich in water to the hydrodechlorination step.

Reacting Gro with isobutene or tert-butanol produces glycerol ethers, which can be added to fuels, also including polyglycerols and glycosyl glycerol. Both high polarity and hygroscopic character of Gro make it unsuitable as a fuel additive in unmodified form; other problems are the Gro polymerization at high temperatures and the partially oxidised into toxic acrolein. Then, glycerol ether formulations can be commercialized as oxygenate fuel additives for use in gasoline engines. The CPS Biofuels company react Gro coming from both biodiesel and ethanol facilities with low molecular weight alkenes supplied by petrochemical producers, for producing glycerol tert-butyl ether (GTBE), a green, renewable, sustainable, non-toxic fuel additive.

Esterification of Gro with carboxylic acids results in monoacylglycerols (MAGs) and diacylglycerol (DAG), which have well established industrial applications, particularly in food and oleochemical industries. MAGs are amphilic molecules and useful as nonionic surfactants and emulsifiers, while DAGs have the advantage of stability to decomposition at cooking temperatures and their human nutritional characteristics compared to triacylglycerol (TAG) clearly indicates the important suppressive effect by DAG on body fat accumulation.

Reacting Gro with nitrating agents, the nitroglycerine which is an explosive and also used as an antianginal drug, can be produced; it is currently produced in modular plants. Another reaction product can also be dinitroglycerol, which treated with a cyclizing agent is converted into glycidyl nitrate, that can be polymerized to poly(glycidyl nitrate) (PGN).

Polyglycerol is a clear viscous liquid, highly soluble in water and in polar organic solvents such as methanol, being nonvolatile at room temperature. Its viscosity increases with the molecular weight. Combined with Amberlyst 35 ion-exchange resin is an excellent catalyst for glycerol alkylation with isobutene. Polyglycerols are commercially available for different applications including from cosmetics to controlled drug release; biocompatibility is an attractive feature of aliphatic polyether structures containing hydroxyl end-groups.


## 3. BIOLOGICAL APPROACHES TO VALORIZE GLYCEROL

The abundance of Gro in nature established that many organisms, mainly microorganisms can metabolize the polyol, utilizing it as a source of carbon and energy. Different metabolic pathways followed by glycerine are found depending on the microorganism. These differences are relevant in considering biological tools to add value to the polyol, because many intermediate metabolites or final products are of utility for specific industries and for development of biorefinery strategies. We will analyze possibilities for using entire microorganisms or enzymes involved in key metabolic steps as biological and molecular tools. Biological strategies can allow utilize Gro for different purposes, mainly: (i) as a raw

substrate for growing microorganisms of strategic value by themselves (i.e. as single cell additive to cattle feeding); (ii) as a raw substrate to grow transgenic cells producing recombinant proteins and enzymes, medicinal drugs, pigments, biosurfactants, or metabolites of high value; or (iii) to convert Gro in key metabolites of value as chemicals by using specific set of enzymes or genetically altered metabolic pathways. Figure 2 schematize about variants in the use of Gro for industrial microbiology.
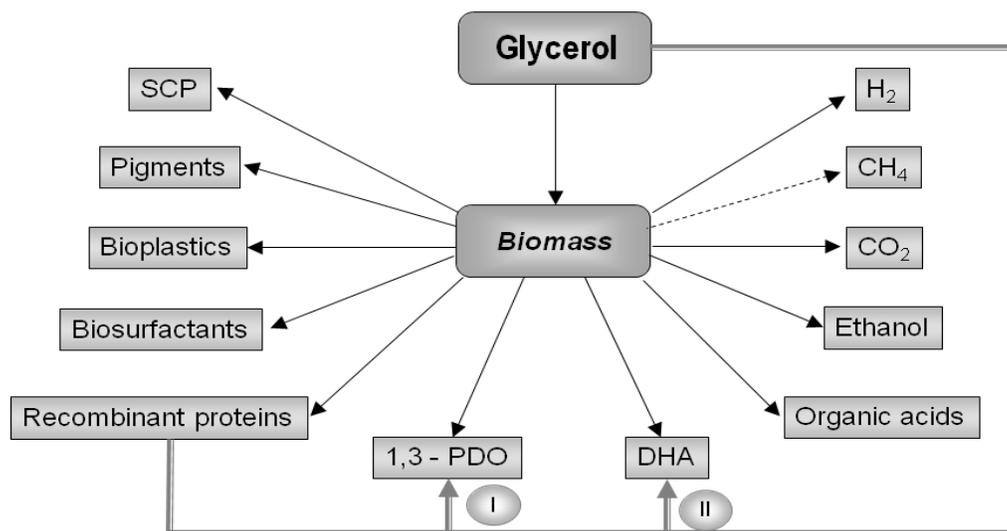


Figure 2. Glycerol substrate in industrial microbiology. References I and II: glycerol conversion to 1,3-PDO and DHA, respectively, by specific enzymes (see biochemical reactions in text).

## 3.1. The Meeting History between Glycerol and Microorganisms

Relationships between Gro and microorganisms were first documented by Pasteur, who reported, in 1858, the polyol presence in yeast culture broth, thus evidencing the capacity of microorganisms to metabolize Gro by fermentation. This scientific finding brought forth the microbial industrial production of Gro, especially during World War I (Wang et al., 2001); creating a successful biotechnological enterprise that short after was gloomed by the development of processes for its chemical synthesis, especially before the World War II. Great relevance that existed for obtain Gro in relation with army conflicts is clear considering the use of nitroglycerin for production of dynamites and explosives. The surplus of Gro originated lately by the rapid expansion of biodiesel production is forcing to manufacture companies to shut down plants for its chemical synthesis (McCoy, 2006). On the other hand, the glut is refreshing the old association between the polyol and microorganisms; but now in the substrate sense. Thus, Gro is visualized as a carbon-energy source for microbial growth, paving the way to produce biomass (Juszczyk and Rymowicz, 2009) recombinant proteins (Giordano et al., 2010), bioplastics (Mothes and Otto, 2009), single cell oils (Papanikolaou et al., 2008) or valuable metabolites (Papanikolaou et al., 2008; Mantzouridou, 2009; Musial and Rymowicz, 2009; Rywinska and Rymowicz, 2009). Thus, the overall scenario for

biological processing of Gro is clearly related with the use of the polyol as a key compound for applications in biorefinery-like procedures.

## 3.2. Glycerol as Carbon and Energy Microbial Source. The First Step to Production

Gro qualifies as a suitable source for carbon-energy satisfying demands for cell proliferation of many chemo-organo-heterotrophic microorganisms (Peters, 2007). Between them, worth to highlight is the enterobacterium *Escherichia coli* and the yeast *Saccharomyces cereviseae*, because they are model organisms profusely employed in bioprocesses producing recombinant proteins or other transgenic or non-transgenic technologies. Other microorganisms able to metabolize Gro aerobically or anaerobically include species form the genus *Klebsiella*, *Clostridium*, *Enterobacter*, *Citrobacter*, and *Lactobacillus* (da Silva et al., 2009). The first condition for bioprocessing of Gro, is its uptake by the cell. Although Gro can cross the plasmatic membrane through passive diffusion, its more effective transport is mediated by specific permeases (see Figs. 3 and 4). *E. coli* permeases belong to the group of aquaporines, which function as a passive carrier (Sanno et al., 1968; Richey and Lin, 1972; Sweet et al., 1990). The permease is also known as the Gro facilitator (Fu et al., 2000), and it acts as a channel with stereo- and enantio-selectivity for many non-ionic compounds including Gro, polyalcohols, and urea derivatives. In *S. cereviseae*, in addition to a channel protein for facilitated diffusion, it was also identified an active mechanism for transport of the polyol (da Silva et al., 2009).

Once inside the cell, Gro can be metabolize by different pathways, depending of the specific microorganism (da Silva et al., 2009). The polyol oxidation for obtaining cellular energy can occur by respirative or fermentative routes, depending on which is the final electron acceptor of the process. Oxidation can also take place via primary conversion to glycerol-3-phosphate (Gro3P) or by the route producing DHA (see Figs. 3 and 4, respectively, for details). Under aerobic respiration (Fig. 3), such as that able to occur in *E. coli*, oxygen is the molecule accepting electrons and full oxidation of Gro to carbon dioxide involves the tricarboxylic acid cycle (TCA). But in anaerobiosis other compounds (such as nitrate or formate) are the chemical species reduced, and this process for energy obtaining is referred as anaerobic respiration. The latter oxidation can also be performed by *E. coli* strains in the absence of oxygen. Oxidative metabolisms for glycerine are fully active in yeasts. The intermediate metabolite Gro3P, produced in the pathway involving Gro kinase, can also serve as a precursor for lipid biosynthesis.

Utilization of Gro as a source of carbon and energy for growing a microorganism employed in a productive process is a relevant strategy in adding value to the polyol, and the associate use for make recombinant proteins is a key option (Giordano et al., 2010). Normally, aerobic conditions are the preferred for developing a bioprocess for recombinant protein production in *E. coli*. In them, higher biomass yield can be obtained, and proteins synthesis is proportional to formed biomass. Cultures under aerobiosis perform maximal substrate oxidation and produce higher energy (ATP) levels. However, and despite the lesser energy obtained by a fermentative process, the latter is the more selected when the aim is the synthesis of different metabolites (Fig. 4). It was in 1936 when probably the first report (but not concluding) about Gro fermentation by *E. coli* was published (Dozois et al., 1936). Then,

employing classical microbial assays, they were detected acid and gas production in fermentative culture conditions of this bacterium. Contemporary studies call in doubt the Gro fermentation by *E. coli* [see comment and references in (Gonzalez et al., 2008)]. However, recent reports vindicate that capability by applying molecular methodologies and fermentation technologies (Dharmadi et al., 2006; Gonzalez et al., 2008; Murarka et al., 2008), as well as modelling (Cintolesi et al., 2012). As it is detailed in Figure 4, in wild type *E. coli* relevant fermentative products are ethanol, hydrogen and carbon dioxide. From the latter, they are relevant ethanol and hydrogen as key biofuels that can be produced by processing Gro by biotechnological processes (Fig. 2).
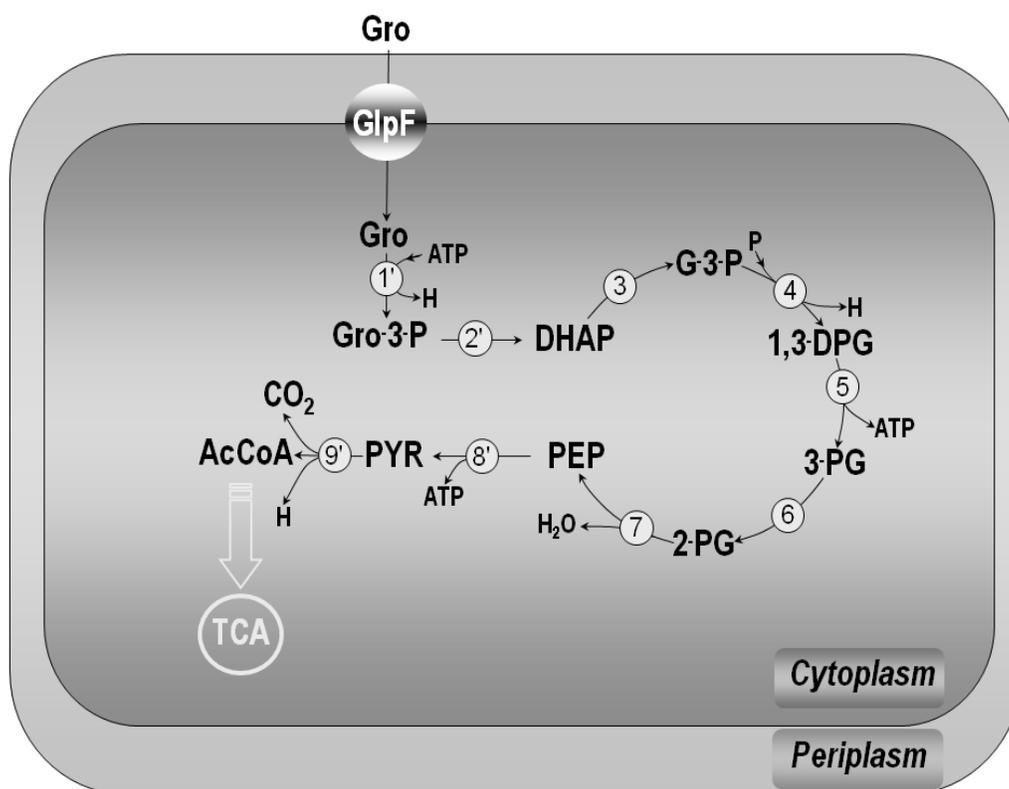


Figure 3. Main intermediaries and pathways during glycerol oxidation via respiration in *E. coli*. References: 1': Glycerol kinase (EC 2.7.1.30); 2': Glycerol-3-phosphate dehydrogenase (EC 1.1.5.3); 3: Triosephosphate isomerase (EC 5.3.1.1); 4: Glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12); 5: Phosphoglycerate kinase (EC 2.7.2.3); 6: Phosphoglycerate mutase (EC 5.4.2.1); 7: Enolase (EC 4.2.1.11); 8': Pyruvate kinase (EC 2.7.1.40); 9': Pyruvate dehydrogenase (EC 1.2.1.51). Gro-3-P: glycerol-3-phosphate; DHAP: dihydroxyacetone phosphate; G-3-P: Glyceraldehyde 3-phosphate; 1,3-DPG: 1,3-diphosphoglycerate; 3-PG: 3-phosphoglycerate; 2-PG: 2-phosphoglycerate; PEP: phosphoenolpyruvate; PYR: pyruvate; AcCoA: Acetyl coenzyme A; TCA: tricarboxylic acid cycle.

In *Klebsiella*, *Clostridium*, *Enterobacter* and *Citrobacter* the polyol can be metabolized by oxidation or by a reductive route involving two enzymatic steps (da Silva et al., 2009). Firstly, Gro is converted to 3-hydroxy-propionaldehyde by Gro dehydratase [an enzyme using cobalamin (vitamin $B_{12}$) as a coenzyme] and related diol dehydratases (Seifert et al., 2001).

The second step is mediated by 1,3-PDO dehydrogenase, catalyzing the NADH-dependent reduction of 3-hydroxypropionaldehyde to 1,3-PDO. This reductive pathway for Gro is unique for obtaining 1,3-PDO by biological fermentation.
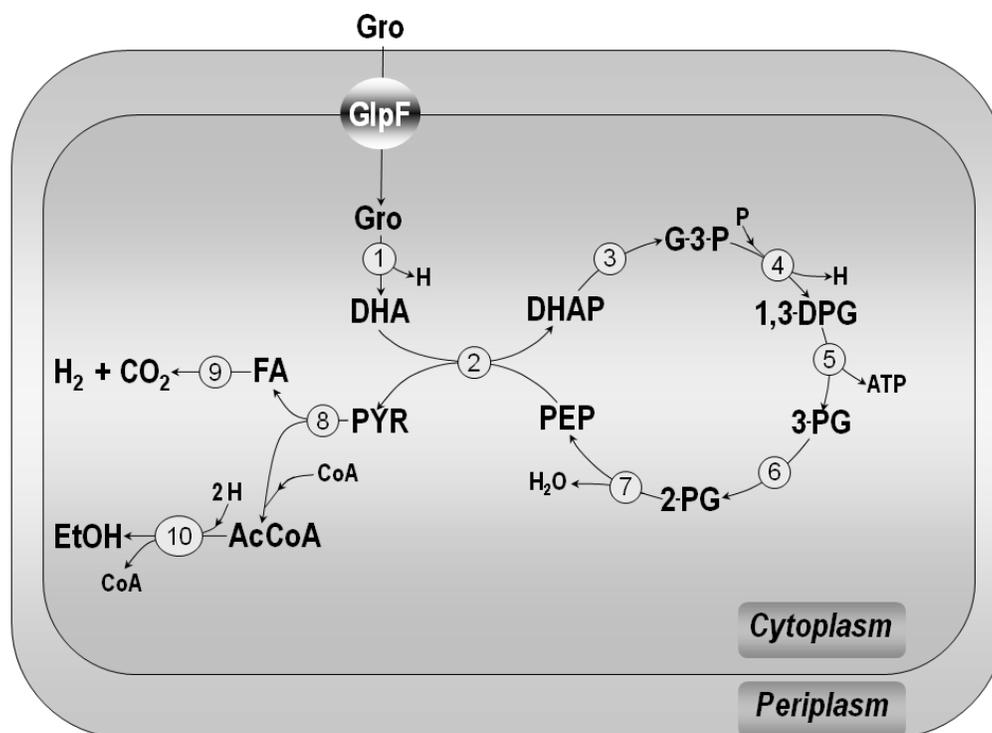


Figure 4. Fermentation pathway of glycerol in *E. coli*. References: Glycerol dehydrogenase (EC 1.1.1.6); 2: Dihydroxyacetone kinase (EC 2.7.1.29); 3: Triosephosphate isomerase (EC 5.3.1.1); 4: Glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12); 5: Phosphoglycerate kinase (EC 2.7.2.3); 6: Phosphoglycerate mutase (EC 5.4.2.1); 7: Enolase (EC 4.2.1.11); 8: Pyruvate formate-lyase (EC 2.3.1.54); 9: Formate hydrogen lyase complex (EC 1.2.1.2 plus EC 1.12.1.2; or EC 1.1.99.33); 10: Alcohol / acetaldehyde dehydrogenase (EC 1.1.1.1; EC 1.2.1.10); Gro: glycerol; DHA: dihydroxyacetone; DHAP: dihydroxyacetone phosphate; G-3-P: Glyceraldehyde 3-phosphate; 1,3-DPG: 1,3-diphosphoglycerate; 3-PG: 3-phosphoglycerate; 2-PG: 2-phosphoglycerate; PEP: phosphoenolpyruvate; PYR: pyruvate; FA: formate; AcCoA: Acetyl-coenzyme A; EtOH: ethanol; P: phosphate; H: $NADH^+ + H^+$ or $NADPH^+ + H^+$.

## 3.3. Relevant Products from Glycerol Bioconversion

As described above, biological processing of Gro may pursue the use of the polyol as the raw material for growing cells. Such cells may be of value by themselves (i.e. for feeding usage; namely SCP for Single Cell Proteins), or because they are genetically modified microorganisms generating recombinant proteins or enzymes of highly added commercial value. Other relevant approach is the biological use of glycerin to obtain key metabolites that are valuable compounds. The latter can be reached by using entire wild-type or transgenic organisms, or enzyme(s) catalyzing key steps in the metabolism of Gro in one specific cell.

### 3.3.1. Bioconversion of Glycerol to Dihydroxyacetone

DHA is one compound that theoretically could be straightforwardly obtained by bioconversion of Gro. The process requires of a unique step catalyzed by Gro dehydrogenase (GroDHase):

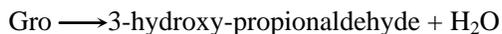$$Gro \ + \ NAD^+ \rightleftharpoons DHA + NADH + H^+$$
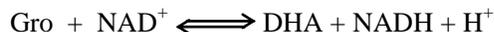
Depending on the microorganism, two type of GroDHases can be found; the soluble enzyme (EC 1.1.1.6), typically occurring in *E. coli* (Asnis and Brodie, 1953; Truniger and Boos, 1994), or the membrane bound enzyme (EC 1.1.99.22) as that present in *Gluconobacter oxydans* (Gatgens et al., 2007). Globally, GroDHases have been identified in different organisms, including bacteria, yeasts and mammals (Ruzheinikov et al., 2001).

Given the high commercial relevance of DHA because it is used in cosmetics and is a key compound for organic synthesis of valuable chemicals, different processes were developed for its production (da Silva et al., 2009). Biological procedures for synthesize DHA are economically more convenient than those of chemical production. *G. oxydans* is one microorganism frequently utilized to convert Gro into DHA with some degree of efficiency (Gatgens et al., 2007). Although different variants were implemented to optimize procedures, the microbial process has as a main trouble the fact that both, the substrate and the product negatively affect because they inhibit bacterial growth (da Silva et al., 2009). A possibility that has not yet completely explored is the development of methodology to use isolated enzymes for transforming the polyol into the hydroxyketone. For the latter it could be convenient to consider the soluble rather than the membrane-bound GroDHase and also some characteristics of the *E. coli* enzyme, as in example that it exhibits thermostability (Asnis and Brodie, 1953). A main obstacle in the use of a system of isolated enzyme is the necessity of regenerating the oxidized form of the coenzyme ($NAD^+$) from NADH, and some strategies have been proposed to solve the problem (Nemeth and Sevella, 2008). However, this type of biotechnological designs are quite promising for improve the production of a valuable chemical as DHA.

### 3.3.2. Bioproduction of 1,3-Propanediol From Glycerol

1,3-PDO is the major product of microbial reductive fermentation of Gro (da Silva et al., 2009). The biological pathway involves two steps. It courses with Gro dehydration catalyzed by vitamin $B_{12}$-dependent Gro dehydratase (EC 4.2.1.30) and associated diol dehydratases (EC 4.2.1.28), followed by the reductive stage mediated by NADH-dependent 1,3-PDO dehydrogenase (EC 1.1.1.202); as respectively detailed by next equations:

$$Gro \longrightarrow 3\text{-hydroxy-propionaldehyde} + H_2O$$

$$3\text{-hydroxypropionaldehyde} + NADH + H^+ \rightleftharpoons 1,3\text{-PDO} + NAD^+$$

The relevance of 1,3-PDO as a commodity chemical, mainly utilized in the industrial production of plastics (such as polyester fibers and polyurethanes) as well as cyclic derivatives, has been highlighted above (see section 2.1.2). Although the high value of the compound, its chemical synthesis is relatively expensive, and the current situation is demanding designs for more convenient biotechnological procedures. Many developments for

the production of 1,3-PDO have been established, with the uses of wild-type or genetically modified microorganisms. For obtaining molecular tools related with 1,3-PDO metabolism, they are important species of the genus: *Clostridium*, *Citrobacter*, *Klebsiella*, *Lactobacillus*, and *Bacillus*, between others (da Silva et al., 2009). In these organisms, the enzymes of the metabolic pathway are coded by genes grouped in the *dha* regulon. The kinetic characteristics of the enzymes involved critically limit the biological production, since they undergo a suicide-inactivation exerted by the substrate (Daniel et al., 1998; Knietsch et al., 2003). Gro dehydratase (strictly glycerol hydro-lyase) and diol dehydratase (specifically D,L-1,2-propanediol hydro-lyase) utilize cobalamin as an essential cofactor, in a reaction proceeding via a radical mechanism that leads to the irreversible cleavage of the Co—C bound present in vitamin $B_{12}$. These enzymes are multisubunit (of the type $\alpha_2\beta_2\gamma_2$) and reactivation of the Gro-inactivated forms involves a heterodimeric protein complex and requires ATP (Daniel et al., 1998).

### 3.3.3. Bioconversion of Glycerol to Commercially Valuable Organic Acids

Organic acids of use in different industries that can be obtained form microbial conversion of Gro include succinic acid, propionic acid, and citric acid (da Silva et al., 2009). As detailed in Figures 3 and 4, the metabolism of the polyol produces phospho-*enol*-pyruvate (PEP), which is the metabolite from which initiates the pathway leading to succinic acid. PEP can be converted to oxaloacetic acid (OAA) by reactions catalyzed by PEP carboxykinase (4.1.1.32) or PEP carboxylase (EC 4.1.1.31), respectively:

$$PEP + CO_2 + ATP \Longleftrightarrow OAA + ADP$$
$$PEP + HCO_3^- \longrightarrow OAA + \text{inorganic orthophosphate (Pi)}$$
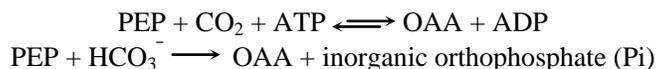
OAA is then reduced by NADH-dependent malate dehydrogenase (EC 1.1.1.37):

$$OAA + NADH + H^+ \Longleftrightarrow \text{malic acid} + NAD^+$$

and malic acid is reversibly dehydrated to fumaric acid (also of value for some industries) by fumarase (or fumarate hydratase, EC 4.2.1.2):

$$\text{malic acid} \Longleftrightarrow \text{fumaric acid} + H_2O$$

Finally, two distinct membrane-bound enzyme complexes (EC 1.3.99.1) can catalyze the interconversion of fumaric acid and succinic acid:

$$\text{fumaric acid} + XH_2 \Longleftrightarrow \text{succinic acid} + X$$
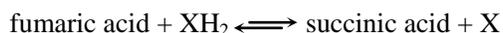
Under anaerobic growth, the enzyme is fumarate reductase, whereas succinate dehydrogenase is involved in aerobic growth. The $XH_2$ electron donor is a component of the membrane. Both complexes are formed by a membrane-extrinsic component containing a FAD-binding flavoprotein and an iron-sulfur protein, plus a hydrophobic membrane anchor protein and/or cytochrome-b (Blaut et al., 1989). In a further step, succinic acid can be converted into propionic acid in a reaction mediated by enzymes of the type propionyl-

CoA:succinate CoA transferase (EC 2.8.3.-) related to the crotonase superfamily (Haller et al., 2000):

$$propionyl\text{-}CoA + succinate \Longleftrightarrow propionate + succinyl\text{-}CoA$$

On the other hand, citric acid is an intermediate of the Krebs cycle, being synthesized by the reaction of citrate synthase (EC 2.3.3.1):

$$Acetyl\text{-}CoA + H_2O + OAA \Longleftrightarrow citrate + CoA + H^+$$

Succinic acid is a chemical of important apply in food and pharmaceutical industries, also being of utility to chemically produce 1,4-butanediol as well as to obtain surfactants, detergents and biodegradable plastics. Microbial making of succinic acid from Gro fermentation currently employs *Anaerobiospirillum succiniproducens*. For propionic acid production, microorganisms useful are *Propionibacterium* spp. and *Clostridium propionicum*; being this acid taken up by industries of biodegradable plastics, thermoplastics, herbicides, arthritis drugs and perfumes. Whereas with respect to citric acid, its application is principally to give fruity flavour to foods and beverages; also serving as additive in detergents, and pharmaceuticals. Biotechnologies to produce citric acid are based in *Aspergillus niger* and the yeast *Yarrowia lipolytica*, with this latter organisms growing conveniently on Gro (da Silva et al., 2009).

### 3.3.4. Other Valuable Bioconversions of Glycerol

As detailed in Figures 2-4, bioconversion of Gro can also produce metabolites (intermediate of final products) of importance as biofuels. Main examples are the production of ethanol and, interestingly, the possibility to obtain hydrogen (considered an ideal clean fuel after that its oxidation gives water: $H_2 + \frac{1}{2}O_2 \rightarrow H_2O$). By fermentation it is also possible to generate methane and similar biogases. Of interest are the studies from Dharmadi et al. (2006) showing that fermentation of Gro by *E. coli* is pH-dependent and that conditions can be settled to improve the process to generate ethanol and hydrogen. On the other hand, glycerine can also be utilized as a substrate for growing of microorganisms producing industrially key derivatives, as in examples: (i) the plastic-like polymer polyhydroxyalcanoate (polyesters accumulated by some bacteria intracellularly as a reserve of carbon and energy); (ii) biosurfactants, of the ionic and non-ionic types, as rhamnolipids produced by *Pseudomonas aeruginosa*; and (iii) pigments, like in the production of the red/orange compound astaxanthin (utilized to feed salmon, trout and crustaceans) or prodigiosin, a red pigment produced by *Serratia marcescens* that is an active immunosupressor and inductor of apoptosis, after which is of value in anti-cancer drugs (da Silva et al., 2009). A main product of biological oxidation is $CO_2$, a gas useful in several industries.

## CONCLUDING REMARKS

Gro is a chemical of wide application in diverse industries that, paradoxically, is experiencing a striking loss in its value in the last decade. The main reason for the latter is the

fact that Gro is a main by-product in the generation of biodiesel, after which the former is experiencing a huge over-offer. The situation created a commitment to find new uses and applications for glycerine and to develop (bio)technological strategies to rise-up its commercial value. The scenario is interesting when it is associated with development of processes of the biorefinery type. In such a way, Gro is a compound with key properties that are suitable for application in procedures for the generation of different goods of value as biofuels, bioplastics, food-additives, and active ingredients in pharmaceutical, cosmetic, and chemical industries. Many strategies to add-value to Gro are currently underway, with basis either in chemical or biological approaches. Besides the relevance that each approach has by itself, it is worth to have an integrated management, thus combining the toughness of the tools each one affords. The latter is a specific biorefinery-like activity, where expertises in the areas of chemistry and biology come together to solve complex technological problems in a framework compatible with a sustainable environment.

## ACKNOWLEDGMENTS

## REFERENCES

Abbadi A, Van Bekkum H (1996) Selective chemo-catalytic routes for the preparation of β-hydroxypyruvic acid. *Applied Catalysis A: General* 148: 113-122.

Adhikari S, Fernando SD, Haryanto A (2009) Hydrogen production from glycerol: An update. *Energy Conversion and Management* 50: 2600-2604.

Adhikari S, Fernando SD, To SDF, Bricka RM, Steele PH, Haryanto A (2008) Conversion of glycerol to hydrogen via a steam reforming process over nickel catalysts. *Energy and Fuels* 22: 1220-1226.

Adhikari S, Fernando S, Gwaltney SR, Filip To SD, Mark Bricka R, Steele PH, Haryanto A (2007a) A thermodynamic analysis of hydrogen production by steam reforming of glycerol. *International Journal of Hydrogen Energy* 32: 2875-2880.

Adhikari S, Fernando S, Haryanto A (2007b) Production of hydrogen by steam reforming of glycerin over alumina-supported metal catalysts. *Catalysis Today* 129: 355-364.

Adhikari S, Fernando S, Haryanto A (2007c) A comparative thermodynamic and experimental analysis on hydrogen production by steam reforming of glycerin. *Energy and Fuels 2*1: 2306-2310.

Agvald P, Adding LC, Artlich A, Persson MG, Gustafsson LE (2002) Mechanisms of nitric oxide generation from nitroglycerin and endogenous sources during hypoxia in vivo. *Br J Pharmacol* 135: 373-382.

Akiyama M, Sato S, Takahashi R, Inui K, Yokota M (2009) Dehydration-hydrogenation of glycerol into 1,2-propanediol at ambient hydrogen pressure. *Applied Catalysis A: General* 371: 60-66.

Amidon TE, Liu S (2009) Water-based woody biorefinery. *Biotechnol Adv* 27: 542-550.

Antuña S, Comelli, R. (2010). *In*. P 20100103278, ARG.

Asnis RE, Brodie AF (1953) A glycerol dehydrogenase from *Escherichia coli*. *J Biol Chem* 203: 153-159.

Balaraju M, Rekha V, Devi BLAP, Prasad RBN, Prasad PSS, Lingaiah N (2010) Surface and structural properties of titania-supported Ru catalysts for hydrogenolysis of glycerol. *Applied Catalysis A: General* 384: 107-114.

Balaraju M, Rekha V, Prasad PSS, Devi BLAP, Prasad RBN, Lingaiah N (2009) Influence of solid acids as co-catalysts on glycerol hydrogenolysis to propylene glycol over Ru/C catalysts. *Applied Catalysis A: General* 354: 82-87.

Bianchi CL, Canton P, Dimitratos N, Porta F, Prati L (2005) Selective oxidation of glycerol with oxygen using mono and bimetallic catalysts based on Au, Pd and Pt metals. *Catalysis Today* 102-103: 203-212.

Bicker M, Endres S, Ott L, Vogel H (2005) Catalytical conversion of carbohydrates in subcritical water: A new chemical process for lactic acid production. *Journal of Molecular Catalysis A: Chemical* 239: 151-157.

Blaut M, Whittaker K, Valdovinos A, Ackrell BA, Gunsalus RP, Cecchini G (1989) Fumarate reductase mutants of *Escherichia coli* that lack covalently bound flavin. *J Biol Chem* 264: 13599-13604.

Buffoni IN, Pompeo F, Santori GF, Nichio NN (2009) Nickel catalysts applied in steam reforming of glycerol for hydrogen production. *Catalysis Communications* 10: 1656-1660.

Cintolesi A, Clomburg JM, Rigou V, Zygourakis K, Gonzalez R (2012) Quantitative analysis of the fermentative metabolism of glycerol in *Escherichia coli*. *Biotechnol Bioeng* 109: 187-198.

Comelli R (2011) Chapter 16 "Glycerol, the co-product of biodiesel: One key for the future bio-refinery". *In* "*Biodiesel Quality, emision and by-products*", .

Corma Canos A, Iborra S, Velty A (2007) Chemical routes for the transformation of biomass into chemicals. *Chemical Reviews* 107: 2411-2502.

Cui Y, Galvita V, Rihko-Struckmann L, Lorenz H, Sundmacher K (2009) Steam reforming of glycerol: The experimental activity of La1-xCexNiO$_3$ catalyst in comparison to the thermodynamic reaction equilibrium. *Applied Catalysis B: Environmental* 90: 29-37.

Chen H, Zhang T, Dou B, Dupont V, Williams P, Ghadiri M, Ding Y (2009) Thermodynamic analyses of adsorption-enhanced steam reforming of glycerol for hydrogen production. *International Journal of Hydrogen Energy* 34: 7208-7222.

Chiodo V, Freni S, Galvagno A, Mondello N, Frusteri F (2010) Catalytic features of Rh and Ni supported catalysts in the steam reforming of glycerol to produce hydrogen. *Applied Catalysis A: General* 381: 1-7.

da Silva GP, Mack M, Contiero J (2009) Glycerol: a promising and abundant carbon source for industrial microbiology. *Biotechnol Adv* 27: 30-39.

Daniel R, Bobik TA, Gottschalk G (1998) Biochemistry of coenzyme B$_{12}$-dependent glycerol and diol dehydratases and organization of the encoding genes. *FEMS Microbiology Reviews* 22: 553-566.

Dasari MA, Kiatsimkul PP, Sutterlin WR, Suppes GJ (2005) Low-pressure hydrogenolysis of glycerol to propylene glycol. *Applied Catalysis A: General* 281: 225-231.

Dharmadi Y, Murarka A, Gonzalez R (2006) Anaerobic fermentation of glycerol by *Escherichia coli*: a new platform for metabolic engineering. *Biotechnol Bioeng* 94: 821-829.

Dou B, Dupont V, Rickett G, Blakeman N, Williams PT, Chen H, Ding Y, Ghadiri M (2009) Hydrogen production by sorption-enhanced steam reforming of glycerol. *Bioresource Technology* 100: 3540-3547.

Dozois KP, Carr CJ, Krantz JC, Hachtel F, Beck FF (1936) Sugar Alcohols. VI: Utilization of Sugar Alcohols and Their Anhydrides by Various Microorganisms. *J Bacteriol* 32: 499-503.

Epure V, Griffon M, Pollet E, Avérous L (2011) Structure and properties of glycerol-plasticized chitosan obtained by mechanical kneading. *Carbohydrate Polymers* 83: 947-952.

Feng J, Fu H, Wang J, Li R, Chen H, Li X (2008) Hydrogenolysis of glycerol to glycols over ruthenium catalysts: Effect of support and catalyst reduction temperature. *Catalysis Communications* 9: 1458-1464.

Fordham P, Besson M, Gallezot P (1995) Selective catalytic oxidation of glyceric acid to tartronic and hydroxypyruvic acids. *Applied Catalysis A, General* 133: L179-L184.

Fu D, Libson A, Miercke LJ, Weitzman C, Nollert P, Krucinski J, Stroud RM (2000) Structure of a glycerol-conducting channel and the basis for its selectivity. *Science* 290: 481-486.

Gandarias I, Arias PL, Requies J, Güemez MB, Fierro JLG (2010) Hydrogenolysis of glycerol to propanediols over a Pt/ASA catalyst: The role of acid and metal sites on product selectivity and the reaction mechanism. *Applied Catalysis B: Environmental* 97: 248-256.

Garcia R, Besson M, Gallezot P (1995) Chemoselective catalytic oxidation of glycerol with air on platinum metals. *Applied Catalysis A, General* 127: 165-176.

Gatgens C, Degner U, Bringer-Meyer S, Herrmann U (2007) Biotransformation of glycerol to dihydroxyacetone by recombinant *Gluconobacter oxydans* DSM 2343. *Appl Microbiol Biotechnol* 76: 553-559.

Giordano PC, Martinez HD, Iglesias AA, Beccaria AJ, Goicoechea HC (2010) Application of response surface methodology and artificial neural networks for optimization of recombinant *Oryza sativa* non-symbiotic hemoglobin 1 production by *Escherichia coli* in medium containing byproduct glycerol. *Bioresour Technol* 101: 7537-7544.

Gong L, Lu Y, Ding Y, Lin R, Li J, Dong W, Wang T, Chen W (2010) Selective hydrogenolysis of glycerol to 1,3-propanediol over a Pt/WO$_3$/TiO$_2$/SiO$_2$ catalyst in aqueous media. *Applied Catalysis A: General* 390: 119-126.

Gong L, Lü Y, Ding Y, Lin R, Li J, Dong W, Wang T, Chen W (2009) Solvent effect on selective dehydroxylation of glycerol to 1,3-propanediol over a Pt/WO$_3$/ZrO$_2$ catalyst. *Cuihua Xuebao/Chinese Journal of Catalysis* 30: 1189-1191.

Gonzalez R, Murarka A, Dharmadi Y, Yazdani SS (2008) A new model for the anaerobic fermentation of glycerol in enteric bacteria: trunk and auxiliary pathways in *Escherichia coli*. *Metab Eng* 10: 234-245.

Guo L, Zhou J, Mao J, Guo X, Zhang S (2009) Supported Cu catalysts for the selective hydrogenolysis of glycerol to propanediols. *Applied Catalysis A: General* 367: 93-98.

Haller T, Buckel T, Retey J, Gerlt JA (2000) Discovering new enzymes and metabolic pathways: conversion of succinate to propionate by *Escherichia coli*. *Biochemistry* 39: 4622-4629.

Hekmat D, Bauer R, Fricke J (2003) Optimization of the microbial synthesis of dihydroxyacetone from glycerol with *Gluconobacter oxydans*. *Bioprocess and Biosystems Engineering* 26: 109-116.

Hirai T, Ikenaga NO, Miyake T, Suzuki T (2005) Production of hydrogen by steam reforming of glycerin on ruthenium catalyst. *Energy and Fuels* 19: 1761-1762.

Huang L, Zhu Y, Zheng H, Ding G, Li Y (2009) Direct conversion of glycerol into 1,3-propanediol over Cu-H $4SiW_{12}O_{40}/SiO_2$ in vapor phase. *Catalysis Letters* 131: 312-320.

Iriondo A, Barrio VL, Cambra JF, Arias PL, Güemez MB, Navarro RM, Sanchez-Sanchez MC, Fierro JLG (2009) Influence of $La_2O_3$ modified support and Ni and Pt active phases on glycerol steam reforming to produce hydrogen. *Catalysis Communications* 10: 1275-1278.

Iriondo A, Barrio VL, Cambra JF, Arias PL, Güemez MB, Navarro RM, Sánchez-Sánchez MC, Fierro JLG (2008) Hydrogen production from glycerol over nickel catalysts supported on $Al_2O_3$ modified by Mg, Zr, Ce or La. *Topics in Catalysis* 49: 46-58.

Johnson DT, Taconi KA (2007) The glycerin glut: Options for the value-added conversion of crude glycerol resulting from biodiesel production. *Environmental Progress* 26: 338-348.

Juszczyk P, Rymowicz W (2009) Characterization of microbial biomass production from glycerin waste by various yeast strains. *In* G Aggelis, ed, *Microbial conversions in raw glycerol*. Nova Science Publishers, Inc., New York, pp 125-135.

Kamm B, Kamm M (2007) Biorefineries-multi product processes. *Adv Biochem Eng Biotechnol* 105: 175-204.

Kamm B, Kamm M (2004) Principles of biorefineries. *Appl Microbiol Biotechnol* 64: 137-145.

Kenji S, Hidehiko, T., Setsuo, F. (1989). *In*. 01225486, JP.

Kimura H, Tsuto K, Wakisaka T, Kazumi Y, Inaya Y (1993) Selective oxidation of glycerol on a platinum-bismuth catalyst. *Applied Catalysis A, General* 96: 217-228.

Knietsch A, Bowien S, Whited G, Gottschalk G, Daniel R (2003) Identification and characterization of coenzyme $B_{12}$-dependent glycerol dehydratase- and diol dehydratase-encoding genes from metagenomic DNA libraries derived from enrichment cultures. *Appl Environ Microbiol* 69: 3048-3060.

Kurosaka T, Maruyama H, Naribayashi I, Sasaki Y (2008) Production of 1,3-propanediol by hydrogenolysis of glycerol catalyzed by $Pt/WO_3/ZrO_2$. *Catalysis Communications* 9: 1360-1363.

Kusunoki Y, Miyazawa T, Kunimori K, Tomishige K (2005) Highly active metal-acid bifunctional catalyst system for hydrogenolysis of glycerol under mild reaction conditions. *Catalysis Communications* 6: 645-649.

Lahr DG, Shanks BH (2005) Effect of sulfur and temperature on ruthenium-catalyzed glycerol hydrogenolysis to glycols. *Journal of Catalysis* 232: 386-394.

Lehnert K, Claus P (2008) Influence of Pt particle size and support type on the aqueous-phase reforming of glycerol. *Catalysis Communications* 9: 2543-2546.

Luo N, Fu X, Cao F, Xiao T, Edwards PP (2008) Glycerol aqueous phase reforming for hydrogen generation over Pt catalyst - Effect of catalyst composition and reaction conditions. *Fuel* 87: 3483-3489.

Ma L, He D, Li Z (2008) Promoting effect of rhenium on catalytic performance of Ru catalysts in hydrogenolysis of glycerol to propanediol. *Catalysis Communications* 9: 2489-2495.

Mantzouridou F (2009) The potential of raw glycerol in the production of food grade carotenoids by fungi. *In* G Aggelis, ed, *Microbial conversions in raw glycerol*. Nova Science Publishers, Inc., New York, pp 101-124.

Maris EP, Ketchie WC, Murayama M, Davis RJ (2007) Glycerol hydrogenolysis on carbon-supported PtRu and AuRu bimetallic catalysts. *Journal of Catalysis* 251: 281-294.

McCoy M (2006) Glycerin surplus. Plants are closing, and new uses for the chemical are being found. *Chemical Engineering* News 84: 7.

Meher LC, Gopinath R, Naik SN, Dalai AK (2009) Catalytic hydrogenolysis of glycerol to propylene glycol over mixed oxides derived from a hydrotalcite-type precursor. *Industrial and Engineering Chemistry Research* 48: 1840-1846.

Miyazawa T, Kusunoki Y, Kunimori K, Tomishige K (2006) Glycerol conversion in the aqueous solution under hydrogen over Ru/C + an ion-exchange resin and its reaction mechanism. *Journal of Catalysis* 240: 213-221.

Mothes G, Otto O (2009) Biotechnological production of bioplastics from raw glycerol. *In* G Aggelis, ed, *Microbial conversions in raw glycerol*. Nova Science Publishers, Inc., New York, pp 65-84.

Murarka A, Dharmadi Y, Yazdani SS, Gonzalez R (2008) Fermentative utilization of glycerol by *Escherichia coli* and its implications for the production of fuels and chemicals. *Appl Environ Microbiol* 74: 1124-1135.

Musial I, Rymowicz W (2009) Biodiesel by-products used as substrates for oxalic acid production by *Aspergillus niger*. *In* G Aggelis, ed, *Microbial conversions in raw glycerol*. Nova Science Publishers, Inc., New York, pp 31-40.

Nemeth A, Sevella B (2008) Development of a new bioprocess for production of 1,3-propanediol I.: Modeling of glycerol bioconversion to 1,3-propanediol with *Klebsiella pneumoniae* enzymes. Appl Biochem Biotechnol 144: 47-58.

Ohara H (2003) Biorefinery. *Appl Microbiol Biotechnol* 62: 474-477.

Pagliaro M, Rossi M (2008) The Future of Glycerol. *New usages for a versatile raw material*. The Royal Society of Chemistry, Cambridge.

Papanikolaou S, Fakas S, Fick M, Chevalot I, Galiotou-Panayotou M, Komaitis M, Marc I, Aggelis G (2008) Biotechnological valorisation of raw glycerol discharged after bio-diesel (fatty acid methyl esters) manufacturing process: production of 1,3-propanediol, citrica acid and single cell oil. *Biomass Bioenergy* 32: 60-71.

Peters D (2007) Raw materials. *Adv Biochem Eng Biotechnol* 105: 1-30.

Porta F, Prati L (2004) Selective oxidation of glycerol to sodium glycerate with gold-on-carbon catalyst: An insight into reaction selectivity. *Journal of Catalysis* 224: 397-403.

Profeti LPR, Ticianelli EA, Assaf EM (2009) Production of hydrogen via steam reforming of biofuels on Ni/CeO$_2$-Al$_2$O$_3$ catalysts promoted by noble metals. *International Journal of Hydrogen Energy* 34: 5049-5060.

Qiao X, Tang Z, Sun K (2011) Plasticization of corn starch by polyol mixtures. *Carbohydrate Polymers* 83: 659-664.

Richey DP, Lin EC (1972) Importance of facilitated diffusion for effective utilization of glycerol by *Escherichia coli*. *J Bacteriol* 112: 784-790.

Rossi CCRS, Alonso CG, Antunes OAC, Guirardello R, Cardozo-Filho L (2009) Thermodynamic analysis of steam reforming of ethanol and glycerine for hydrogen production. *International Journal of Hydrogen Energy* 34: 323-332.

Roy D, Subramaniam B, Chaudhari RV (2010) Aqueous phase hydrogenolysis of glycerol to 1,2-propanediol without external hydrogen addition. *Catalysis Today* 156: 31-37.

Ruzheinikov SN, Burke J, Sedelnikova S, Baker PJ, Taylor R, Bullough PA, Muir NM, Gore MG, Rice DW (2001) Glycerol dehydrogenase. structure, specificity, and mechanism of a family III polyol dehydrogenase. *Structure* 9: 789-802.

Rywinska K, Rymowicz W (2009) Citric acid production from raw glycerol by *Yarrowia lipolytica* Wratislavia 1.31. *In* G Aggelis, ed, *Microbial conversions in raw glycerol.* Nova Science Publishers, Inc., New York, pp 19-30.

Sánchez EA, Comelli, R. A. (2012) Hydrogen by glycerol steam reforming on a nickelealumina catalyst: Deactivation processes and regeneration. *International Journal of Hydrogen Energy.* doi:10.1016/j.ijhydene.2011.12.088.

Sánchez EA, D'Angelo MA, Comelli RA (2010) Hydrogen production from glycerol on Ni/Al$_2$O$_3$ catalyst. *International Journal of Hydrogen Energy* 35: 5902-5907.

Sanno Y, Wilson TH, Lin EC (1968) Control of permeation to glycerol in cells of Escherichia coli. *Biochem Biophys Res Commun* 32: 344-349.

Saxena RK, Anand P, Saran S, Isar J (2009) Microbial production of 1,3-propanediol: Recent developments and emerging opportunities. *Biotechnology Advances* 27: 895-913.

Schell C, Riley C, Petersen GR (2008) Pathways for development of a biorenewables industry. *Bioresour Technol* 99: 5160-5164.

Seifert C, Bowien S, Gottschalk G, Daniel R (2001) Identification and expression of the genes and purification and characterization of the gene products involved in reactivation of coenzyme B$_{12}$-dependent glycerol dehydratase of *Citrobacter freundii*. *Eur J Biochem* 268: 2369-2378.

Slinn M, Kendall K, Mallon C, Andrews J (2008) Steam reforming of biodiesel by-product to make renewable hydrogen. *Bioresource Technology* 99: 5851-5858.

Sweet G, Gandor C, Voegele R, Wittekindt N, Beuerle J, Truniger V, Lin EC, Boos W (1990) Glycerol facilitator of *Escherichia coli*: cloning of glpF and identification of the glpF product. *J Bacteriol* 172: 424-430.

Teruyuki N, Yoshinori, K. (1989). *In*. 1168292, JP.

Truniger V, Boos W (1994) Mapping and cloning of gldA, the structural gene of the Escherichia coli glycerol dehydrogenase. *J Bacteriol* 176: 1796-1800.

Valliyappan T, Ferdous D, Bakhshi NN, Dalai AK (2008) Production of hydrogen and syngas via steam gasification of glycerol in a fixed-bed reactor. *Topics in Catalysis* 49: 59-67.

Vasiliadou ES, Heracleous E, Vasalos IA, Lemonidou AA (2009) Ru-based catalysts for glycerol hydrogenolysis-Effect of support and metal precursor. *Applied Catalysis B: Environmental* 92: 90-99.

Vaudagna SR, Comelli RA, Fígoli NS (1997) Influence of the tungsten oxide precursor on WO$_x$-ZrO$_2$ and Pt/WO$_x$-ZrO$_2$ properties. *Applied Catalysis A: General* 164: 265-280.

Wang X, Li S, Wang H, Liu B, Ma X (2008) Thermodynamic analysis of glycerin steam reforming. *Energy and Fuels* 22: 4285-4291.

Wang K, Hawley MC, DeAthos SJ (2003) Conversion of glycerol to 1,3-propanediol via selective dehydroxylation. *Industrial and Engineering Chemistry Research* 42: 2913-2923.

Wang ZX, Zhuge J, Fang H, Prior BA (2001) Glycerol production by microbial fermentation: a review. *Biotechnol Adv* 19: 201-223.

Wang S, Liu H (2007) Selective hydrogenolysis of glycerol to propylene glycol on Cu-ZnO catalysts. *Catalysis Letters* 117: 62-67.

Yaylayan AV, Harty-Majors S, A. Ismail A (1999) Investigation of dl-glyceraldehyde–dihydroxyacetone interconversion by FTIR spectroscopy. *Carbohydrate Research* 318: 20-25.

Yuan Z, Wang J, Wang L, Xie W, Chen P, Hou Z, Zheng X (2010) Biodiesel derived glycerol hydrogenolysis to 1,2-propanediol on Cu/MgO catalysts. *Bioresource Technology* 101: 7088-7092.

Yuan Z, Wu P, Gao J, Lu X, Hou Z, Zheng X (2009) Pt/solid-base: A predominant catalyst for glycerol hydrogenolysis in a base-free aqueous solution. *Catalysis Letters* 130: 261-265.

Zhang B, Tang X, Li Y, Xu Y, Shen W (2007) Hydrogen production from steam reforming of ethanol and glycerol over ceria-supported metal catalysts. *International Journal of Hydrogen Energy* 32: 2367-2373.