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New trends in bioprocesses for lignocellulosic biomass and CO$_2$ utilization

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A B S T R A C T

Innovation and technology are seen today as key points in the transition to a greener and more sustainable economy. In this sense, the development of new processes able to result in reduced emissions and levels of CO$_2$ in the atmosphere has been considered essential to support this transition and, at the same time, promote economic growth. Several techno-economic and life cycle assessment studies have shown promising data when sugars derived from lignocellulosic biomass are used as carbon source for fermentation processes. The use of CO$_2$ as carbon source for fermentation is another smart concept for reusing this molecule while minimizing its emissions to the atmosphere. However, the large-scale implementation of biomass-based and CO$_2$-based processes still require significant research efforts to result in robust and cost-competitive technologies. This paper discusses some promising approaches able to advance this research area including potential strategies for process intensification (enzymatic hydrolysis using high solid loading, simultaneous saccharification and fermentation, fermentation with downstream process integration), robust microbial strains for application in biomass-based and CO$_2$-based bioprocesses, microbial co-cultivation systems, greener technologies for lignocellulosic biomass fractionation, among others. A critical evaluation of sustainability aspects including techno-economic and life cycle assessment is also provided. Overall, this study contributes with information on innovative trends able to advance the development of greener bioprocesses.

1. Introduction

Provisional figures released by the World Meteorological Organization suggested that 2020 was one of the three warmest years on record globally. This exceptional global heat is driven by greenhouse gas (GHG) emissions. Like in the previous years, concentrations of the major GHGs (CO$_2$, CH$_4$, and N$_2$O) continued to increase in 2019 and 2020, reaching new record highs [1]. Due to this scenario, countries around the world are taking actions to reduce their GHGs emissions in an important battle against climate change. In this sense, special attention has been given to the development of new industrial processes able to result in reduced carbon emissions, as well as in promoting an enhanced use of renewable resources in order to reduce fossil fuel dependency, protecting the climate and the environment. Today, plant substrates, also called as lignocellulosic biomass, are seen as one of the most promising materials to replace fossil resources in the production of fuels and chemicals with reduced GHGs emissions [2]. The interest in such raw materials is

further boosted by the fact that they are not only of value for the fuel and chemical industries, but they can also be relevant for several other industrial sectors including food, feed, pharmaceutical and materials, where they can directly provide valuable compounds such as protein, amino acids, oligosaccharides, and phenolics, or can be used to obtain sugars for fermentation purposes. Due to the potential applications and expected benefits, the development of new biomass-based technologies is seen nowadays as one of the main drivers of our society to move towards a more sustainable future with reduced GHGs emissions and a more appropriate use of natural resources.

Several techno-economic and life cycle assessment studies have shown promising data when biomass-derived sugars are used in fermentation processes [3–6]. However, some important points still have to be improved to create technologies with enough robustness for large-scale implementation. One of the points requiring improvements is the biomass fractionation step, which is still expensive due to the high energy demand. In addition, this step usually requires the use of strong

chemicals (like sulfuric acid, sodium hydroxide or ammonia, among others) for an efficient disruption of the cell wall structure, which is not an environmentally friendly option [7]. Another point requiring improvements is the fermentation step to convert sugars from the biomass-based hydrolysate medium. Due to the complexity of these media (presence of different types of sugars and several inhibitors derived from the pretreatment step), the sugar's conversion yields from hydrolysates are in general low and make these processes non-competitive when compared to the chemical routes currently employed on an industrial scale [8]. Improving the performance of the microbial strains for an efficient conversion of sugars from complex substrates (hydrolysates produced from lignocellulosic biomass) is an important question to be solved to support the large-scale implementation of these bioprocesses. Finally, biomass feedstocks are also composed by other relevant compounds besides sugars, which may include lignin, protein, and even lipids. Valorization of these compounds in a biorefinery perspective could be a potential alternative to improve the economic feasibility of the sugar's conversion processes, while would also contribute for zero-waste generation [9].

Besides the establishment of new processes using lignocellulosic feedstocks to replace fossil resources, another promising option to help reduce GHG emissions is the development of technologies for CO_2 utilization. The idea in this case is to turn waste CO_2 emissions into valuable products, such as chemicals and fuels [10]. Currently, fixation and conversion of CO_2 for the production of biobased compounds is an emerging area of interest. Besides this option, another alternative for CO_2 utilization would be in the step of biomass pretreatment. In this case, CO_2 could be considered a more sustainable option when compared to the use of strong chemicals usually employed for biomass fractionation [11]. Both concepts of CO_2 utilization still require significant efforts to make CO_2-based processes a reality in a large scale.

In this paper, the most promising trends for the development of biomass-based and CO_2-based bioprocesses are presented and discussed. Focus is given to aspects that can positively impact the efficiency and sustainability of the process technology, including principles of process intensification, simultaneous saccharification and fermentation, fermentation using co-cultivation of microorganisms, microbial conversion of CO_2, and CO_2 utilization for biomass fractionation. Finally, potential techno-economic and environmental aspects related to the use of these promising technologies are also discussed.

2. Process intensification: an alternative to improve biomass conversion yields with reduced operational costs

The concept of process intensification, which originated in the chemical process industry, was created with the aim of reducing capital costs of the production processes through the reduction in equipment size. Over the years, this concept has been expanded and today it comprises four main pillars: spatial (structure), thermodynamic (energy), functional (synergy), and temporal (time) (Fig. 1). Spatial refers to maximizing the homogeneity of the system, which is done by promoting systematic physical interferences to induce homogeneous molecular collisions/diffusion. Energy means to maximize the thermodynamic driving forces and transfer area to increase the effectiveness of the reaction. Synergy relates to the fusion of multiple functions to promote a significantly better performance compared to the sequential application of individual functions. Finally, time translates into kinetic improvements to maximize the reaction rates and effectiveness of molecular events [12].

Despite originated in the chemical industry, due to the potential to improve process performance and reduce costs, this concept has also been applied in different areas. Application of process intensification approaches to bioprocesses, for example, has been done with the main aim of maximizing the three key fermentation performance indicators: titer, productivity, and yield. In this case, intensification of fermentation processes corresponds to maximize homogeneity (structure), relieve transport phenomena limitations (energy), arrange smart integration (synergy), and improve kinetics (time) [13].

Process intensification principles have also been considered to advance the techno-economic potential of lignocellulosic biorefineries. This has been done through strategies such as use of high solid loading for enzymatic hydrolysis of biomass, simultaneous saccharification and fermentation processes, and fermentation with downstream process integration. All these examples relate to the four main pillars of process intensification (Fig. 1). The spatial approach, in particular, can be explained through the use of customized equipment or system to improve the process efficiency. The reactor configuration is in fact one of the main points affecting the efficiency of biomass processing. A simulated plug-flow reactor (PFR) or a combination of continuous stirred-tank reactors (CSTRs) in series, for example, can be more effective for enzymatic hydrolysis when using customized impellers and baffles on
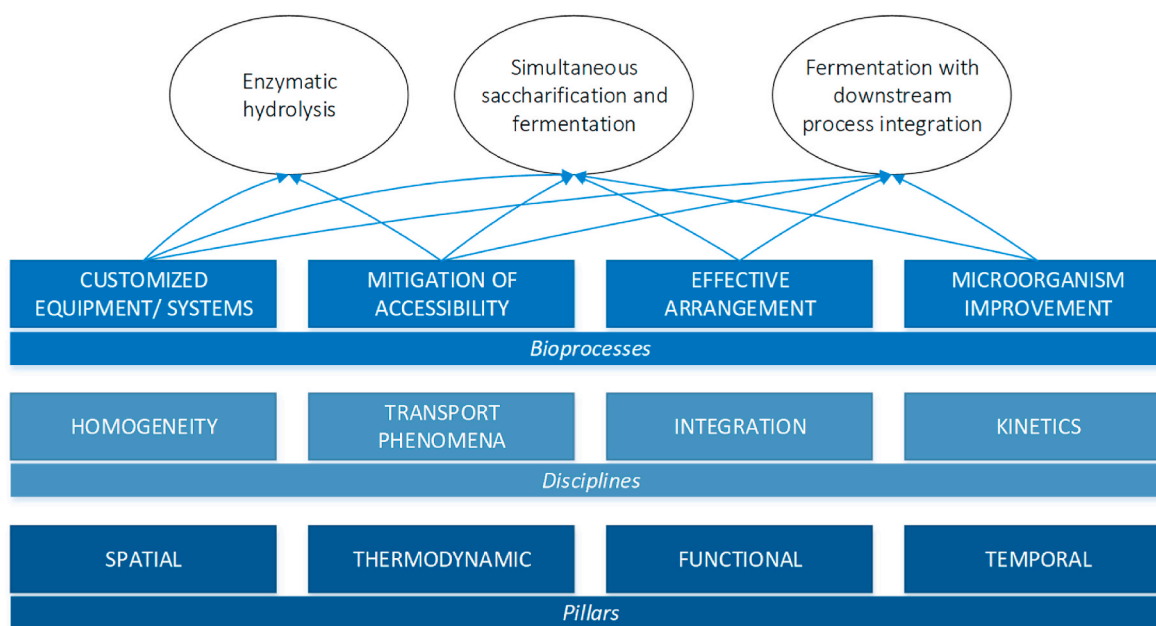

Fig. 1. The process integration concept applied to biomass bioprocessing.

each stage [14,15]. Such systems can potentially improve the enzymes accessibility to the biomass structure.

A feasible industrial implementation of simultaneous saccharification and fermentation processes requires overcoming important issues such as homogeneity, transport phenomena, integration and kinetics. Homogeneity can be solved by equipment customization to improve transport phenomena. However, to design an effective system for such application, the right combination of on-line measurement, big data & analytics, and model prediction is necessary to adjust/control the process parameters on real-time, as well as to keep the conversion rate at the maximum values during both, saccharification and fermentation steps, regardless of the variation in the composition of the raw material [9].

Currently, the use of microbial strains with improved ability to convert sugars into the product of interest is also a strategy that has been used for bioprocess intensification. Different techniques including metabolic engineering, synthetic biology and evolutionary engineering can be applied to generate strains with enhanced conversion rates and robustness for industrial scale application [16]. Robust strains are also key elements for application in integrated processes such as simultaneous saccharification and fermentation, and fermentation with downstream process integration, since they are able to tolerate the non-conventional conditions applied in these systems. Besides the development of robust cell factories, the integration of unit operations for a combined fermentation with downstream process, for example, also requires special attention and complex design rules to result in an efficient performance [17].

2.1. Enzymatic hydrolysis using high solid loading

Use of high solid loading for enzymatic hydrolysis has been proposed as an alternative to improve the feasibility of lignocellulosic biorefineries by reducing the capital and operational costs. A high solid loading process corresponds to a system where the ratio between insoluble solids and liquid is such that very little to no free water is present in the slurry during the saccharification reaction. Such characteristics are commonly observed when using solid amount higher than 15 wt%. Therefore, to obtain a successful performance of high solid loading enzymatic hydrolysis systems, some important aspects need to be properly addressed, among of which, understanding the water constraint effects, as well as the inhibitory effects of others molecules released from biomass structure or of the reaction product on enzyme activity, are some of the most important [18,19]. Additionally, an appropriate equipment must be used to obtain a feasible operation at high solid content, which should be selected by taking into account the type of lignocellulosic biomass (pretreated or not) and process configurations (including the amount of solid) to be used. Enzymatic hydrolysis of alkaline pretreated biomass at 20 wt% solid content, for example, resulted in an increased glucose yield (from 70 to 76%) when a stirred reactor designed with a segmented helical stirrer was used instead of the standard stirrer [15]. In fact, the impeller geometry significantly affects the performance of enzymatic hydrolysis with high solid loading. In this sense, a double helical impeller has shown better results of mixing time, energy consumption and sugars released compared to inclined blade or marine impellers, when operated either in batch or fed-batch modes [20]. Customization of equipment and selection of an appropriate operating mode can address issues related to solids homogenization and transport phenomena, making feasible the operation at high solid loading.

Besides mechanic and mass transfer constraints of operation at high solid loadings, enzymes inhibition by end-products is another challenging aspect to overcome in these systems. Hydrolysis of cellulose by the action of commercial cellulolytic enzymes (composed mainly by endoglucanases, cellobiohydrolases and β-glucosidases enzymes) results in cellulase inhibition when high concentration of sugars is reached in the medium [21]. The mechanism of inhibition by end-products is still not well elucidated. A recent study on the inhibition action at the

solid-liquid interface explained the type of surface inhibition by the development of a novel steady-state kinetic approach [22]. However, additional efforts are required to better understand the inhibition mechanism. Knowledge about it will make it possible to enhance the enzyme mixture strategy, as well as will support an improved operational control in fed-batch process, for example. Additionally, it will also allow to advance the development of more efficient enzyme cocktails and, consequently, will benefit the viability of biomass-based processes, being also of interest to biorefineries. In this sense, the development of tailor made cellulase cocktails has attracted great attention in the last years to produce enzymes for specific processes. Some important criteria considered to produce such cocktails include the characteristics of the substrate, optimum temperature, adsorption effect, reaction time, solid loading, and final product concentration [9,23].

Briefly, some important points to be considered for an efficient enzymatic hydrolysis using high solid loading include: 1) Use of customized equipment/system to attend the rheological properties of the mixture while promoting an efficient contact between substrate and enzymes with minimal energy requirement for mixing; 2) Combination of an appropriate operational mode with feeding strategies, synchronized by on-line measurements for optimum process control; 3) Use of enzymatic cocktails with improved characteristics to promote hydrolysis of biomass (combined enzymes and/or tailor made cocktails).

2.2. Simultaneous saccharification and fermentation

The simultaneous saccharification and fermentation (SSF) process was created with the purpose of alleviating the end-product inhibition during enzymatic hydrolysis of cellulose [24]. Today, however, this process has been mainly used to improve the techno-economic feasibility of lignocellulosic biorefineries, especially for second-generation ethanol production, since it can also provide other important benefits such as reduction of capital investment and operational costs, and improvement of the overall process efficiency. A techno-economic study of ethanol production from softwood, for instance, resulted in higher ethanol yield (70%) and lower production cost (0.57 USD/L) when the SSF configuration was used instead of the separate hydrolysis and fermentation, which gave an ethanol yield of 62% and production cost of 0.63 USD/L [25]. Further improvements of the SSF process can still be achieved through process intensification using high solid loading and customized reactors. Such systems allow increasing the final product concentration, reducing the costs related to downstream process. In this sense, a customized vertical ball reactor has been recently proposed for SSF of high solid loading biomass (24% w/v), for example. When applied for SSF of rice straw pretreated by sequential deacetylation and diluted acid processes, in a fed-batch mode using a commercial cellulase cocktail and a thermotolerant *Kluyveromyces marxianus* strain, this system resulted in an ethanol production of 52.3 g/L, productivity of 1.10 g/L.h, hydrolysis yield of 71%, and fermentation yield of 67% [26].

One of the main challenges of SSF processes is to define the optimum temperature, since it must be compatible with both processes, enzymatic hydrolysis and fermentation. In that case, thermotolerant strains able to work under temperatures higher than 40 °C are the most suitable candidates for application in SSF. For non-thermotolerant strains, a promising approach to solve the temperature issue is to submit the strain to adaptive laboratory evolution (evolutionary engineering) in order to improve its performance at higher temperatures. The use of evolved strains able to work at temperatures closer to the optimal for the enzymes can potentially increase the sugars consumption and product formation during SSF. This strategy has been shown to be useful for improving the production of both biofuels and biochemicals by SSF [27, 28]. In a different perspective, improving the enzyme activity for an efficient performance at lower temperatures could also be an option to overcome the temperature issue in SSF processes. Although still little investigated, a recent study has shown that the application of moderate electric field accelerates the enzyme activity below the optimal

temperature. Application of 8 and 12 V/cm during the enzymatic hydrolysis with commercial enzymes at 30 and 40 °C caused a significant increase (34–123%) of released sugars compared to the control experiment without application of electric field [29]. According to the study, the electrophoretic motion introduced by the moderate electric field is similar in magnitude to the molecular motion introduced through the increased temperature.

Another alternative to solve the temperature issue in SSF processes is to promote changes in the process configuration, as for example, performing a sequential conversion of xylose and glucose using a fed-batch simultaneous saccharification and co-fermentation. When applied for ethanol production from steam-pretreated wheat straw, this variant of the SSF strategy resulted in an efficient conversion of both, xylose and glucose sugars, reaching ethanol yield of 92% even using a temperature not optimum for the action of enzymes [30]. In summary, combination of process strategies and microbial improvement can significantly enhance the efficiency of SSF processes, also impacting the economic feasibility of the production process due to the reduced requirement of equipment and reduced operational costs. For the future, the achievements and important findings already reported on SSF for ethanol production could be used to guide the development of SSF for the production of other valuable biobased products.

2.3. Fermentation with downstream process integration

For the development of an efficient process technology, production and downstream steps should be developed together in order to design the optimum operation conditions and minimize interface problems. In addition, fermentation processes are commonly hampered by a series of problems caused by the accumulation of products in the fermenter. Integration of both steps, fermentation and product separation, can overcome this problem. Moreover, fermentation with downstream process integration is a useful approach to remove a product that, at a certain concentration, may inhibit the microbial metabolism, reducing the fermentation efficiency as a consequence. By removing the product as soon as it is formed, the product formation can be accelerated, and higher titers and yields can be achieved. Some examples of reaction-separation processes include systems such as hybrid reaction-membrane separation (membrane filtration, membrane electrodialysis, membrane pervaporation, or membrane distillation), extractive fermentation, reactive distillation, and adsorptive distillation [31].

A successful example of fermentation with downstream process integration has been reported for the production of butanol by *Clostridium acetobutylicum* ATCC 824. Application of a two-stage continuous cultivation system coupled to a hydrophobic pervaporation unit for simultaneous product recovery resulted in a significant productivity increase (from 0.13 to 0.30 g/L.h) due to the alleviation of product inhibition and increased concentration of substrate used [32]. Another case of success is for the production of succinic acid. In this case, fermentation using an acid resistant engineered strain of *Escherichia coli* combined with reactive extraction resulted in no needed of pH adjustment and, as a consequence, no gypsum formation [33]. Moreover, when an integrated separation process composed by single-step batch reactive extraction followed by a two-step back extraction and final cracking and crystallization was used, succinic acid was obtained with high yield (>99%) and purity (99.5%) [34]. These examples clearly show that the equipment choice and strategy to be used for integration of the fermentation and downstream processes steps have to be evaluated case by case, taking into account the product to be produced. In addition, modeling tools and techno-economic assessment can support the prediction of capital and operation costs, being also useful to help in the decision of the most suitable equipment/strategy to be used on a large scale.

3. Co-cultivation of microorganisms: a promising approach to convert complex substrates

Lignocellulosic biomass has a huge potential to be used as feedstock for a sustainable production of fuels and chemicals through fermentation. However, the recalcitrance of these materials is one of the major hurdles in their efficient utilization. Solutions to overcome this recalcitrance and release sugars for fermentation have been proposed by means of pretreatment, which is usually performed by chemical or physicochemical methods, followed by enzymatic hydrolysis [7,35]. In this sense, microbial research has been mainly focused on the production of cheaper enzymes and in a more efficient utilization of biomass derived sugars [36,37].

As discussed before, the performance of a fermentation process can be improved by using microbial strains with enhanced ability to convert sugars into product. The classical methods used for strain improvement (e.g. metabolic engineering, synthetic biology) are often limited to promote modifications by increasing, adding and/or deleting an intracellular process [37]. Another option and also regularly applied method is the adaptive laboratory evolution (ALE) in which the microorganism is exposed to stress conditions to induce beneficial mutations [8]. At the end, the evolved strain has ability to do conversions or endure conditions that the wild-type counterpart do not naturally have [38]. However, even considering the significant advances in these research fields, a profitable utilization of lignocellulosic biomass has not yet been reached.

A relatively new strategy to increase the effectiveness of biotechnological biomass usage is to employ a combination of two or more different microbial species for bioprocessing. This co-cultivation approach can potentially alleviate some of the problems associated to the lignocellulose use. The general idea of this concept is to take advantage of the specialized ability of two or more organisms and create a synergistic effect. Since multiple strains are used in a single process, a broader variation in beneficial characteristics can be selected. Optimization of a co-cultivation process could then be performed by selecting the right strains to be combined, instead of engineering one do-it-all strain.

Co-cultivation of microorganisms has already been utilized for centuries to produce sake, for example. In sake fermentation, a consortium of *Aspergillus oryzae* and *Saccharomyces cerevisiae* is commonly used to produce high concentration of ethanol. In this process, *A. oryzae* secretes enzymes that hydrolyze the rice starch into glucose, while *S. cerevisiae* then converts glucose into ethanol. Due to the continuous supply and uptake of glucose during the process, the enzyme's inhibition by the product is reduced. Additionally, catabolite repression might be reduced by the constant low concentration of sugars, increasing the overall fermentation efficiency [39]. Sake fermentation is thus a prime example of how co-culturing of multiple species can have an overall synergistic effect, beneficial to the bioprocess.

In the case of biomass conversion, co-cultivation might offer solutions to three major problems inherent to the use of lignocellulose. The first is the need for a pretreatment step to overcome the recalcitrance of these materials [7]. During pretreatment, high temperatures, pressure and acidic or alkaline conditions are often combined, which allow an efficient disruption of the biomass structure. However, during the application of these harsh conditions, some degradation reactions also occur leading to the formation and release of different compounds to the reaction medium. Degradation products are formed from the distinct components of lignocellulose and such compounds may cause severe inhibition of the microbial metabolism during fermentation of biomass-based media (hydrolysates) depending on their concentration in these media [7]. Co-cultivation of microbial strains could be an option to minimize the inhibitory effect of these compounds, then favoring the product formation.

The second major hurdle preventing wide scale lignocellulosic biomass usage is the need for expensive cellulase enzymes. These

enzymes break down cellulose from pretreated biomass releasing fermentable sugars. Cellulase enzymes can account for up to 48% of process costs in biomass processing [40]. Co-cultivation can be applied in this case to produce the enzymes necessary for biomass deconstruction and simultaneously promote the conversion of released sugars into the desired product (SSF configuration).

A third factor impeding an efficient utilization of biomass in bioprocesses is the presence of both hexose and pentose sugars in biomass hydrolysate. For an efficient and simultaneous co-fermentation of hexose and pentose sugars, extensive efforts in metabolic and evolutionary engineering of microorganisms are required since most microorganisms can only ferment either one of these sugars at a time, most commonly the hexoses, due to the catabolite repression effect [41,42]. Co-cultivation might furthermore increase the overall yield of a process. In brief, co-cultivation can play a vital role in alleviating problems such as presence of inhibitory compounds and mixture of sugars, making the use of lignocellulosic biomass more profitable. Some examples of recent studies reporting these three potential applications of co-cultivation systems for biomass conversion are summarized in Table 1.

3.1. Co-cultivation for hydrolysate detoxification and improvement of product yield

The focus of most lignocellulose-based fermentation processes is to reach an efficient conversion of carbohydrates. However, as biomass has a complex structure, a variety of other non-carbohydrate compounds are also present in the hydrolysates together with sugars, which are not efficiently or not used at all during fermentation [53]. Lignin, for example, a complex heteropolymer consisting of mostly aromatic compounds, is one of the major structural components in lignocellulose. Depolymerization of lignin into its subunits during pretreatment leads to the generation of phenolic compounds that can inhibit the microbial metabolism [54]. A bacterial species, *Rhodococcus opacus,* has been recently found to tolerate and utilize lignin derived aromatic compounds [45]. Owing to this unique ability, it could play a vital role in the valorization of lignin. Co-cultivation of *R. opacus* during the fermentation of biomass hydrolysates, for example, could convert these toxic aromatic compounds into a high value co-product. Additionally, by consuming such compounds, *R. opacus* would reduce the toxicity of the fermentation broth, benefiting the formation of the main product by the other strain. Moreover, this bacterium is able to accumulate large amounts of intracellular lipids [55], which would bring additional benefits to the bioprocess through the generation of an extra co-product.

Besides the aromatic/phenolic compounds, other degradation products formed during the biomass pretreatment step include furan derivatives (furfural and hydroxymethylfurfural) from carbohydrate breakdown. Furan derivatives have been reported to be degradable by the bacterial species *Bacillus subtilis* DS3 [43]. So, this strain could also be a potential candidate for application in the detoxification of biomass hydrolysates through co-cultivation systems.

Carbohydrates in lignocellulosic biomass consist of pentose and hexose sugars. However, so far, fermentation processes have been mainly focused on the conversion of hexoses, as the number of microbial strains with ability to efficiently convert pentoses is limited. When both sugars are present in a medium, microorganisms tend to utilize hexoses first, and will only consume pentoses when hexose is no longer available [41]. This mechanism is called carbon catabolite repression (CCR) and is a method used by the cells to reduce the energy needs. When CCR is active, the synthesis of enzymes used in the catabolism of other sugars is repressed. Co-cultivation with a naturally pentose fermenting strain could alleviate this problem. In this case, the main organism could still utilize hexoses, while the secondary organism would ferment the pentoses. Then, the CCR would be avoided. *Pachysolen tannophilus* is an example of yeast capable of using xylose to produce ethanol. This yeast has already been tested in a co-cultivation with *S. cerevisiae* to produce ethanol from a medium containing mixture of xylose and glucose [49]. Indeed, the sugars conversion yield was improved when the co-cultivation was used instead of the single species fermentation. However, this process still requires improvements since the produced ethanol caused inhibition to *P. tannophilus*.

3.2. Co-cultivation for simultaneous enzyme production and product formation

One of the most expensive steps in biomass processing is the enzymatic hydrolysis of cellulose. The main reason for this is the high cost of cellulase enzymes, which can account for 20% up to 48% of the total processing costs [40]. Several yeast and fungal strains are known to produce significant amounts of extracellular cellulase. A good cellulase producing strain is, for example, *A. oryzae*. This strain is able to produce cellulases and endoxylanases when grown in lignocellulosic materials, which makes it a potential candidate for use in the hydrolysis of lignocellulosic biomass [56]. Additionally, it also produces proteases, which could reduce the need of additional nitrogen sources during fermentation. Recently, co-cultivation of *A. oryzae* with *S. cerevisiae* was found to be very promising for use in the production of ethanol from brewer's spent grains, without requiring the addition of extra cellulases for enzymatic hydrolysis of the biomass [39]. Promising results have also been reported for the co-cultivation of extremely thermophilic co-cultures of *Caldicellulosiruptor* and *Thermoanaerobacter*, which did not require addition of cellulases to the system and resulted in an efficient conversion of both pentose and hexose sugars into ethanol as main

Table 1
Potential applications of co-cultivation strategies for biomass conversion.

Purpose	Substrate	Product	Microorganism used	Reference
Detoxification	Furfural	–	*Bacillus cereus*	[43]
	Furfural	–	*Bacillus subtilis*	[43]
	Furfural & Hydroxymethylfurfural	–	*Amorphotheca resinae* ZN1	[44]
	Furfural & Hydroxymethylfurfural	–	*Cupriavidus basilensis* HMF14	[52]
Detoxification with improvement of product yield	Lignin derived aromatics	Lipids	*Rhodococcus opacus*	[45]
Simultaneous cellulose hydrolysis and fermentation	Avicel & pretreated biomass	Ethanol	*Caldicellulosiruptor* & *Thermoanaerobacter*	[46]
	Brewer's spent grains	Ethanol	*Aspergillus oryzae* & *Saccharomyces cerevisiae*	[39]
	Unbleached hardwood kraft pulp	Acetone, Butanol, Ethanol	*Clostridium saccharoperbutylacetonicum* & *phlebia*	[47]
	Avicel	Ethanol	*Clostridium thermocellum* & *Thermoanaerobacterium saccharolyticum*	[48]
Simultaneous pentose and hexose usage	Glucose and xylose	Ethanol	*Zymomonas mobilis* & *Pachysolen tannophilus*	[49]
	Hexose and pentose	Ethanol	*Candida shehatae* & *Saccharomyces cerevisiae*	[50]
	Hexose and pentose	Ethanol	*Pichia stipitis* & *Brettanomyces clausenii*	[51]

fermentation product [46].

These examples demonstrate the potential of co-cultivation systems for use on the simultaneous production of cellulase and conversion of sugars into product. By reducing the need of adding cellulase enzymes to the process, a significant cost reduction can be achieved. Additionally, the synergistic effects of using multiple strains can also result in an increased consumption of substrate and product formation, bringing additional benefits to the process. Overall, these findings open up new opportunities to advance the development of a lower cost process for enzymatic hydrolysis of biomass and also bring new perspectives for biorefineries. It is also worth noting that performing the production of cellulase enzymes and fermentation in only one step is closer to the development of a consolidated bioprocess for biomass conversion, which has been much envisioned in order to simplify the process and reduce costs. Finally, the possibility of integrating different process steps with impact in the economic feasibility of the global process also makes the co-cultivation strategy a way of promoting process intensification.

3.3. Strain improvement for specific co-cultivation applications

An added benefit of co-cultivation systems compared to single species fermentation is a potentially reduced need for genetic modifications. A co-cultivation process can be efficiently performed by just selecting the right complementary strains. In some cases, the conditions of the process should also be fine-tuned as the two species may have different optimal conditions. So, a balance between the two has to be found. Even when the two ideal strains are combined in the right conditions, genetic modifications might still be an option for further optimization. In general, the same methods used in regular cell factory engineering could be applied. An increase in product yield, for example, will have beneficial effects on any process, with a single species or multiple strains. This can be done by finding bottlenecks in a species' metabolism and alleviating them or by suppressing non-relevant energy intensive processes in the cell.

When two strains are combined to achieve simultaneous cellulose hydrolysis and fermentation, for example, modifications could be focused on making cellulose hydrolysis as efficient as possible. This improvement could come from increased cellulase production and/or efficiency gains of the cellulase enzymes. Additionally, reduction of sugar uptake by the cellulolytic organism, like knockdown of *pyruvate decarboxylase*, would increase the glucose availability for the main product formation [47]. Finally, genetic tools could be used to make the ideal process conditions of both species more in line with each other, and/or to increase process efficiency. ALE could also be considered to improve the robustness of the strains before application in the co-cultivation systems.

4. CO$_2$ utilization in bioprocesses

Numerous studies have shown that unprecedented climate change and global warming are largely due to the increased concentration of GHG, particularly CO$_2$, in the atmosphere. Among the main causes for this worrying increase in the level of atmospheric CO$_2$, anthropogenic emissions, that is, those originated from human activities, can undoubtedly be highlighted, especially from burning of fossil fuels for heat, electricity, and transportation [57]. In this regard, the atmospheric concentration of CO$_2$ has steadily increased since preindustrial times, from about 280 parts per million (ppm) at the beginning of the Industrial Revolution [58] to a current level of 414 ppm, measured on December 2020 [59]. On the other hand, catastrophic consequences such as rising sea levels, increased ocean acidity, melting of polar ice caps and glaciers, droughts and floods, loss of biodiversity on the insect, animal and plant kingdoms and drop in food production, have been predicted if the CO$_2$ emission rate continues at the same current level and the average global temperate surpasses that of the preindustrial era by 2 °C [60]. Therefore, there is an urgent need to not only remove the CO$_2$ that has already been

emitted into the atmosphere, but also to reduce further emissions of this greenhouse gas. To this end, fossil fuels should be avoided and replaced by renewable feedstocks to produce sustainable bioproducts such as biofuels and biochemicals, while the atmospheric CO$_2$ in excess should be captured, stored and/or reused to provide both environmental and economic benefits.

Different Carbon Capture and Storage (CCS) approaches have been considered to reduce the concentration of CO$_2$ in the atmosphere. Some of the capture techniques are based on physical and/or chemical processes such as adsorption, absorption, membrane separation, cryogenic distillation and chemical looping combustion. After separating the CO$_2$ from other flue gases, it can be stored either in the ocean or underground. However, storing CO$_2$ underground has raised some concerns over costs and stability, as the long-term risks associated with this process are not fully known. For instance, the release of CO$_2$ stored in the oceans could lead to high acidification levels affecting the marine biota [61].

A more attractive solution is the Carbon Capture and Utilization (CCU) approach, in which CO$_2$ is sequestered and then converted into commercially valuable chemicals and fuels, or used directly either as a greener alternative to more traditional solvents in the food and beverage industries, for example, or in fire protection systems, among other applications. These non-conversion uses of CO$_2$ as well as its utilization as a feedstock for chemical production have the potential to reduce CO$_2$ emissions by at least 3.7 GT/y (approximately 10% of the current annual CO$_2$ emissions), both directly and by reducing the use of fossil fuels [61].

4.1. Microbial conversion of CO$_2$: potential and perspectives

Several processes have been reported for CO$_2$ utilization including biological and enzymatic conversion, photo- and electro-chemical reduction, as well as chemical and solar-thermal/catalytic processes [61]. Among these, biological CO$_2$ mitigation systems have attracted much attention as an environmentally benign solution to bioremediate atmospheric CO$_2$ with simultaneous generation of useful products in a sustainable way. These processes involve the use of photosynthetic and non-photosynthetic microorganisms such as microalgae, cyanobacteria, bacteria and archaea (Table 2), which have different metabolic pathways, conversion mechanisms and abilities to accumulate/produce valuable metabolites [62].

4.1.1. CO$_2$ conversion by photosynthetic microorganisms

Most of the photosynthetic microorganisms assimilate CO$_2$ primarily through the Calvin-Benson-Bassham cycle, converting the inorganic carbon into complex organic compounds. Significant amounts of lipids and starch, for example, which can be subsequently used to produce biodiesel and ethanol, respectively, have been found in microalgal biomass after optimization of the CO$_2$ fixation conditions [63]. Photosynthetic bacteria are also interesting microorganisms to be used on CO$_2$ utilization since they are able to produce multiple products such as hydrogen, organic acids, alcohols, among others. One of the major groups of photosynthetic organisms includes a variety of bacteria of the phylum Proteobacteria (e.g. Purple photosynthetic bacteria), which have the ability to store carbon in the form of polyhydroxyalkanoates (PHAs) - biodegradable polymers used as bioplastics [64].

4.1.2. CO$_2$ conversion by non-photosynthetic microorganisms

Non-photosynthetic microorganisms such as mixed anaerobic bacteria (e.g., methanogenic bacteria, acetogenic bacteria, acidogenic bacteria, *Actinobacteria* and *Crenarcheota* (both aerobic and anaerobic)) perform gas fermentation through different CO$_2$ fixation pathways: the Wood-Ljungdahl (WL), the reductive Tricarboxylic acid cycle (rTCA), the reductive acetyl-Co A (rACoA), the dicarboxylate-4-hydroxybutyrate (DC-4HB) and the 3-hydroxypropionate bicycle (3-HP-Fuchs-Holo), depending on the microbial taxa [65]. Among the non-photosynthetic strains, acetogenic bacteria (most of which belong to the genera

Table 2
Examples of microbes capable of assimilating CO_2 and their carbon fixation pathways.

Organisms				Carbon fixation pathways
Photosynthetic organisms (trap light energy (photons) and store it as chemical energy)	Oxygenic organisms (produce oxygen as by-product of photosynthesis)	Microalgae	*Chlamydomonas reinhardtii; Chlorella sorokiniana; Chlorella vulgaris; Dunaliella salina; Dunaliella tertiolecta; Nannochloropsis oculata; Scenedesmus dimorphus; Scenedesmus obliquus*	Calvin-Benson-Bassham (CBB) cycle
		Cyanobacteria (a.k.a blue-green bacteria or blue-green algae)	*Arthrospira platensis; Nostoc punctiforme; Anabaena cylindrica*	Calvin-Benson-Bassham (CBB) cycle
	Anoxygenic organisms (unable to produce oxygen)	Photosynthetic bacteria	Purple sulfur bacteria; Purple non-sulfur bacteria; Green sulfur bacteria; Green non sulfur bacteria; Heliobacteria	Calvin-Benson-Bassham (CBB) cycle; reductive Tricarboxylic acid (rTCA) cycle; 3-hydroxypropionate (3-HP) bicycle
Non-Photosynthetic organisms	Aerobic organisms	Crenarcheota	*Sulfolobus solfataricus*	dicarboxylate-4-hydroxybutyrate (DC-4HB) cycle; 3-hydroxypropio-nate-4-hydroxybutyrate (3 HP–4HB) cycle
	Anaerobic organisms	Acido/Acetogenic bacteria	*Acetobacterium woodii; Clostridium aceticum; Clostridium autoethanogenum; Clostridium carboxidivorans; Clostridium ljungdahlii; Moorella thermoacetica; Sporomusa acidovorans*	Wood-Ljungdahl (WL) pathway; reductive Tricarboxylic acid (rTCA) cycle
		b-Proteobacteria	*Cupriavidus necator (Ralstonia eutropha)*	Wood-Ljungdahl (WL) pathway; Calvin-Benson-Bassham (CBB) cycle
		Crenarcheota	*Acidianus ambivalens*	3-hydroxypropionate-4-hydroxybutyrate (3 HP–4HB) cycle; dicarboxylate-4-hydroxybutyrate (DC-4HB) cycle
		Euryarchaeota	*Methanobacterium beijingense; Methanoculleus receptaculi; Methanopyrus kandleri*	Wood-Ljungdahl (WL) pathway

Clostridium and *Acetobacterium*) have been highlighted as potential candidates for use on CO_2 conversion since they are able to handle variable gas compositions with low susceptibility to poisoning by sulfur, chlorine, and tars, besides presenting high metabolic efficiency and high product specificity [66,67].

Although a number of microorganisms have been identified as potential candidates for use on CO_2 conversion to chemicals and other valuable products, usually the microbial strains do not have suitable growth characteristics, thermal stability and tolerance to inhibitors for industrial-scale application. Diverse acetogenic bacteria, for example, have been suggested as promising biocatalysts to utilize C1 gases such as CO_2; however, their industrial application is limited because of their slow growth rates and low productivity when using C1 gases as sole carbon and energy sources [68]. To overcome this problem, the use of enabling technologies such as genetic engineering, metabolic engineering, synthetic biology and directed evolution has been considered to improve the strain's phenotypes in order to result in an enhanced growth rate, robustness to extreme cultivation conditions, or even to expand their repertoire of chemical syntheses, which in the end may result in a more economically feasible industrial process. Selecting and/or engineering strains for a high production of the desired metabolite is fact a critical step to establish an efficient and cost-competitive microbial route for CO_2 conversion. Optimizing the culture conditions to increase product yield and carbon fixation is also essential to maximize the CO_2 capture and utilization by the microorganism. In a recent study, for example, the conversion of CO_2 to ethanol by *Clostridium autoethanogenum* was significantly improved when the fermentation medium was supplemented with a small amount of CO [69].

In summary, several obstacles still need to be overcome to result in an efficient microbial conversion of CO_2 into bioproducts, capable of being implemented on a large scale. In addition, the cost of the process is another critical factor to be considered for implementation of these technologies. In this sense, a strategy that can potentially reduce the cost of CO_2-based bioprocesses is the use of extremely thermophilic/high CO_2 tolerant strains [70], since many industrial processes produce CO_2 at high temperature. Moreover, using microorganisms able to work under high temperatures reduces the risk of contamination during fermentation, contributing to reduce or even eliminate costs associated

with preventing contamination.

4.2. CO_2 utilization as a green technology for biomass fractionation

In recent decades, the growing concern about the security of oil supply and global warming, in particular, has increased the interest in establishing efficient processes for lignocellulosic biomass utilization on the production of biofuels and other biobased products while targeting net zero CO_2 emissions. Various of these processes consist of obtaining monosaccharides from a certain feedstock, which are subsequently used as carbon source to produce the desired molecule by fermentation or catalytic conversion. To produce fermentable sugars from lignocellulose, an initial step of pretreatment is required to disrupt the recalcitrant structure of the raw material, separating its major components (cellulose, hemicellulose and lignin). The main purpose of the pretreatment stage is to increase the accessibility of hydrolytic enzymes to cellulose and, if relevant, to hemicellulose, which are then depolymerized in the subsequent stage of enzymatic hydrolysis [7].

Several pretreatment techniques have been proposed to disrupt the lignocellulose structure, which usually employ acids (e.g., sulfuric or phosphoric acid), alkaline solutions (e.g., sodium hydroxide, lime or ammonia) or liquid hot water, as well as physicochemical methods such as ammonia fiber explosion (AFEX) or steam explosion [71]. However, these techniques still present key drawbacks that hinder their application at commercial scale, such as the high energy demand, the need to use large amounts of expensive chemicals that affect the economic viability of the process, as well as the difficulties for recovering and reusing chemical agents with corrosive properties [72,73]. In this context, supercritical carbon dioxide ($scCO_2$) explosion has attracted plenty of attention as a viable and greener alternative compared to other biomass pretreatment techniques [74].

Supercritical fluids refer to any substance that is above its critical temperature and pressure, and exhibit unique physicochemical properties such as liquid-like solvating power and gas-like mass transfer. In turn, CO_2 is an abundant gas, non-toxic, non-flammable, recyclable, and exhibits low critical temperature and pressure (31.1 °C and 73.8 bar, respectively) [75]. In addition, CO_2 is emitted from a variety of industrial production processes including brewing, ethanol fermentation and

cement production [76]; therefore, it can be supplied in large quantities and at relatively low cost.

4.2.1. Supercritical CO₂-based process for extraction of high value compounds

The potential of scCO$_2$ technique has been demonstrated in numerous studies on the extraction and fractionation of valuable compounds from natural resources. Supercritical fluid extractions using CO$_2$ as solvent has been widely used, for example, to recover lipids from biologic materials such as seaweeds, seeds, nuts, soybean and sunflower, among others. Other noteworthy examples include the extraction of pigments from vegetables and flavors from herbs [77]. This mature high-pressure technology has reached the commercial scale in food and pharmaceutical industries, with applications ranging from coffee decaffeination, hops extraction, up to the extraction of phytochemicals from plants [78]. Some recent applications of this technology include the extraction of bioactive compounds from different biomass sources such as agricultural by-products [79], microalgae [80] and organic wastes [81,82].

4.2.2. Supercritical CO₂-based process for lignocellulosic biomass pretreatment

The interest in using scCO$_2$ as an alternative approach for pretreatment of lignocellulosic materials has increased in recent years due to the several advantages that this method presents when compared to the conventional pretreatment techniques, as for example, no need of using toxic compounds, no generation of hazardous chemical wastes, and possibility of solvent recycling. Additionally, CO$_2$ can be easily removed

from the products by depressurization of the system [72]. Therefore, the implementation of this environmentally friendly pretreatment method associated with the extraction of high-value compounds from lignocellulosic materials deserves special attention as a strategy to enhance the economic viability and sustainability of biomass-based biorefineries.

Biomass pretreatment under supercritical conditions involves the reaction of CO$_2$ with water from the wet material and subsequent formation of carbonic acid, which then promotes a partial hydrolysis of the hemicellulose fraction and weakens the binding of cellulose and hemicellulose with lignin. In addition, the presence of water in the form of moisture also favors biomass swelling, opening its pores and enhancing the CO$_2$ penetration. Finally, a rapid depressurization process creates a physical "explosion effect", resulting in highly disrupted biomass fibers with higher surface area accessible to enzymes in the subsequent hydrolysis step [83]. The improvement in the enzymatic digestibility of scCO$_2$ pretreated biomass compared to untreated biomass has been reported for different lignocellulosic materials (Table 3).

It is worth noting that the selection of optimal pretreatment conditions depends on the biomass composition, in particular, the lignin content [84]. Biomass containing high lignin content requires more severe pretreatment conditions. Furthermore, besides the moisture content of the biomass, other variables such as temperature, pressure and time should also be optimized to result in maximum sugar yield after enzymatic hydrolysis [85]. On the other hand, the effect of other parameters including the depressurization time and solid loading on the digestibility of scCO$_2$ pretreated biomass by cellulolytic enzymes has not been completely elucidated till date and needs to be better studied.

Another relevant aspect to highlight is the possibility of intensifying

Table 3
Sugar yield after enzymatic hydrolysis of different types of biomass untreated or pretreated by supercritical CO$_2$ technology.

Biomass	Digestibility untreated biomass (%)	Digestibility pretreated biomass (%)	Reactor volume (ml)	Biomass amount (g)	CO$_2$ pressure (MPa/bar/psi)	Temp. (°C)	Moisture (%)	Holding time (min)	Depressurization time (s)	Reference
Aspen (hardwood)	9.73	56.8	50	1.5	21.4/214/3100	165	73	30	*	[87]
Big bluestem grass	17.0	66.0	25	2	20/200/2900	170	*	60	*	[88]
Corn cob	12.0	62.0	30	1.2	20/200/2900	170	50	30	*	[89]
Corn cob	34.9	39.6	30	1.5	15/150/2175	100	50	30	15	[85]
Corn stalk	16.6	46.4	30	1.2	20/200/2900	170	50	150	*	[89]
Corn stalk	26.0	27.4	30	1.5	15/150/2175	100	50	30	15	[85]
Corn stover	12.0	30.0	94.7	5.0	24.1/241/3500	150	75	60	*	[83]
Corn stover	36.0	85.0	25	2	20/200/2900	160	*	60	*	[88]
Guayule bagasse	*	77.0	500	*	27.6/276/4000	200	75	30	*	[90]
Oil palm trunk	12.31	42.77	5000	100	35/350/5076	100	60	60	180	[91]
Rice straw	27.7	32.4	10	*	30/300/4351	110	*	30	180	[92]
Rice straw	34.6	36.6	30	1.5	15/150/2175	100	50	30	15	[85]
Southern yellow pine (softwood)	8.24	17.6	50	1.5	21.4/214/3100	165	73	30	*	[87]
Soybean hull	27.0	50.0	250	15	8.6/86/1250	130	66.7	30	*	[84]
Sugarcane bagasse	13.4	61.3	50	3.0	20.6/206/2988	180	80	60	*	[93]
Switchgrass	10.4	81.0	25	2	20/200/2900	160	*	60	*	[88]
Wheat straw	8.52	20.8	150	2.0	12/120/1740	185	*	30	*	[94]

* Not reported.

the biomass fractionation step by integrating the scCO$_2$ pretreatment with the enzymatic hydrolysis in the same step. Since enzymatic hydrolysis is an expensive and time-consuming step, its integration with scCO$_2$ pretreatment can potentially lead to economic and efficiency gains. This strategy has already been tested for simultaneous scCO$_2$ pretreatment (without rapid depressurization) and hydrolysis of sugarcane bagasse [86] and resulted in higher cellulase activity and increased digestibility of the pretreated biomass. However, the optimal operation conditions should vary according to the lignocellulosic material and enzyme cocktail used. Moreover, studies are also required to better understand the mechanisms behind the degradation of lignocellulosic biomass by simultaneous pretreatment and enzymatic hydrolysis using scCO$_2$ as reaction medium.

5. Sustainability potential and perspectives of biomass-based and CO$_2$-based bioprocesses

Sustainability indicators have gained an increased importance as a tool to demonstrate the technological, economic, environmental and social impact of a process technology, being also a useful instrument for decision-making. Today, with the concern in reducing CO$_2$ emissions, such indicators have been widely used in combination with other disciplines to provide information, as for example, on the potential costs and environmental impacts of new biobased technologies. In practical terms, the development of low-cost and low-carbon manufacturing processes strongly depends on the use of appropriate strategies that may lead to these results. Process intensification through the realization of different steps of a process in a single stage, is a nice way to minimize equipment, potentially reducing processing time and costs. However, in practice this has not always happened [17]. In fact, for the creation of such robust processes, a simultaneous evaluation of the sustainability and process performance indicators considering the global production chain is necessary to optimize the full economic and climate potential. Moreover, combining different processes requires adaptation of each individual step to also work efficiently under these new conditions, and this will require the use of new types of equipment with different characteristics, new strains for bioprocesses, etc.

Another alternative to make biomass-based processes more economically feasible is through the integration of different production chains in a biorefinery. By implementing this approach, installations can be optimized and used for the processing of the different biomass fractions, which can also facilitate the scale-up [8]. Moreover, by adding value to the different fractions of the biomass, a more appropriate and effective use of the feedstock will be provided, contributing to the zero-waste concept. This will also positively impact the environment. Furthermore, studies on life cycle assessment have demonstrated that both the production of biobased chemicals and biofuels can deliver significantly less GHG emissions compared with the non-biobased alternatives [3,4]. However, recent findings on sustainability assessment of biorefineries have shown that the success of biorefineries depends not only on the efficient production of fuels and chemicals, but also on the quality and quantity of biomass available. Aspects including the complex conversion processes and uncertainty in the supply and sources of biomass are considered major challenges to overcome in order to improve the commercialization of bioproducts produced in biorefineries [9].

Besides the biomass-based bioprocesses, great attention is also being given nowadays to the development of CO$_2$-based bioprocesses as an alternative to reduce GHG in the atmosphere. Today, the interest in such technologies is big, but still many research efforts are required to make these processes robust and feasible for industrial implementation. Anyway, an interesting way to contribute to the economic viability would be, for example, to take CO$_2$ directly from emitting industries. Power plants, as well as iron, steel and cement industries, for example, emit millions of tons of CO$_2$ per year, being responsible for a significant percentage of the global CO$_2$ emissions (cement industry counts for 5% of the total; iron and steel, about 6%) [95]. Such industries could be potential suppliers of CO$_2$ for bioprocesses. This would also contribute with a greener solution to these companies to deal with the excess of CO$_2$ produced.

Another option to obtain CO$_2$ would be from fermentation processes. This would open opportunities for CO$_2$ integration in biorefineries for example, since CO$_2$ is produced during the sugar's fermentation step. In a general perspective, the CO$_2$ produced from fermentation could be recovered for further utilization on the production of other biobased products by fermentation, or even for application in the step of biomass fractionation, as discussed before. In both cases, a sustainable solution to the excess of CO$_2$ would be provided. However, regardless of the application, techno-economic and life cycle assessment should always be used to quantify the economic and environmental impacts associated to the technology developed.

6. Conclusions

Urgent actions are needed to reduce the emissions and levels of CO$_2$ in the atmosphere in order to promote a sustainable development. To achieve such goals, CO$_2$ can be either released in smaller amounts through the development of greener process technologies based on the use of lignocellulosic biomass as a feedstock instead of oil, or it can be captured and used as carbon source for the production of biomolecules. Both approaches are promising and have received considerable attention and investments in science and technology to result in deployable technologies. Both, biomass-based and CO$_2$-based technologies have potential to result in less CO$_2$ in the atmosphere, contributing to a better climate, benefiting the environment, and creating basis for new business opportunities. However, important techno-economic aspects still need to be improved to obtain robust technologies for industrial application. Implementation of process intensification concepts can potentially result in reduced costs due to a better utilization of installations and equipment, and improved process performance in general. Selection and/or engineering of suitable microbial strains is another critical point to improve the efficiency of both, biomass and CO$_2$ bioconversion. In this sense, the use of modern genetic tools and techniques for strain improvement as well as the use of co-cultivation systems can offer new opportunities to maximize the utilization of such carbon sources even in presence of inhibitory compounds or under extreme process conditions (like high temperature, low pH, etc.). Finally, for further directions of future research it is essential to combine these tools and concepts with techno-economic and life cycle analyses, in order to obtain indications on the cost-competitiveness and environmental impact of the new proposed technology.

Credit author statement

Solange I. Mussatto: Conceptualization, Writing- Reviewing and Editing, Supervision, Project Administration, Funding Acquisition; **Celina K. Yamakawa**: Investigation, Writing- Original draft preparation; **Lucas van der Maas**: Investigation, Writing- Original draft preparation; **Giuliano Dragone:** Investigation, Writing- Original draft preparation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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