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TITLE: Hybridization of Sugar-Carboxylate-Syngas Platforms for the Production of Bio-alcohols from Lignocellulosic Biomass (LCB) - A State-of-the-art Review and Recommendations

AUTHOR DETAILS

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ABSTRACT

Lignocellulosic biomass (LCB), the most abundant renewable feedstock for bioenergy generation, is commonly converted to second generation bioalcohols, the main drop-in fuels for petroleum gasoline, through three technologies based on sugar, carboxylic acid and syngas platforms. The hybridization of either any two or three platforms altogether is a novel concept aimed at improvement of yield and quality (high heating value) of bioalcohols. This article reviews the present status of the integration techniques of hybrid platforms with an overall assessment of their advancement with respect to their individual counterpart as well as the challenges involved. It has been indicated that to extract the maximum benefit of hybridization, research studies should be spurred in the fields of kinetic analysis of all thermochemical and biochemical processes, microbial interaction, optimization of process parameters (pH, temperature), performance analysis of engine for the utilization of mixed product bioalcohols, sustainability analysis through the development of mathematical models for lab-scale operations and process simulation models for large scale units along with life cycle assessment. Moreover, pyrolysis of LCB has been identified as a unique central process for the supply of all intermediate compounds, namely, sugar, carboxylic acid and syngas during the hybrid networking of three platform technologies. In this context, the scheme of CONVER-B, a joint research project under the INNO-INDIGO partnership program, aiming at sustainable integration of the platforms to produce bio-alcohols from LCBs leaving zero effluent simultaneously with carbon sequestration potential has been introduced and discussed.

Keywords: Bioalcohol; Lignocellulosic Biomass; Sugar-Carboxylate-Syngas platforms; Hybridization; Challenges; Recommendations

1. Introduction

Lignocellulosic biomass (LCB) derived biofuels and bioproducts are the key driver in the path of transition towards the bio-based economy all over the world, establishing absolute alliance among energy, society and environment [1,2]. It is well established that bioenergy, one of the preeminent components of bio-economy, will be mainly dependent on LCB as the chief renewable resource (feedstocks), mostly due to their worldwide abundance [1,3]. Being the most abundant feedstock appearing as residues of agriculture, forestry and as effluents from food, textile, pulping and other industrial processing, LCB could be used in biorefineries to generate myriads of renewable bioproducts; biofuels being the supreme

product [4-7]. One of the advantages of using LCB as feedstock for bioenergy generation is that it totally eliminates the upsetting social issue of 'food vs. fuel' competition [4,8]. Conventionally, lignocellulosic wastes are converted to biofuels (gaseous: biogas/biomethane; biohydrogen; bio-syngas and liquid: bio-alcohols and bio-oil) either through biochemical or thermochemical routes [9-14]. As reported in the latest survey by the International Energy Agency (IEA), conventional biofuel production reached 143 billion litres (4% increment on a year-on-year basis), in the year 2017, having an equivalent energy value of 83 Mtoe [15]. Analyzing the ongoing trend of world biofuel production, IEA forecasted a 15% growth estimating to be 165 billion litres (total energy value 97 Mtoe) by 2023, 119 billion litres (approximately two-third) of which will come from bioethanol alone [15]. This fact is already being implemented globally and is reflected in the renewable energy action plans of different countries. The Indian government has planned to achieve a target of 10% blending of fossil transport fuels with bioethanol and biodiesel by 2017 and raised the target to 20% beyond 2017 [16]. The European Union (EU) has set a new binding target, in form of renewable energy directive II (RED II), to acquire at least 14% of their transport fuels from renewable resources by 2030 [17]. In Finland, the target is set about 20%, by 2020 [18]. Bio-alcohols, namely; ethanol, butanol, hexanol etc. are already proven suitable for the use in spark-ignition engines as low-emission transport fuels and hence, are the biofuels of current interest [19-22]. A recent study has shown that corn-based biobutanol can save 39-56% automobile fossil fuels and reduce CO_2 emissions by 32-48% in comparison to gasoline [23]. This transition can only be sustained by employing strategic planning and utilization of LCB feedstocks in technologically advanced frameworks ensuring maximum conversion to biofuels, minimization of waste generation and reutilization of all residues.

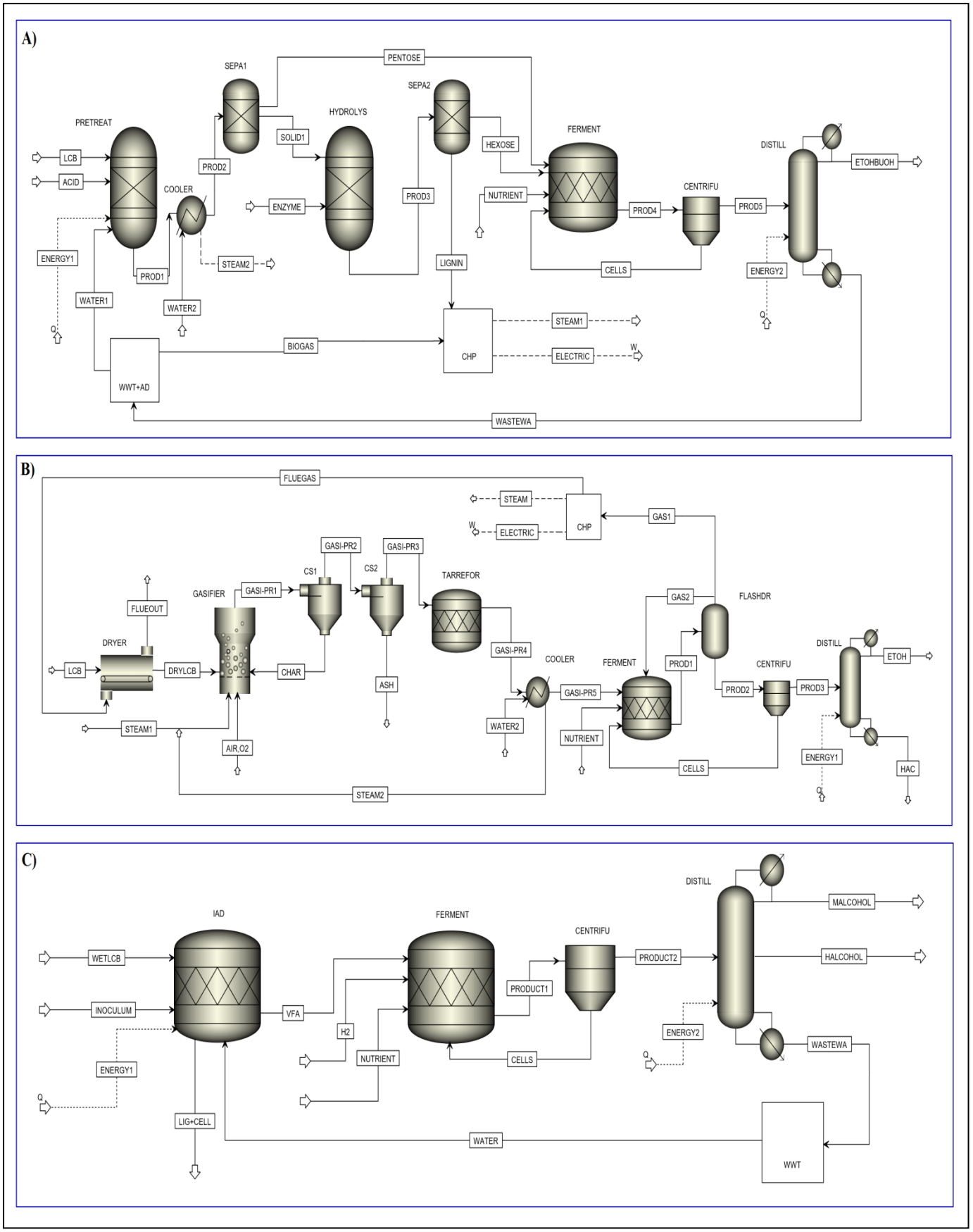
There are three pathways, namely sugar platform (SP), carboxylate platform (CP) and syngas platform (SyP), mainly used for the conversion of LCB feedstocks to bio-alcohols [24-26]. The names of the platforms are derived from those of the intermediate precursors through which organic or agricultural wastes are ultimately converted to bioalcohols. The sugar platform directly coverts 6-carbon and 5-carbon sugar/carbohydrate compounds obtained from LCBs through pretreatment and enzymatic hydrolysis, to different bio-alcohols as the major end product [24]. Conversely, in syngas and carboxylate platforms, as described by many researchers, alcohol generation from LCBs is mediated through formation of energy-rich precursor molecules and their mixtures, namely bio-syngas and mixed carboxylic acids, respectively [25,26]. Due to the astounding diversity of composition of lignocellulosic

wastes, having different distribution of cellulose, hemicellulose and lignin, it appears that none of the platforms can provide unique solution individually for the generation of bio-alcohols from LCBs, in general. Recently, few research articles demonstrated that the combination of any of the two platforms among sugar, carboxylate and syngas ones can improve the quality and productivity of bio-alcohols from LCB [27-30]. Although many informative review articles are available on individual platforms, comprehensive review on the performance and prospects of combination/hybridization of different platforms for the generation of alcohols from LCBs is rare [25,31-34]. In this article, the present status of this briskly evolving 'hybrid technology' in the field of bio-alcohol production from LCB is thoroughly revisited from all crucial perspectives. Ultimately, a novel "zero effluent" concept, namely; 'CONVER-B', of cascading of the three platforms particularly focusing on bio-alcohol production from LCB with carbon storage capability is recommended with the projection of higher energy efficiency compared to that of stand-alone platforms.

2. Analysis of three platforms

2.1 Working principles and microorganisms

The conventional working principles of the three platforms, namely; SP, CP and SyP, are technically summed up and depicted respectively in Figure 1A, 1B and 1C, for simple understanding. The platforms fundamentally differ from each other regarding various operational aspects. According to the representation of Figures 1A, 1B and 1C, all three platforms convert lignocellulosic biomass to bioalcohols as the chief end-product.


A)
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GAS1
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GASI-PR3
CS1
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TARREFOR
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FLASHDR
FLUEOUT
DRYER
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WETLCB
PRODUCT2
HALCOHOL
INOCULUM
VFA
PRODUCT1
H2
ENERGY2
WASTEWA
NUTRIENT
CELLS
ENERGY1
LIG+CELL
WATER
WWT

Figure 1: Major processes, material streams and unit operations of the three individual platforms; A) Sugar Platform (SP); B) Syngas Platform (SyP); C) Carboxylate Platform (CP); PRETREAT: Pretreatment; COOLER: Cooling unit; SEPA: Separation/Filtration unit; HYDROLYS: Enzymatic hydrolysis; FERMENT: Fermenter; CENTRIFU: Centrifugation unit; DISTILL: Distillation tower; CHP: Combined Heat and Power; WWT + AD: Waste water treatment plant with anaerobic digestion unit; LCB: Lignocellulosic Biomass; PROD1(SP): Pretreated LCB; PROD2 (SP): Cooled pretreated LCB; SOLID1(SP): Solid fraction of cooled pretreated LCB; PROD3 (SP): Enzymatically hydrolyzed LCB; NUTRIENT: Nutrient medium for fermentation; PROD4 (SP): Fermentation products; CELLS: Microbial cells; PROD5 (SP): Cell free fermentation products; ETOHBUOH: Ethanol, butanol; WASTEWA: Waste water; CS: Cyclone separator; TARREFOR: Tar reformer; FLASHDR: Flash drum for liquid-gas separation; DRYLCB: Dry lignocellulosic biomass; O_2: Oxygen; GASI-PR: Gas-solid mixture; PROD1, PROD 2 and PROD 3 (SyP): Fermentation products; ETOH: Ethanol; HAC: Acetic acid ; IAD: Incomplete anaerobic digestion; WETLCB: Wet lignocellulosic biomass; LIG + CELL: Lignin and cellulose; VFA: Volatile fatty acid; H_2: Hydrogen; PRODUCT1 and PRODUCT 2 (CP): Fermentation products; MALCOHOL: Medium alcohol; HALCOHOL: Higher alcohol; Energy 1 and 2: Input energy streams.

It is clear from Fig. 1A that in the conventional sugar platform, lignocellulosic biomass is first pretreated through hydrothermal processing using dilute mineral acids (alkali, mineral salts, organic solvents and some other catalysts are also used in different cases, acid pretreatment is considered as a representative process for all) whereby the hemicellulose portion of LCB is primarily converted to pentose sugars, namely, xylose and/or arabinose and a small part of cellulose is converted to glucose [34-37]. The cellulose present in the solid part of pretreated mass is subsequently hydrolysed to glucose using enzymes [35, 36, 38]. After hydrolysis, the hydrolysed mass is filtered. The filtrate containing simple carbohydrates like pentoses and hexoses, is fermented to generate alcohols, namely, ethanol and butanol [36] and the solid filter cake containing lignin is considered either as waste stream or directly combusted to generate electricity and steam in combined heat and power (CHP) unit [39,40]. The liquid stream is passed through a centrifuge and the cell concentrate is recycled Pure alcohol is recovered in distillation column [39,40]. The bottom liquid effluent from the distillation column is usually passed through waste water treatment plant (WWT) and the

treated water is recycled. The solid waste from the WWT is sometimes utilized in an anaerobic digester (AD) to generate biogas [39,40]. All these possible units have been presented in Figure 1A. The high temperature pretreatment and the alcohol recovery (distillation) steps require energy.

As depicted in Figure 1B, in syngas platform the pre-dried LCBs are first converted to syngas in a gasifier using steam and air mixture and the gas-solid mixture is subsequently separated in series of two cyclones [41-43]. While the char part is recycled to the gasifier, the ash, obtained in the solid streams of the second cyclone appears as waste. Exit gas stream from the second cyclone is sometimes passed through reformer for enrichment of syngas [41]. The syngas is subsequently fermented to ethanol [41,44]. The combined outlet stream of unconverted syngas and alcohol-rich liquid product from the fermenter is usually passed through a flash drum and the two phases are separated [37]. The gas stream is bifurcated in to two lines, one recycled to the fermenter and the rest directed to a CHP [41]. Electricity and steam are generated in the CHP and steam is utilized in the gasifier, as much as possible. All these units have been included in the general schematic representation for SyP in Figure 1B. The waste heat of flue gas from the CHP is exchanged to dry the LCB fed to the gasifier [41]. In case of carboxylate platform for alcohol generation, usually the carboxylic acids produced by acidogenic mixed microbiomes of anaerobic digestion (AD) processes are converted to alcohols either through catalytic thermochemical processes or by the combination with other bioprocesses responsible for reduction of acids to alcohols [25,45,46]. In this strategy, acetoclastic methanogenesis during anaerobic digestion is deliberately inhibited so that volatile fatty acids (VFAs) are mainly formed and hence it can be termed as an incomplete AD (IAD) process [47]. It is understandable that with selective suppression of the methanogens, any AD microbiome can be turned to an acidogenic microbiome that can be used in a CP. Energy requirement of the IAD process is mainly for the mild pretreatment of feedstock which can be fully avoided for low-lignin containing simple feedstocks [4]. On the other hand, the subsequent bioprocess generating alcohol requires energy for separation of alcohols through distillation. The basic principle of carboxylate platform extended up to bio-alcohol generation has been depicted in Fig. 1C. For both SyP and CP the methods of alcohol recovery and water treatment processes are similar to those in the SP. In another option of carboxylate platform, medium chain fatty acids (MCFAs) can be microbially derived through reversed β-oxidation of short chain fatty acids (SCFAs) produced during IAD [48]. The MCFAs can be further converted to higher alcohols with high carbon numbers and larger

calorific values in subsequent fermentation processes [48]. The 'MIXALCO' processes converting carboxylic acids to corresponding alcohols through thermochemical routes also belong to carboxylate platform [49,50]. In the subsequent sections, discussions have been provided on basics of individual platforms in a comparative way.

In all three platforms, production of bioalcohol from simple sugar, VFAs or bio-syngas is mediated by specifically dedicated microorganisms exhibiting different metabolic ability and performance. Different strains of the solventogenic yeast *Saccharomyces cerevisiae* are most popularly used in the sugar platform to produce bioethanol utilizing simple hexose (C6) sugars [51,52]. However, wild strains of *S. cerevisiae* cannot ferment pentose (C5) sugars, such as xylose, arabinose etc. genenerated through the de-polymerization of hemicellulose during the pretreatment of LCBs [53]. To tackle this problem, pentose-fermenting bacterial strains, namely; *Zymomonas mobilis* and genetically engineered strains of *Escherichia coli* etc. and another yeast, *Pichia* (or *Scheffersomyces*) *stipitis*, are also gaining interest recently [54-57]. For the production of butanol in the sugar platform, *Clostridium acetobutylicum* is extensively used to convert the carbohydrate monomers (C5 and C6) generated from lignocellulosic biomass [58-61]. Although *C. acetobutylicum* is the most typically used butanol producing strain in the sugar platform, lately, *C. beijerinckii*, *C. pasteurianum* and *C. saccharoperbutylacetonicum* are also being used by many researchers [62-64]. Research studies using combination of yeasts (*S. cerevisiae* and/or *P. stipitis*) and bacteria (*clostridial strains, E. coli or Z. mobilis*) as co-cultures for the simultaneous consumption and connversion of both hexoses and pentoses to alcohols have recently been reported in many studies on SP [65-69]. In the carboxylate platform, a mixture of cellulolytic and acidogenic consortia of IAD processes act on the holocellulose (cellulose and hemicellulose) fraction directly to produce SCFAs, which can in turn be converted to alcohols through biological processes using homoacetogenic microorganisms like *Clostridium ljungdahlii* having reducing capabilities [25]. On the other hand, SCFAs can be further converted to MCFAs under the action of reactor microbiomes and higher alcohols can be produced through the microbial reduction of the latter acids. For the production of MCFAs the presence of chain elongating bacteria, e.g., *Clostridium kluyveri* has to be ensured [25]. In the conventional syngas platform, syngas (H_2 + CO) is converted by the acetogenic bacterium, *Clostridium ljungdahlii* to ethanol and acetic acid [70,71]. Some other pure strains acetogenic/carboxidotrophic bacteria namely; *Clostridium carboxidivorans, Clostridium ragsdalei, Clostridium autoethanogenum* etc. are also being used recently for conversion of

syngas to bioalcohols [72-75]. Among these bacteria, *C. carboxidivorans* can also produce higher alcohols than ethanol, namely; butanol and hexanol from syngas [72,76]. Besides these pure strains, some recent studies reported use of mixed culture for syngas fermentation producing mixed alcohols like, ethanol, propanol and butanol [77,78].

2.2 Feedstocks and requirement for pretreatment

The lignin content of the LCB feedstocks is a vital factor for all platforms because of its direct contribution to the recalcitrance [79]. In a recent publication, the Indian lignocellulosic feedstocks have been categorized according to their lignin content; high lignin (1-10%), medium lignin (10-20%) and low lignin (>20%) to provide a basis for the selection of most viable conversion routes [4]. The primary aim of the SP and CP is to overcome the lignin barrier to gain access to the cellulose and hemicellulose fractions of the LCBs (Figure 1A and 1C). Selection of proper LCB feedstocks based on the lignin content for the particular platform is a prerequisite, which can facilitate better conversion and complete utilization of the LCB feedstocks.

As per the requirement of the bioprocesses involved in the SP and CP, the use of LCBs containing low, and low-to-medium lignin content are suitable for the sugar and carboxylate platforms respectively. As the microorganisms involved in SP can only accept hexose (glucose) and pentoses (xylose, arabinose), mainly low lignin LCBs [56-67], requiring mild delignification and easy pretreatment steps for the conversion of cellulose and hemicellulose to respective monomeric simple sugar are suitable. On the other hand the bioavailability of cellulose and hemicellulose for the action of cellulolytic bacteria in the reactor microbiome is the major decisive factor for the efficient conversion of LCBs in CP [9]. To avoid energy intensive delignification process prior to the entry in CP, medium and preferably low lignin LCBs are acceptable in carboxylate or volatile fatty acid platform using an IAD microbiome [80]. This is limited in dry biomass and at high lignin content. The SyP is not influenced by the lignin content since the gasification prior to fermentation is a thermochemical process that can accept any carbon source as its reactant/feedstock [80]. Although dry LCBs are not preferred in SP and CP, the feedstock for SyP should be preferably dry. Therefore, any dry lignocellulosic biomass can be handled by this platform irrespective of the lignin content. The SP essentially requires extensive pretreatment and enzymatic hydrolysis steps of LCB feedstocks [57,60-64]. Besides the conventional high temperature acid/alkali pretreatment, use of ultrasonication, microwave treatment, extraction with ionic liquid, organoslv

pretreatment, eutectic solvent etc. have also been demonstrated to be effective [80,81]. Literature review reveals that mild pretreatment of agricultural waste like rice straw and wheat straw yields better yield of product in CP used for biogas generation [82,83]. However, many CP are run without pretreatment of biomass [84]. Currently, biochemical pretreatment of LCBs employing lignocellulolytic microorganisms are also attracting interest due to their functional effectiveness and better techno-economic-environmental attributes than the conventional pretreatments [85]. In SyP, absolutely no pretreatment step is required.

2.3 Array and yields of product, by-products and residues

From SP, usually ethanol and butanol are obtained by using pure culture of dedicated solventogenic microorganisms, namely yeast and Clostridia, respectively, as already mentioned in section 2.2 [54-64]. In case of butanol production through this platform, acetone and ethanol are also produced as solvent by-products. A study by Wu et. al., 2008, revealed that a bio-butanol plant with a capacity of 2589.12 Mg/d (1Mg/d = 1 t(metric)/d) corn grain produces about 466 Mg/d butanol with additionally 261.6 Mg/d and 11.28Mg/d of acetone and ethanol, respectively, as by-products [23]. This study also envisaged that 0.22L biobutanol can be obtained from 1kg of corn. The yield of ethanol using solventogenic yeast is about 0.41 L/kg when dry-milled corn grain is used as feedstock [86]. A study on syngas platform showed that using the gaseous product generated from a gasification unit consuming 1200 Mg/d switchgrass, a subsequent bioethanol plant produces 298.77Mg/d ethanol [87]. Holtzapple and Granda, 2009, reported a comparative theoretical analysis of the potential of SP, CP and SyP, for ethanol production from standard LCB [49]. However, in this study the Sp and CP have been defined a bit differently from the conventional ones. Contribution of lignin as the substrate in these two platforms is included with the help of an additional gasification step generating H_2 as reducing agent for CO_2 (sugar platform) and acetic acids (carboxylate platform) producing enhancing overall ethanol yield [49]. On the basis of a mole of standard biomass, composed of 31.7% lignin ($CH_{1.12}O_{0.377}$) and 68.3% holocellulosic (cellulose + hemicellulose) polysaccharides ($C_6H_{10}O_5$) on ash free basis, 3 moles of ethanol is produced through SP and CP, whereas, the SyP produces 2.5 moles ethanol [49]. It has been estimated that both sugar and carboxylate platforms have the equal ethanol production potential of 175 gallon/ton, whereas, the ethanol production potential of syngas platform is about 145 gallon/ton standard biomass [49]. The ethanol production potential of the syngas platform is less because of the partial oxidation of carbon present in the biomass feedstock

during the generation of syngas in the gasification process. The yield of volatile fatty acids (acetic + propionic + butyric acids in the ratio 6:1:3 to 5:1:5) in the CP ranges from 0.118-0.61 g/g volatile solids [45].

During the production of biobutanol from conventional feedstocks, residues amounting to 31.1% of the total solids are formed, which are called DDGS (dried distillers grains with solubles) and comprise the solid wastes from upstream (cooking) and downstream (separation) processes [23,88]. Previous literature indicates that DDGS corresponds to 40% energy content of corn fed to the system [23]. Moreover, fatty acids generated in the fermenter are not recovered or utilized. The SyP generates 74 Mg/d ash and/or char and 49 Mg/d cell cake in a 1200 Mg/d switchgrass-based plant [87]. Since the first step in the alcohol production through carboxylate platform is IAD process (Fig. 1C), the feedstocks are generally silages, spent grains and other wet biomass solid. Digestate, generated in the IAD, is usually used as fertilizer after neutralization and does not generate much revenue [89]. As reported in the literature, the solid part of the digestate generated from co-digestion of lignocellulosic biomass (groats: 9%; olive oil cake: 29%; triticale: 57% w/w) and chicken manure (5% (w/w)) contains about 35% carbohydrates and 21% Klason lignin at the end of the process and can be a potential feedstock for further energy conversion [90]. The carbohydrate part in the residue is rich in cellulose as the digestion rate of hemicellulose is much higher than the former [90]. Thus it can be delignified and hydrolysed enzymatically to release simple sugars and fed to an alcohol fermentation system for production of ethanol (or butanol, not reported yet) [91,92]. Since digestate is rich in lignin, it can also be used in the syngas platform for conversion to syngas and eventual fermentation to ethanol [93]. This can be particularly useful if lignin-rich feedstock has to be handled in carboxylate platform.

2.4. Energy analysis

Using the Mueller and Cuttica model, it was estimated that the energy consumption is about 0.63kWh/L butanol production from corn grain, when cooking and gas stripping/distillation were used as pretreatment and downstream processing steps, respectively [23,88]. Cooking constituted the major share (69.9%) of energy consumption while the other energy consuming steps being fermentation and product recovery altogether accounted for 30.1% of the total energy consumption. In case of SP for alcohol production, while waste lignin stream is sometimes directly combusted for the generation of electricity, the distillation bottoms are also utilized to generate biogas, which is subsequently utilized in energy generation [39,40].

Sometimes a part of energy can be exported after being utilized for alcohol production. In other cases a part of energy has to be imported for alcohol production even after utilization of electricity produced in the plant. The energy efficiency (η) of ethanol plant has been defined according to the situations (with export/import) as follows [40]:

In case of energy export:

$$\eta = \frac{Y_{bioethanol} \times CV_{bioethanol} + Y_{electricity}}{100 \times CV_{lignocellulose}}$$

In case of energy import:

$$\eta = \frac{Y_{bioethanol} \times CV_{bioethanol} - E_{import}}{100 \times CV_{lignocellulose}}$$

Where, Y = yield

In case of SyP for ethanol production, the overall energy efficiency on the higher heating value (HHV) basis is 41.95%, syngas generation and fermentation step having energy efficiencies of 66% and 63.5%, respectively [87]. The significance of different energy efficiencies (η) in this platform has been defined as follows:

$$\eta_{gasification} = \frac{Output\ flowrate\ of\ syngas\,(Nm^3/h) * HHV_{syngas}(MJ/Nm^3)}{Input\ flowrate\ of\ biomass\,(kg/h) * HHV_{syngas}(MJ/kg)}$$

$$\eta_{fermentation} = \frac{Output\ flowrate\ of\ ethanol\,(m^3/h) * HHV_{syngas}(MJ/m^3)}{Input\ flowrate\ of\ syngas\,(Nm^3/h) * HHV_{syngas}(MJ/Nm^3}$$

$$\eta_{overall} = \frac{Output\ flowrate\ of\ ethanol\,(m^3/h) * HHV_{syngas}(MJ/m^3)}{Input\ flowrate\ of\ biomass\,(kg/h) * HHV_{syngas}(MJ/kg)}$$

In the efficiency calculation, the energy consumed during any process has not been considered. The most energy intensive step is the downstream processing, i.e., distillation of ethanol, consuming 31.27MWh when a plant having a capacity of 1200 Mg/d is run using switchgrass as the feedstock [87]. As mixed bacterial consortia are used in the CP, sterilization and pretreatment of the feedstock can be avoided and hence energy is saved. On the other hand, the separation step could be energy intensive since the platform produces

mixed carboxylic acids [25,45]. However, systematic report on energy analysis of alcohol production through CP is not much available.

Table 1 represents the comparative performance of different individual platforms with respect to material and energy balances as well as cost.

Table 1: Performance of individual platforms from the perspectives of material and energy balances and economic analysis.

Platform	**Feedstock**	**Plant capacity***	**Alcohol**	**Pretreatment/precursor process**	**Process of recovery**	**Material balance**	**Energy balance**		**Economy**	**Refs.**
						Alcohol yield	**Whether in-house energy generated from lignin**	**Energy efficiency (%)**	**Production price****	
SP	Corn stover	2200 dry ton/day	Ethanol	Dilute acid pretreatment and enzymatic hydrolysis	Distillation followed by molecular sieve adsorption	79 gallon/ton of dry feed	Yes	Not mentioned	$ 2.14 /gallon	[39]
SP	Mexican lignoc	2000 t/da	Ethanol	Pretreatment and	Distillation followe	48.6-74.1 gallon/ton	Yes	20.7-48.9	$ 2.05 /gallon	[40]

	ellulosics	y		enzymatic hydrolysis	d by molecular sieve adsorption					
SP	Bamboo	2000 Mt/d)	Ethanol	Hot water pretreatment and enzymatic hydrolysis	Distillation followed by molecular sieve adsorption	147-198 ML/year	Yes	Not mentioned	$ 0.589 /L	[94]
SP	Corn stover	729692 ton/year	Butanol	Dilute acid pretreatment	Vacuum distillation	155 L/ton	Yes	44	$ 1.5-1.8 /L	[95]
SP	Corn stover	126 kt/year	Butanol	Dilute acid pretreatment	Pervaporation and nanofiltration	0.21 kg/kg	No	Not mentioned	€ 1.09 /kg	[96]
SyP	Switchgrass	(2206 MT/day	Ethanol	NA	Distillation	282 L/ton	NA	Not mentioned	$ 1.32 /L	[97]
SyP	Sugarcane bagas	100 ton/h	Ethanol	NA	Distillation	0.225 ton ethanol/ton feed	From gas (25.5	43%	$ 0.69 /L	[41]

	se						MW)			
CP	Korean food waste	(500 tons/day	Mixed alcohol		Not clearly mentioned	0.25-0.46 g/g	NA	Not mentioned	$ 0.48 /L ethanol equivalent	[45]

[*]Plant capacities have been presented as reported in the respective references.

[**]Production costs have been presented as reported in the respective references.

3. Integration of platforms for bio-alcohol production - State of the art

The principal processes, feedstocks, microbes, patterns of generation of products, by-products and residues, energy efficiencies and conversion data of individual SP, CP and SyP have been indicated in Table 1. The advantages and disadvantages of the individual platforms meant for production of bio-alcohols, as discussed in section 2 are compiled in Table 2. Although each platform involves combination of different processes having inherent merits and demerits, only the major ones affecting the sustainability of three production platforms are mentioned in a collective manner.

Table 2: Advantages and disadvantages of different platforms for bio-alcohol production.

Platform	Advantages	Disadvantages
Sugar platform (SP)	• Specificity of end products (the bio-alcohols). • Maturity of technology. • High yield of alcohol (ethanol production by yeast). • Simultaneous generation of H_2 (another biofuel) when butanol is produced (ABE fermentation pathway). • Maximum conversion of the sugars to bio-alcohols.	• Necessity of energy intensive pretreatment, in case of LCBs. • Necessity of expensive enzymes for hydrolysis. • Generation of lignin-rich waste streams. • Prominent end-product inhibition exerted by the bio-alcohols on the solventogenic

		microorganisms.
Syngas platform (SyP)	• Capability to directly convert recalcitrant lignin. • No requirement of pretreatment. • No requirement of external addition of enzymes. • Specificity of end products (ethanol and acetic acid). • Scope of waste heat recovery to self-sustain the gasification process.	• Necessity of catalyst in the gasification process to maintain the quality of syngas. • Mass transfer limitation in syngas fermentation. • Chance of intoxication of the microorganism/s by traces of condensable volatiles (tar) present in the syngas. • Generation of ash during gasification and necessity of ash handling
Carboxylate platform (CP)	• Requirement of very moderate or no pretreatment. • No requirement of external addition of enzyme or chemical catalyst. • No requirement of sterilization of feedstock. • High yield of VFAs and MCFAs as precursors for alcohol production. • Biogas production from the	• The technology is at a nascent state. • The interaction between the microorganisms involved in carboxylate platform is yet to be explored. • Mixed products (fatty acids of varying chain length). • Stringent requirement of

	digestate and residue of downstream processing is possible.	pH control and suppression of methanogenesis during IAD for generation of SCFAs and MCFAs as end products. • Requirement of hydrogen or other electron donors for the conversion of carboxylic acids to alcohols.

The disadvantages encountered in an individual platform, as listed in table 2, can be tackled through the strategic inter-integration of the platforms with each other. Through the integration of different platforms, the by-product or effluent streams of one platform can be (re)used as the feed for another platform. The availability of literature on the integration of platforms for bio-alcohol production is scarce. So far, four types of twinning of platforms have been reported according to their operating sequence, namely; 1) carboxylate platform followed by sugar platform (CP-SP); 2) syngas platform followed by carboxylate platform (SyP-CP); 3) carboxylate platform followed by syngas platform (CP-SyP) and 4) sugar, carboxylate and syngas platforms operated in sequence (SP-CP-SyP). Although from the classical definition of carboxylate platform, carboxylic acids or volatile fatty acids generated through incomplete AD are precursors, in this review, the studies based on the volatile fatty acid intermediates produced from other bioprocesses using mixed or pure strains are also considered. The reactions occurring in all individual and hybrid platforms, under review, are presented in Table 3.

Table 3: Reactions involved in individual and hybrid platforms.

Conversion	**Reactions**	**Platform**
Glucose to ethanol	$Glucose \rightarrow 2Ethanol + 2CO_2$	SP, CP-SP(I), SP-CP- SyP
Xylose to ethanol	$3Xylose \rightarrow 2Ethanol + 5CO_2$	SP, CP-SP(I), SP-CP- SyP

Glucose to butanol	$Glucose \rightarrow Butanol + 2CO_2 + H_2O$	SP, CP-SP(II), SP-CP- SyP
Xylose to butanol	$6Xylose \rightarrow 5Butanol + 10CO_2 + 5H_2O$	SP, CP-SP(II), SP-CP- SyP
Glucose to acetone	$Glucose + H_2O \rightarrow Acetone + 3CO_2 + 4H_2$	SP, CP-SP(II)
Xylose to acetone	$Xylose \rightarrow Acetone + 2CO_2 + 2H_2$	SP, CP-SP(II)
Syngas to acetic acid	$4H_2 + 2CO_2 \rightarrow acetate^- + H^+ + 2H_2O$ $4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2$	SyP, SyP-CP, SyP-CP- SyP
Syngas to ethanol	$acetate^- + H^+ + 2H_2 \rightarrow ethanol + H_2O$ $CH_3COOH + 2CO + H_2O$ $\rightarrow ethanol + 2CO_2 + H_2O$	SyP, SyP-CP, SyP-CP- SyP
Glucose to acetic acid	$Glucose \rightarrow 3Acetic\ Acid$	CP, CP-SP (II), SyP-CP(1-stage)
Xylose to acetic acid	$2Xylose \rightarrow 5Acetic\ Acid$	CP, CP-SP (II), SyP-CP(1-stage)
Glucose to butyric acid	$Glucose \rightarrow Butyric\ Acid + 2CO_2 + H_2$	CP, SyP-CP(1-stage), SyP-CP-Syp, Sp-CP- Syp
Xylose to butyric acid	$6Xylose \rightarrow 5Butyric\ Acid + 10CO_2 + 5H_2O$	CP, SyP-CP(1-stage), SyP-CP-SyP, Sp-CP- SyP
Acetic acid to butyric acid	$ethanol + Acetic\ Acid\ n \rightarrow butyrate^- + H_2O$	SyP-CP(1-stage), SyP-CP- SyP, SP-CP- SyP
Butyric acid to caproic acid	$ethanol + Butyric\ Acid \rightarrow caproic\ acid + H_2O$	SyP-CP(1-stage), SyP-CP- SyP, SP-CP- SyP
Acetic acid to ethanol	$acetate^- + H^+ + 2H_2 \rightarrow ethanol + H_2O$	SP, CP-SP(I,II)
Butyric acid to butanol	$butyric\ acid + 2H_2 \rightarrow n - butanol + H_2O$	SP, CP-SP(I,II), SyP-CP(1-stage and 2-stage), SP-CP-SyP

Caproic acid to hexanol	$caproic\ acid + H^+ + 2H_2 \rightarrow n-Hexanol + H_2O$	SyP-CP(1-stage), SyP-CP- SyP, SP-CP- SyP

Different pros and cons of the reported research outcomes on integration of platforms are being revisited. Their operating strategies are presented in Figures 2 to 7 and the salient features of their performances are being discussed in the following sections.

3.1. CP-SP hybrids

Two types of CP-SP hybrids have been reported in the literature. In strategy-I, represented in Figure 2, digestate from the acidogenic stage of the IAD process, using mildly pretreated lignocellulosic biomass, is enzymatically hydrolyzed prior to conversion through the sugar fermentation process [84,85]. According to strategy-II of CP-SP hybridization, represented in Figure 3, supernatant from the carboxylate platform is directly fed to sugar platform. In some reported articles, instead of coupling carboxylate platform producing solely carboxylic acid through IAD, partial AD process generating biogas up to a period less than the saturation level has been considered as the representative one [100,101]. Partial AD is followed so that some of the hydrolysable sugar polymers (cellulose, hemicellulose) remain unconverted in the digestate. Hence, it can directly be introduced to enzymatic hydrolysis process and the severity of overall pretreatment step is reduced [101]. It is expected that the liquid effluent of the partial AD would contain some carboxylic acid and sugar monomers. In another report, instead of IAD, a stable mixed culture of cellulolytic, xylanolytic and acidogenic bacteria including butyrate producing ones has been used to generate carboxylic acid from lignocellulosic feedstocks [27]. The carboxylic acid fed to the sugar platform is expected to be converted to alcohol in solventogenic phase of clostridial bacteria used in the sugar platform for butanol production [27].

3.1.1. CP-SP hybrid (Strategy-I)

In a recent research study, a partial AD process has been used as a pretreatment step for the sugar platform [101]. Diverse lignocellulosic waste, namely, rice straw, sycamore and pinewood were used as feedstocks. It was claimed that for rice straw, sycamore and pinewood, the ethanol yield was increased from 32%, 19% and 10.7% of the theoretical value to 69.5%, 40% and 22.1% respectively due to this pretreatment process instead of conventional ones [101]. The maximum increase of yield in case of rice straw was explained

by its lowest lignin content facilitating the hydrolytic activity of the AD microbiome. On the other hand, the presence of guaiacyl type lignin in pinewood was pointed out to be responsible for minimum increase of yield in case of pinewood [101]. The investigators also showed that most of the hemicellulose was utilized to form methane during the AD process of rice straw. The increase of cellulosic content of pretreated biomass, particularly for rice straw was also identified through the determination of crystallinity index [101]. Taking both biogas and ethanol into account, the total energy yield from rice straw and pinewood was determined to be 8.2MJ/kg and 4.1MJ/kg. Although partial AD process is supposed to generate (not discussed) liquid effluent rich in VFA and enzymatic hydrolysis step of solid digestate of partial AD process would have produced lignin rich solid residue, no attempt was made to utilize these wastes. There is a scope for the addition of ABE fermentation, a representative of SP, if the liquid effluent is rich in butyric acid. Otherwise, the carboxylic acids in the liquid effluent can be fed to a SyP to generate higher alcohols. Similarly, lignin rich waste can be introduced to a SyP so that alcohol can be generated by using the syngas produced through gasification of the solid residue.

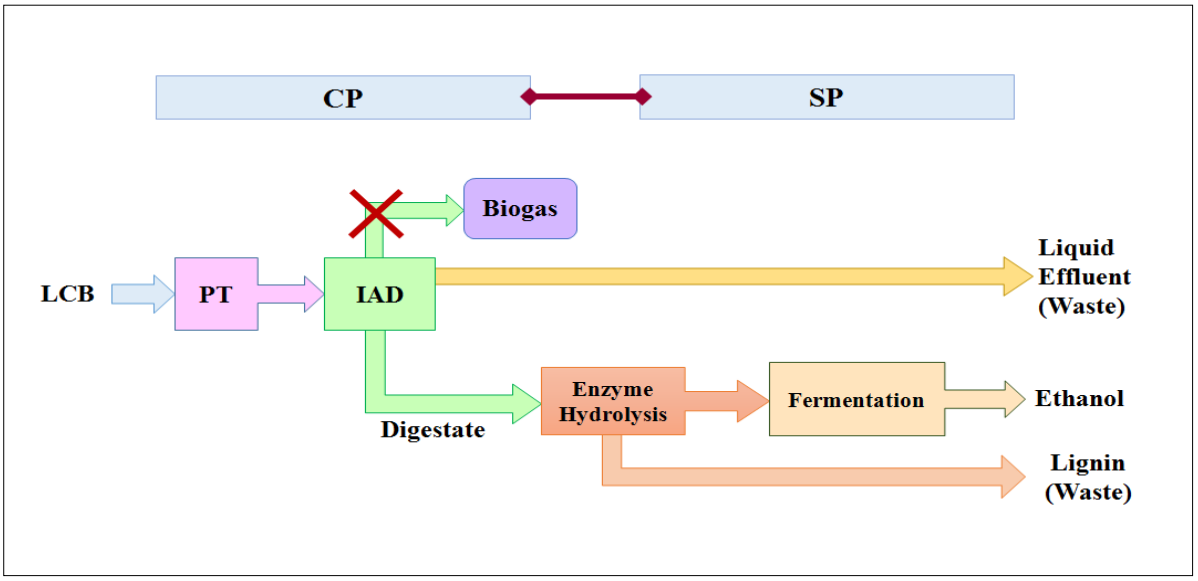

Figure 2: Scheme of CP-SP hybrid (Strategy-I).

Anaerobic digestion process using rumen fluid of cattle was also claimed to be effective in the enhancement of ethanol production from rice straw [102]. The group used solid digestate from the rumen fluid digestion process and the results were very similar to those using the inoculum obtained from partial AD process in biogas plant [101]. It is notable that the acidogenesis conducted by rumen microbiome produced significant amount of VFAs (Acetic

418 acid: 159g, Propionic acid: 83, Butyric acid: 24) when 2.5% loading of rice straw was used
419 [102]. Inhibition of methanogens from this microbiome may turn it into a major acidogenic
420 microbiome producing VFAs/SCFAs that can be ultimately converted in their corresponding
421 bio-alcohols.
422
423 **3.1.2. CP-SP hybrid (Strategy-II)**
424 In a very recent attempt, rice straw was converted to biobutanol through a two-stage
425 fermentation process [27]. The first stage represented the CP in which pretreated (alkali
426 treated) rice straw was predominantly converted to VFAs, mainly, butyric acid and hydrogen
427 under the action of a mixed culture, named as DCB17, dominated by cellulolytic, xylanolytic
428 and butyric acid producing bacteria [103]. In the second stage, the butyric acid rich
429 supernatant of the first stage (SFS) was co-fermented with enzymatically hydrolyzed
430 pretreated rice straw (HPRS) using *C. beijerinckii* NCIMB 8052 to butanol [27]. The ratio of
431 SFS:HPRS used was 2:8 on mass basis. Bio-butanol was produced along with hydrogen,
432 acetone and ethanol as co-products. In comparison to butanol production of 80.3 g and 146g
433 per kg rice straw, respectively, by *C. acetobutylicum* NRRL B-591 and a mixed culture
434 dominated by clostridia from pretreated and enzymatically hydrolyzed rice straw reported in
435 two other studies [104,105], 230g butanol/kg rice straw was obtained in this integrated CP-SP
436 process [27]. The specific energy yield (9633.7kJ/kg) of the two-stage process was much
437 higher than that obtained using clostridial microflora (8043.5kJ/kg) considering both butanol
438 and hydrogen as the energy carriers in the products [27,104]. Studies showed that the
439 enhancement of bio-butanol yield is highly influenced by the continuous supply of butyric
440 acid, which down-regulates the enzymatic activities of acetate kinase, acetate
441 phosphotransacetylase, phosphate butyryl transferase during solventogenesis in ABE
442 fermentation [106-108].

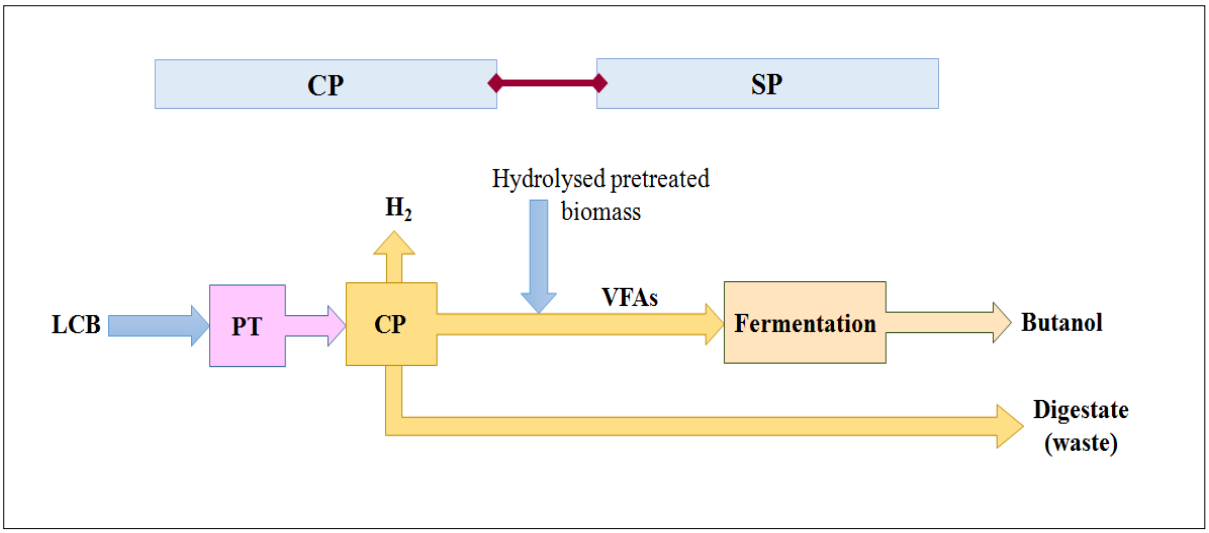

Figure 3: Scheme of CP-SP hybrid (Strategy-II).

Thus, the synergistic integration of carboxylate and sugar platforms not only enhances the butanol production, it also eliminates the necessity of the application of costly enzyme cocktails that is used in the conventional sugar platform. However, even after this integration, a considerable portion of biomass fraction is lost as digestate of the acidogenic stage (CP) and concentrated lignin portion during conventional enzymatic hydrolysis in the sugar platform. Thus, further attention is needed to facilitate utilization of these energy-rich effluents. A syngas platform using a thermochemical step, namely, gasification or pyrolysis could have been beneficial for recalcitrant lignin rich part. Similarly, the digestate of the acidogenic stage could have been utilized for biogas generation through AD process, the digestate of AD to be used as fertilizers. However, no investigation has been reported in this respect.

3.2. SyP-CP hybrids

The SyP-CP hybrids can be categorized as two stage and single stage ones. In the two-stage category, represented in Figure 4, the product stream of syngas platform is fed to the carboxylate platform for further conversion of short chain fatty acids through chain elongation to MCFAs which can in turn be used as precursors for production of higher bio-alcohols. Steinbusch et. al., 2008, reported the conversion of different volatile fatty acids to corresponding alcohols by a mixed culture at high hydrogen pressure and low pH [109]. In the single stage hybrids, represented in Figure 5, both the syngas fermentation and the biochemical processes involved in CP occur simultaneously in a single reactor for the

production of MCFA. Medium chain fatty acids are ultimately converted to bio-alcohols by syngas fermenting microbes, namely, *C. ljungdahlii* or *C. autoethanogenum* present in the microbiome of the integrated system.

3.2.1. Two-Stage SyP-CP hybrids

Vasudevan et. al., 2014, reported the sequential combination of SyP and CP, whereby, the effluent of the syngas fermentation process containing acetic acid and ethanol was fed to an anaerobic bioprocess driven by open reactor microbiome [28]. The mixed acidogenic consortia led to chain elongation of carboxylic acids (from acetic acid to n-caproic acid via n-butyric acid) in the presence of ethanol prevailing in the SyP effluent. The well established reversed β-oxidation pathway was proposed to be responsible for the chain elongation in this two stage hybrid of SyP and CP [28]. Although their aim was to produce n-caproic acid as the major end product, the fermentation effluent was more enriched in n-butyric acid. While the maximum value of n-caproic acid production rate reached only $1.7 gL^{-1}d^{-1}$, n-butyrate production rate as high as $20 gL^{-1}d^{-1}$ was achieved [28]. The self-inhibitory nature of n-caproic acid even at low concentration on the chain elongating microbes and hyper-sensitivity of the microbiome to pH were identified as the inherent causes for the high n-butyrate titer [28]. The maintenance of favorable pH and the avoidance of methanogenesis were suggested to be the necessities for chain elongating reactions to proceed. In another attempt, instead of mixed consortia, a pure strain of *Clostridium kluyveri* was used successfully for the integration of SyP with CP [29]. Continuous extraction of medium chain fatty acids, namely n-caproic acid, from the reactor was used to maintain its concentration below inhibitory level. *C. kluyveri* also produced n-caprylic acid in this system. The chain-elongation performance of *C. kluyveri* was observed to be better at a low ethanol to acetate ratio (3:1) and neutral pH [29]. Although these studies were not focused on alcohol production as the end-product, it was envisaged that by maintaining high partial pressure of hydrogen ultimate conversion to higher alcohols would have been possible, using mixed culture and low system pH [110,111]. In another study conducted by Kucek et. al., 2016, the enhancement of specific production of n-caprylic acid was investigated [112]. A basal medium containing substrate ratio of 15 (COD basis) obtained by mixing high ethanol to low acetate was fed to an UAB with continuous product extraction and inoculated with a reactor microbiome grown on effluent of ethanol rich beer fermentation. From the 186 days operation of the UAB highest n-caprylic acid productivity of 19.4 g COD/L/d was achieved [112]. The microbe *Rhodocyclaceae* K82 sp. was identified as the major chain elongating bacterium in the microbiome with a relative abundance of 70.8%.

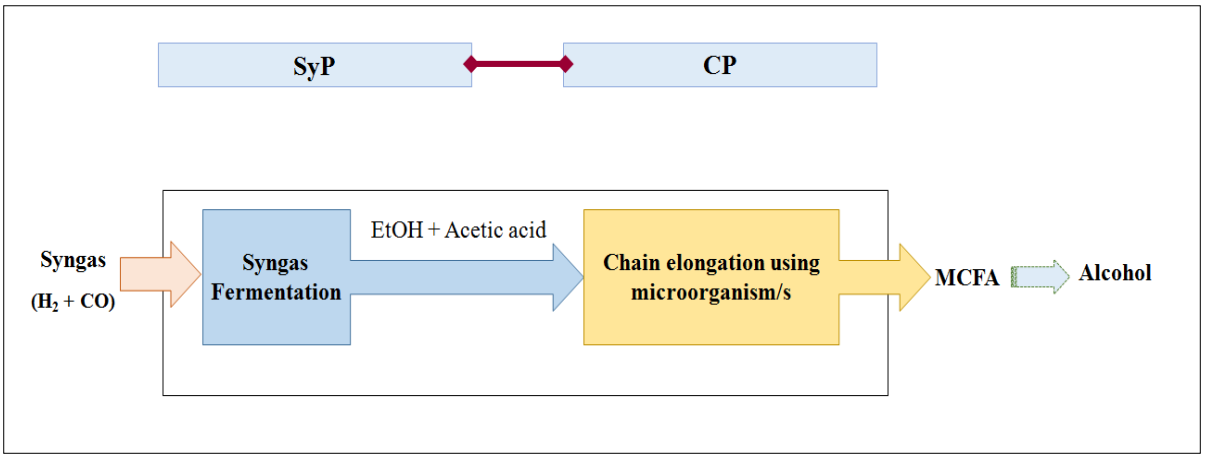

Figure 4: Scheme of two stage SyP-CP hybrid.

It is expected that the excess hydrogen produced in the thermochemical step (gasification/pyrolysis) of syngas platform can be used to produce higher alcohols from MCFAs by the activity of potential microbiomes. The hybrid processes, similar to MixAlco type hybridization in which the MCFA are thermochemically converted to ketones and ultimately to corresponding alcohols, can also be used [49,50,113].

3.2.2. Single-Stage SyP-CP hybrids

Diender et. al., 2016, studied the performance of production of MCFA and higher alcohols (butanol, and hexanol) by co-culturing *C.kluyveri and C. autoethanogenum* using synthetic medium and syngas [114]. They reported that although the feeding of acetate (and ethanol) was not a pre-condition for growth of the co-culture, it drastically enhanced the higher alcohol production.

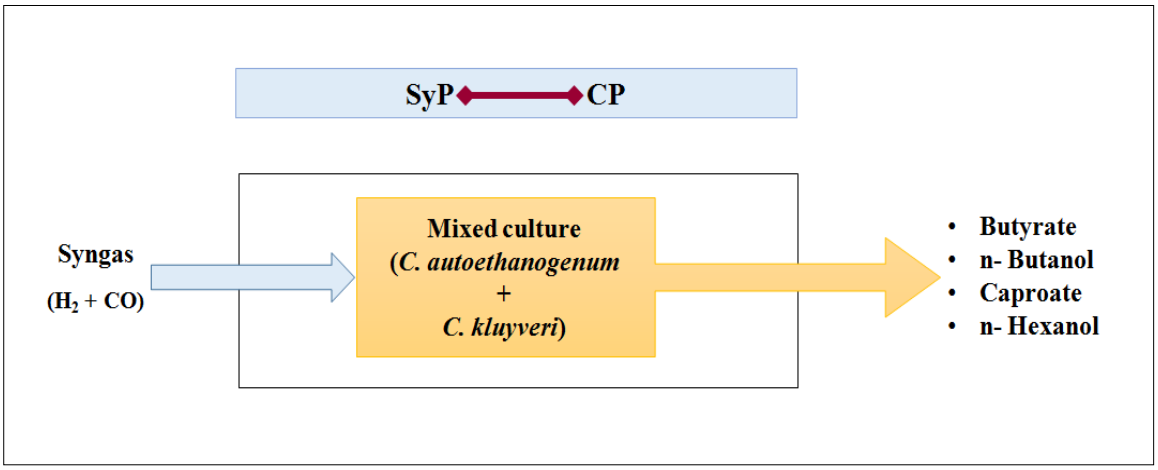

Figure 5: Scheme of single stage SyP-CP hybrid.

They reported that the production rates of butanol and hexanol were 3.5± 0.69 and 2.0 ± 0.46 mmol/l/d, respectively, and those of butyrate and caproate were 8.5 ± 1.1 and 2.5 ± 0.63 mmol/l/d, respectively [114]. pH sensitivity and toxicity of produced caproate were suggested to be major constraints for chain elongation. Like many other mixed culture driven bioprocesses as demonstrated by Ghosh et. al., 2016, in this system a clear commensal interaction can be noticed between *C. kluyveri* and *C. autoethanogenum*, where the latter organism protects the former from being intoxicated by the CO [115]. Ganigué et. al., 2016, studied the production of mixed fatty acids and higher alcohols using a syngas-enriched mixed culture [111]. They observed that although low pH triggered the production of alcohols, it was detrimental towards the growth of chain elongating bacteria, i.e. *C. kluyveri*. Richter et. al., 2016, aimed at analyzing the production patterns of higher alcohols like butanol, hexanol and even octanol by co-culturing syngas fermenting and chain elongating bacteria, namely *C. ljungdahlii* and *C. kluyveri*, in a single reactor [30]. The optimal pH range was observed to be very narrow (pH at 6.0, but not ideal) due to inherent discrepancies between the pH optima of the two organisms [30]. Therefore, it was suggested to use organisms with very close optimum values of pH for overcoming this problem. The study found that a competitive relationship exist between the organisms for the substrate, namely SCFAs, as one organism (*C. ljungdahlii*) uses it for reduction to alcohols and the other one (*C. kluyveri*) for chain elongation [30].

3.3. Multistage cascading of different platforms

3.3.1. SyP-CP-SyP cascading

From literature review it is clear that a multistage process, namely, SyP-CP-SyP type hybrids, whereby, the carboxylate platform is integrated with the syngas platform performing ethanol and higher alcohol production and reduction of MCFAs has a high prospect [30,116,117].

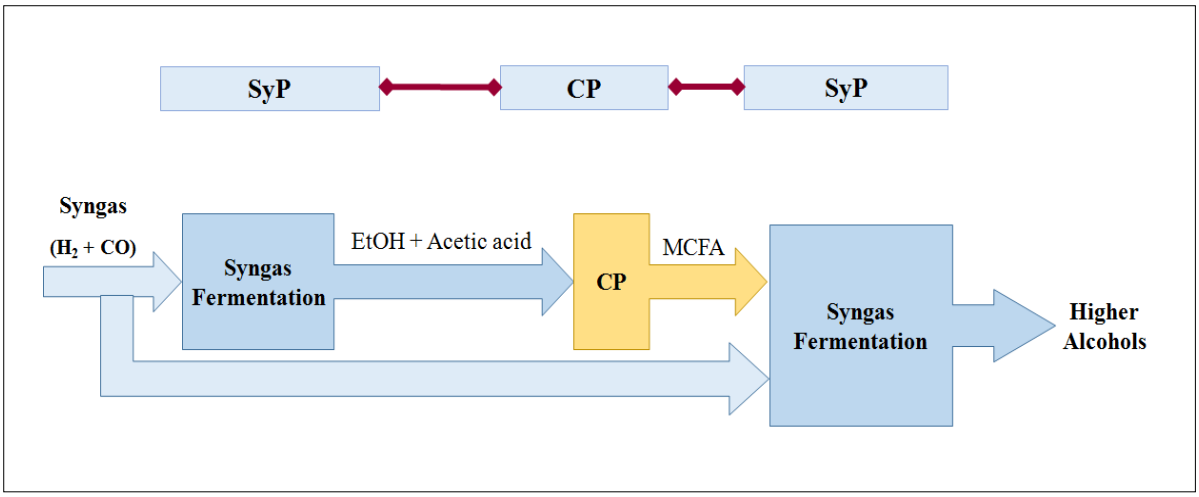

Figure 6: Scheme of multi-stage hybridization of SyP-CP-SyP.

In SyP-CP-SyP cascading, represented in Figure 6, the products (ethanol and acetic acid) of the first syngas platform are fed to the carboxylate platform for their conversion to medium-chain carboxylates (MCFAs) using a chain elongating microorganism and ultimately the product MCFAs of Carboxylate platform are fed to the second syngas fermenter for the reduction of fatty acids to corresponding bio-alcohol. The problem occurring in single stage integration of SyP-CP (Figure 5) due to pH discrepancy should disappear if separate reactors are used. More facts are to be revealed before taking the strategic decision on optimal combination.

3.3.2. SP-CP-SyP cascading

A contemporary study reported the integration of three platforms whereby the carboxylate platform was preceded and succeeded by sugar and syngas platforms, respectively [118]. The SP-CP-SyP hybrid process is depicted in Figure 7.

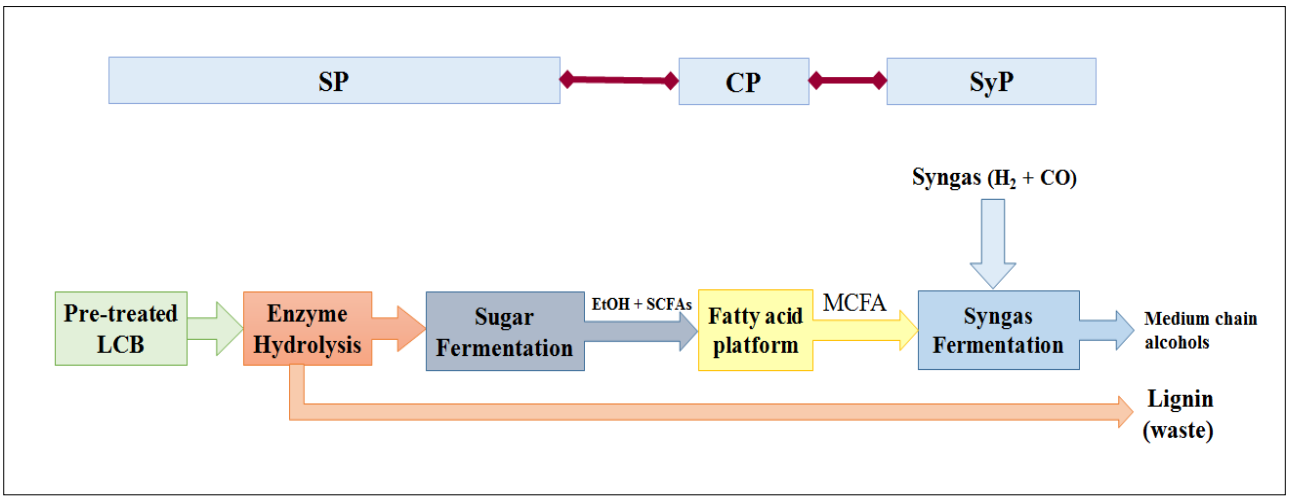

Figure 7: Scheme of multi-stage hybridization of SP-CP-SyP.

The reactor effluent of the yeast driven sugar platform using corn kernel as the feedstock was fed to a carboxylate platform where mixed fatty acids with varying chain length (C2-C6) were generated and continuously extracted from the reactor. The carboxylate platform effluent was fed to the syngas fermenter where a mixture of corresponding alcohols was produced exploiting the reductive power supplied by the syngas by *C. ljungdahlii* [118]. This process ensured the sequential utilization of the liquid effluent of sugar and carboxylate platforms as well as decreased the expenses incurred for the synthetic fermentation medium for the syngas platform. Higher alcohols, namely butanol and hexanol, were obtained in the ultimate product stream from the last stage of the integrated process [118].

A few research studies have also been reported on SP-CP combinations for the generation of MCFA from LCB [119,120]. Kucek et. al., 2016, collected the liquid effluent 'wine lees' from a winery and used it as the source of nutrients and substrate in an upflow anaerobic bioreactor (UAB) for production of MCFAs, namely, n-caproic and n-caprylic acids. The wine lees is rich in residual ethanol with a concentration of 180.5 gCOD/L (40% ethanol, COD basis) which served as the major electron donor for the chain elongation process [119]. The UAB was inoculated with a chain-elongating reactor microbiome adapted to ethanol rich beer fermentation effluent. The UAB was operated at acidic pH of 5.2 with continuous extraction of products by pertraction. Maximum MCFA productivity of 3.9 g COD/L/d was obtained corresponding to 67% MCFA yield at an organic loading rate of 5.8 g COD/L/d of diluted wine lees [119]. The microbiome analysis revealed that the MCFA production in this study was conducted by the microbial members of *Bacteroides* sp., *Oscillospira* sp. and *Clostridium* sp. The typical chain elongating bacterium *C. kluyveri* was not present in the microbiome [119]. Scarborough et. al., 2018, in a very recently performed study attempted to incorporate the unfermented carbohydrate (mainly xylose) rich part originating from the distillation stage of a preceding ethanol fermentation process as substrate for MCFA production [120]. The ethanol fermentation was conducted by a mutant strain of *Saccharomyces cerevisiae* Y128 using pretreated and enzymatically hydrolysed switchgrass. Analysis of the fermentation samples showed that the glucose fraction was almost fully consumed, but there was significant xylose (47% utilized) remained as unconverted carbohydrate in the fermenter [120]. Post-fermentation the effluent of the fermenter was processed in a glass distillation unit for alcohol recovery and the bottom product was recovered as the xylose and organic rich stillage. This stillage was fed to a bioreactor

588 inoculated with sludge of an acid-phase WWTP. The microbes present in the sludge
589 conducted mixed culture fermentation of the stillage and produced MCFAs, mainly caproic
590 and caprylic acids as the end products with productivity values of 2.6 ± 0.3 g/L/day and 0.27
591 ± 0.04 g/L/day [120]. Community analysis of the microbiome of the MCFA reactor identified
592 *Lactobacillus, Pseudoramibacter* and *Roseburia* as the most abundant microbial species and
593 hence the major of producer MCFA. Based on these results it was proposed by the
594 investigators that *Lactobacillus* produced lactate and acetate by hetero-fermentative
595 conversion of xylose of stillage. Afterwards, the lactate is used as the initial precursor for
596 chain elongation to the MCFAs by *Pseudoramibacter* and *Roseburia* [120]. The liquid
597 effluent generated after MCFA recovery was processed in an AD unit to biogas. The biogas,
598 the leftover bio-solids of the AD process and lignin residues generated at any point of the
599 process was converted to heat and electricity using a CHP unit. The economic analysis of this
600 system (generating ethanol, electricity and MCFAs) indicated that due to the utilization of the
601 stillage for the MCFA production the minimum ethanol selling price was 1.76 USD/gallon,
602 which was 18% reduced price of ethanol (2.15 USD/gallon) obtained from another similar
603 study (generating ethanol and electricity) which does not recover and use the stillage [120]. It
604 is expected that integration of these type of SP-CP hybrids can also be extended to a SP-CP-
605 SyP cascade just by the addition of a syngas fermentation process either in single or in
606 combination with the CP stage.

607 **4. Limitations of the present integration strategies for hybrid platforms**

608 Figures 2-7 represent different strategies of integrating sugar, carboxylate and syngas
609 platforms. The foregoing discussions clearly demonstrated the up-to-the-minute status of the
610 hybridization of the platforms for bioalcohol production from lignocellulosic biomass. The
611 performance of different hybrid platforms from the perspective of processes involved, the
612 advantages and the products have been compared, at a glance, in Table 4.

613 **Table 4: Comparative performances of different hybrid strategies.**

Strategy	**Overall Processes involved**	**Input stream**	**Pretreatment**		**Enzymatic hydrolysis**	**Advantage**	**Alcohol production performance**	**Ref.**
			Process	**Advantage**				
CP-	CP:	CP: LCB	CP: Not	Complete	CP: Not		Much	[101

SP -I	Acidogenesis of LCB in IAD of IAD **SP**: Fermentation of digestate and enzymatically hydrolysed LCB	SP: Digestate from CP	required SP: CP	conversion of hemicellulose in CP. Energy saving due to avoidance of energy intensive acid pretreatment.	required SP: Required	Increase in glucose yield due to increase in the concentration of crystalline cellulose.	higher than conventional SP.	,102]
CP-SP II)	**CP**: Acidogenesis of LCB in IAD **SP**: Fermentation of VFA-rich effluent of IADand enzymatically hydrolysed LCB	CP: LCB SP: LCB + Liquid effluent from CP	CP: Low temperature (50^{o}C) alkali pre-treatment SP: Acid pretreatment + enzymatic hydrolysis	Same as strategy I	CP: Not required. SP: Required only for direct LCB stream.	Enzyme cost reduced due to usage of liquid effluent from CP	Much higher than conventional SP due to direct input of liquid effluent from CP.	[27, 103]
Sing	Simulta	Simulate	Not required	NA	NA	Chain	Medium	[114

le stage SyP-CP	neous conversion of syngas to ethanol and acetic acid and production of higher alcohols through reduction of MCFAs produced though chain-elongation	d syngas + synthetic medium				elongation of SCFAs to MCFAs and reduction to alcohols in a single reactor	chain alcohols (butanol, hexanol) are produced.	]
SyP-CP-SyP cascade	Syp: Conversion of syngas to ethanol and acetic acid CP: producti	SyP (First): Syngas CP: Acetic acid and ethanol from first SyP SyP	Not required.	NA	NA	Chain elongation of short chain fatty acids and reduction to alcohols with continuous removal of inhibitory products (caproic acid	Medium chain alcohols (hexanol, octanol) are produced.	[30, 116]

	on of MCFAs through chain-elongation production of higher alcohols through reduction of MCFAs produced though chain-elongation	(Second): MCFA from CP				etc.) and lower necessity of pH control with respect to single stage system		
SP-CP-SyP cascade	SP: Conventional fermentation of LCBs CP: Chain elongation of VFAs in SP effluent in to	SP: LCB CP: ethanol and stillage SyP: MCFA from CP	SP: Conventional	NA	Conventional	Full utilization of effluents of SP with conversion to higher alcohols	Medium chain alcohols (butanol, hexanol) are produced.	[118]

	MCFAs Syp: Reduction of MCFAs to higher alcohols							

From the review of the current scenario it is revealed that the solid digestate of the carboxylate platform, proceeding via incomplete AD, can be used in the sugar platform after enzymatic hydrolysis and without an energy-intensive pretreatment process. Carboxylate platform can also serve as a complementary process for the syngas platform and vice-versa for the production of higher alcohols. The hybridization of the three platforms has been claimed to be one of the best combinations regarding production of bio-alcohols [118]. However, an in-depth scrutiny further reveals some facts that should also be incorporated in future research endeavors attempting hybridization of SP, CP and SyP. From the analysis of Figures 2-7, it is evident that in the CP-SP combinations, studied so far, only few attempts have been made to utilize the liquid effluent and solid digestate of the carboxylate platform. The anaerobically pre-treated digestate can be used as a source of simple sugar and the liquid product of this process can act as precursors for butanol in a subsequent sugar platform, other than ethanol production. In case of SyP-CP and SyP-CP-SyP combinations, most of the previous studies have been focused on simulated systems where the starting points of SyP-CP are syngas and bioreactor-ready medium containing externally added carboxylic acid [30,118]. In fact, when complex lignocellulosic compounds are to be handled in reality, even in these attractive combinations there will be generation of digestate in the carboxylate platform and ash in the syngas platform, if gasification is used as the thermochemical process. Although the synergistic integrations of AD and pyrolysis (PY) in the form of AD-PY, PY-AD and AD-PY-AD have been successfully studied for the generation of biogas, no attempt has been made to incorporate these for bio-alcohol generation [90, 121,122]. However, pyrolysis processes can be incorporated in the syngas platform in place of gasification [123]. Besides the utilization of pyrogas in the syngas platform, other products of pyrolysis, namely, pyro-liquid and pyro-char can also be incorporated in the sugar and carboxylate platforms respectively leading to a hybrid system with zero effluent. The

interesting characteristics of pyrolysis along with the general definition of the process are provided in the following section.

5. Special characteristics of pyrolysis and its prospective role in hybrid platforms

Pyrolysis is a thermochemical process popularly used to effectively convert lignocellulosic biomass to pyro-oil, pyro-gas and pyro-char in the temperature range of 300-900^{o}C [124-128]. While cellulose and hemicellulose are converted to anhydrous-sugars, namely, levoglucosan, cellobiosan etc. and acetic acid, respectively, lignin is converted to phenols and other aromatic compounds and char is produced constituting unconverted solid fractions [129-133]. Some recent studies show high yields of pyro-gas (27.8-34.8% (w/w), pyro-oil (31-53.5 % (w/w)) and pyro-char (18.7-34.2 % (w/w)) during pyrolysis of lignocellulosic agro-waste (rice straw and sugarcane bagasse) and forest wastes (pine wood) [134-136]. The sugars and acids appear in the aqueous phase of pyro-oil. The pyro-gas is mainly constituted of CO, CO_2 and H_2, closely resembling the syngas [123]. Some special characteristics of the pyrolysis process have been tabulated (Table 5) and assessed to judge its suitability to be employed as a candidate process of a hybrid platform for bio-alcohol production.

Table 5: Special characteristics of pyrolysis process regarding hybridization.

Characteristics	**Refs.**
• Pyro-gas can be used in the syngas platform for bio-alcohol generation.	[123]
• Levoglucosan can be converted to glucose and fermented to bio-alcohols.	[133, 137-141]
• Cellobiosan can be converted to reducing sugars.	[142,143]
• Acetic acid can be converted to acetyl-CoA, the central precursor for the production of fatty acids and in turn alcohols by many organisms.	[144]
Pyro-char can be used • as an enhancer of acidogenic step in AD (and IAD processes). • as a biosorbent in alcohol recovery from bioprocesses	[145-147] [148]

• for soil amendment and hence carbon sequestration	[149,150]

From Table 5, it can be inferred that PY can be incorporated in the integration efforts, which will fulfill the zero-effluent criterion of a circular economy [151,152]. By incorporation of PY, besides the syngas, anhydrous sugars and acetic acid in the aqueous pyro-liquid (APL) can be fermented to alcohol using potential microorganism, while biochar and the non-aqueous phase of bio-oil can be obtained as valuable by-products in conventional ways [133-148]. Although it has been identified by many researchers that pyrolysis can serve as a potential process for the generation of intermediates, namely, sugar, syngas and carboxylic acid for SP, SyP and CP respectively, information on its incorporation in hybridization is not available. In one patent (US20120073199A) a low temperature long residence time pyrolysis process was claimed to convert high-lignin LCB to pyro-liquid, which can be fermented to bio-ethanol using yeast [153]. The Table 6 provides some reported facts on conversion of APL, obtained from fast pyrolysis of biomass to bioethanol.

Table 6: Production of ethanol from levoglucosan (LG) of LCB-derived pyro-oil.

LCB feedstock And Pyrolysis Temperature	**LG content of pyro-oil %(w/w) and APL (g/L) used for Pretreatment/direct fermentation**	**LG to Glucose %(w/w)**	**Microorganism**	**Ethanol yield**	**Ref.**
Red oak 500 ^{o}C	2.8 36±1 g/L	NA*	*E. coli* KO11 + *lgk*	0.235 g/g LG 0.65±0.08 g/L	[137,132]
Scots pine 450 -500 ^{o}C	7.8 87 g/L	216	*S. cerevisiae* T2	0.46 g/g glucose	[138, 154]
Waste cotton cellulose 400 **^{o}C**	43 104.3 g/L***	166**	*S. cerevisiae* 2.399	0.45 g/g glucose 16.1 g/L	[139]

			Z. mobilis 10232	0.44 g/g glucose 1.8 g/L	
			Pichia sp. YZ-1	0.42 g/g glucose 15.1 g/L	

[*]Directly fermented as per description in reference.

[**] Calculated from corresponding values in reference.

[***]Calculated from corresponding value (10.43 % (w/w)) in reference.

It is clearly indicated that the anhydro-sugar, namely levoglucosan present in APL is converted, either directly or indirectly via hydrolysis to glucose and ultimately to ethanol. It is thus expected that the enrichment of levoglucosan in APL is directly related to increase in alcohol yield. A recent research article on pyrolysis of oak wood suggests that low concentration oxygen in the sweeping gas leads to higher yield of hydrolysable sugar [155]. It was recommended that the AD of pyro-liquid for the generation of biogas could be another option for producing biomethane as the biofuel [153]. Some studies also suggest that biochar obtained through pyrolysis enhances the yield of ethanol during fermentation of syngas [156]. The addition of biochar is beneficial during the chain elongation reaction using ethanol and. acetic acid. Biochar has been reported to enhance the yield of caproic acid up to 21.1 g/L in comparison to 14.4 g/L obtained for control in absence of biochar [157]. Although there is also a scope for the co-fermentation of carboxylic acid (mainly acetic acid) present in the aqueous part of pyro-liquid with pyro-gas, containing mainly CO, H_2 and CO_2, to higher alcohols in presence of chain elongating and syngas-fermenting bacteria either in cascades or in single stage, the invention did not consider this avenue.

6. Recommendations

Although, hybridization of platforms for bio-alcohol production from LCB seems to be a very captivating research field in recent times, it involves challenges of biochemical reaction kinetics, complex mixed microbial systems, reactor design with efficient process control strategies, thermochemical reactions, mass transfer limitations, recycling of enzymes and catalysts, product recovery, utilization of bio-alcohols in engines either solely or as drop-in

fuels, economic viability, energy and environmental sustainability, and hence entails an interdisciplinary approach.

Analyzing the combination of different platforms for bio-alcohol production from a holistic approach, the incorporation of the following research objectives can be useful in upgrading the present status:

- Coupling of platforms for the utilization of wastes from all individual platforms in as many ways as possible.
- Ensuring implementation of zero-waste concepts by incorporating processes like pyrolysis generating products (char) for carbon sequestration.
- Enhancing overall energy efficiency by incorporating AD processes generating biogas from the effluents of all platforms.
- Improvement of each individual platform (sugar, carboxylate and syngas) from the perspective of microbial and thermochemical reaction kinetics, better reactor design, mass transfer enhancement (syngas platform), process/bioprocess control, knowledge on microbial interactions, re-use of hydrolytic enzymes (sugar platform), catalyst design (gasification/pyrolysis) for improved gas yield, efficient downstream processing for bio-alcohol recovery.
- Development of microscopic and macroscopic mathematical models, whatever necessary, from basic material and energy balances to predict hybrid system performance and facilitation of scaling up.
- Development of process simulation models for the prediction of performance of large scale units.
- Testing of compatibility of bio-alcohols with gasoline engines.
- Process performance based life cycle assessment (LCA).

Keeping all these issues in consideration, a research strategy exploring the possibility of coupling platforms at any stage to generate bio-alcohols from lignocellulosic biomass with zero effluent is recommended from the perspective of economic, environmental and energy sustainability.

6.1. CONVER-B: An advanced model of hybridization of SP-CP-SyP platforms

The collaborative project CONVER-B (the acronym for the INNO-INDIGO research project, “Development of efficient biomass conversion routes for biofuel production and utilisation”) among the present research groups from India, Finland and Germany focuses on the

hyphenation of the three platforms using this strategy as the blueprint [158]. It is intended to produce bio-alcohols, particularly higher ones (butanol, hexanol etc.) simultaneously, with biogas, hydrogen, non-aqueous pyro-oil and pyro-char as by-products with zero-waste generation from various agro-wastes as well as distillery waste, silage etc. Economic viability as well as energy and environmental sustainability will also be examined along with the proof-of-concept study of usability of the biofuels in existing gasoline engines. The conceptual framework of the research scheme 'CONVER-B' is represented in Figure 8.

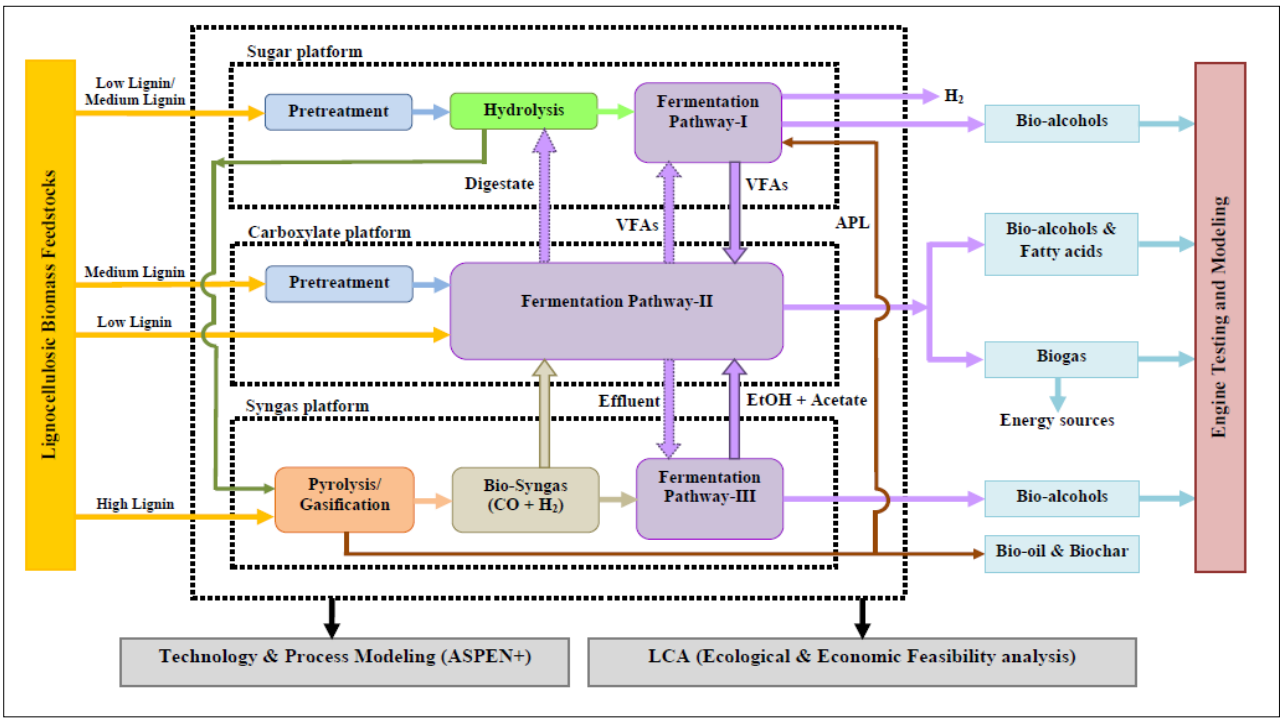

Figure 8: Framework of the CONVER-B research scheme of hybrid platforms producing bio-alcohol from LCBs.

The scheme of project has been designed in such a manner that most of the challenges of different hybrid platforms, as discussed in section 4, can be addresses. Table 7 highlights the strategies of CONVER-B to address different shortcomings of existing hybrid cascades.

Table 7: Challenges of hybrid platforms and addressing strategies in CONVER-B scheme.

Platform	Challenges	Addressing Strategy in CONVER-B
CP-SP-I	Unutilized VFA-rich liquid stream from IAD	Chain elongation of VFA to MCFA by combining with SyP

	Unutilized Lignin-rich solid from enzyme hydrolysis step	Pyrolysis/gasification of lignin stream and utilization of syngas and aqueous part of pyro liquid in SyP and SP respectively.
CP-SP-II	Unutilized digestate of IAD process Unutilized Lignin-rich solid from enzyme hydrolysis step	Digestate of IAD is converted to biogas in an anaerobic digester Lignin rich stream is fed to syngas platform
Single stage SyP-CP	Studies are based on simulated syngas and VFA medium Microbial interaction (commensalism, mutualism etc.) among the microorganisms are not studied	Real syngas generated from pyrolysis / gasification of LCB is studied The VFA rich effluent obtained through acidogenesis in IAD of LCB is used. Microbial interaction between the microorganisms of Syp and CP is studied
2-stage SyP-CP, SyP-CP-SyP, SP-CP-SyP	Studies are based on simulated syngas and VFA medium	Real syngas generated from pyrolysis / gasification of LCB is studied The VFA rich effluent obtained through acidogenesis in IAD of LCB is used.

It is expected that the research outcomes of the project can be utilized to develop sustainable alcohol production units on hyphenated platforms running on various LCB feedstocks having diverse composition regarding cellulose, hemicellulose and lignin as well as elemental analysis (C,H,N,O,S). It is clear that although the presence of nitrogen and sulfur is beneficial for fermentative processes, thermochemical processing, particularly, gasification of LCBs rich in S and N is expected to generate SO_X and NO_X. While high moisture content is a must

for biochemical processing, thermochemical processes become energetically inefficient when wet biomass is to be handled. The project is aimed to follow a zero-effluent criterion as each waste biomass can be converted by the best-suited approach within the overall combined process. Implementation of the research outcomes will be particularly useful for making the energy balance of rural regions of India positive, i.e., surpassing the consumption of energy by its supply from locally available agro-wastes and hence for the overall up-gradation of societal standard of those regions [4]. In Germany, the agricultural biogas sector is well developed [159,160]. However, the conversion of energy crops to biogas, which is burned in combined heat and power (CHP) plants, is economically not viable without subsidies. The implementation of advanced biorefinery concepts as envisaged in the CONVER-B approach by re-fitting the existing biogas infrastructure will open up new perspectives for the biogas sector after feed-in tariffs for biogas-generated electricity run out, and more sustainable value added chains can be established in the agricultural sector. As Finland is rich in forest residues, the implementation of outcomes of CONVER-B will be highly beneficial from the perspective of establishment of biorefineries in future [161].

The results from twinned platforms clearly indicate that a mixture of alcohols would be produced particularly when the carboxylate platform relying on microbiomes is used [30,162]. Therefore, the assessment of performance of existing engines with mixtures of alcohols or their blends with gasoline by experiments and through modeling is crucial [163-166]. Although recent studies showed that the correlation between process parameters such as pH, temperature etc. with the dynamics of active microorganisms in the mixed consortia or the reactor microbiome governs the process efficiency of the carboxylate platform, data in this direction is scarce [167-170]. Since in many cases single stage arrangements for the integration of different biochemical steps are used, studies on interactions between microorganisms of different platforms are essential. Attempts of mathematical and process modeling of multi-platform systems for the prediction of performance of large scale installations or life cycle assessment have not yet been made despite their essentiality for future development of sustainable bio-alcohol units based on lignocellulosic waste. Therefore, future research should address these shortcomings.

7. Conclusions

C-5 and C-6 carbohydrates, carboxylic acids and syngas are important precursor compounds which are ultimately converted to bio-alcohols in three popular pathways, namely, sugar,

carboxylate and syngas platforms. As all the platforms involve some technical loopholes, the research studies on the hybridization of any two or all three of the platforms are gaining interest. The present article focuses on the analysis of pros and cons of each platform along with the review of the present state-of-art of the hybrid platforms for the first time. It is revealed that higher alcohols can be generated by the combination of syngas and carboxylate platforms through the utilization of reducing and chain elongating properties of microorganisms present in the former and latter ones respectively. It has been identified that the correlation between process parameters such as pH, temperature etc. with the dynamics of active microorganisms in the mixed consortia or the reactor microbiome governs the process efficiency of the carboxylate platform, and hence in the hybrid platform. Pyrolysis has been earmarked as a potential process to be used in syngas, sugar and carboxylate platforms simultaneously. From the thorough analysis of the present status of hybrid platforms important objectives for future research studies in this area have been presented. Ultimately the blueprint of research project, CONVER-B, an INDO-EU project taking care of all aspects of hybridization of SP, CP and SyP pathways for the generation of bio-alcohols from lignocellulosic wastes ensuring zero effluent has been highlighted.

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