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OPINION

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Hybrid electrosynthesis as non-genetic approach for regulating microbial metabolism towards waste valorization in circular framework

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Abstract

Biogenic waste (solid/liquid/gaseous) utilization in biological processes has disruptive potential of inclining towards carbon neutrality, while producing diverse products output. Anaerobic fermentation (methanogenesis and acidogenesis) routes are crucial bioprocesses for production of various renewable chemicals (carboxylate platform/organic acids, short/medium chain alcohols, aldehydes, biopolymers) and fuels (methane, hydrogen, hythane, biodiesel and electricity), while individual operations posing process limitations on their conversion efficiency. Advantageous benefit of using the individual bioprocess technicalities is of utmost importance in the context of sustainability to conceptualize and execute integrated waste biorefinery. The opinion article intends to document/familiarize the waste-fed biorefinery potential with application of hybrid advancements towards multiple product/energy/renewable chemical spectrum leading to carbon neutrality bioprocesses. Unique and notable challenges with diverse process integrations along with electrochemical/interspecies-redox metabolites-materials synergy/enzymatic interventions are specifically emphasized on application-oriented waste feedstock potential towards achieving sustainability.

INTRODUCTION – WASTE FED BIOREFINERY

Climate change is a constant alarming concern in the context of ever-increasing environmental pollutants with anthropogenic activities and green-house gas emissions (Jiang et al., 2019). Mitigation needs to be emphasized in the context of sustainability and aligning to the advancements in carbon capture, utilization and storage (CCUS) processes (Venkata Mohan et al., 2016). Biogenic waste (solid/liquid/gaseous) has huge potential to capture market, when cohesively involved with individual advantages of the bioprocesses in an integrated manner (Venkata Mohan et al., 2016; Venkata Mohan & Katakojwala, 2021). Non-genetic biological approaches could be economically viable with higher reach for execution at variable operational scales for multiple products benefit (renewable chemicals, energy and fuels) with carbon neutralization (Tharak & Venkata Mohan, 2022). Hence, the advantage of biocatalysts with regulation on the process limitations and mechanism towards maximizing products conversion using waste feedstock could be considered a challenge to address in the context of waste biorefinery.

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Biological processes are considered sustainable options in waste utilization/conversion towards renewable chemicals, energy and fuels production (Tharak & Venkata Mohan, 2022; Van et al., 2020). Anaerobic fermentation (AF)/anaerobic digestion (AD) are potential bioconversion processes for treating diverse biogenic waste/wastewaters resulting in energy/product spectrum generation (Van et al., 2020; Zhao et al., 2020). Individual bioprocesses have conversion efficiency and system stability limitations, resulting in decreased overall process performance and products output (Baek et al., 2018; Zhao et al., 2020). Mechanism-oriented regulations with process integrations relative to metabolic/electrochemical/enzymatic/material interventions are crucial for specified products generation (Bertsch et al., 2016; Chen et al., 2020; Cotton et al., 2020; Jiang et al., 2019; Lovley & Holmes, 2022). In this domain, the residual organics/inorganics conversion/utilization to increase the overall energy/product value requires acidogenesis and methanogenesis process integrations along with hybrid advancements based on required products orientation (Borrel et al., 2016). Acidogenesis process could be beneficial in establishing a carboxylate platform in terms of short/medium chain fatty acids production (Cotton et al., 2020). Unregulated acidogenesis could lead to methanogenesis (CH_4 production) during biological processes, which is beneficial yet leads to unspecified product output with lesser process understanding (Detman et al., 2018; Van et al., 2020). Hence, process regulations at individual level could be a criterion for establishing/understanding the biological mechanisms with metabolic/electrochemical/enzymatic/material interventions with channelized integrations towards carbon neutrality, residual carbon utilization with maximized products generation (Berghuis et al., 2019; Martins et al., 2018; Singh et al., 2020). Photosynthesis integration can also be aligned to acidogenesis and methanogenesis to achieve self-sustainability, while maximizing resource recovery with carbon neutral footprints/near zero carbon discharge (Venkata Mohan et al., 2016). Photosynthetic integrations could additionally benefit in biomass, biodiesel, bioethanol, edible oil, protein, carbohydrates, nutraceuticals and pigments, while deoiled biomass can be used for biofertilizer and biochar/bioelectrode preparations (Jiang et al.,2019; Venkata Mohan et al., 2016).

Conceptual circularity framework with domestic and industrial concerns is an accountable scenario with current environmental issues (Venkata Mohan & Katakojwala, 2021). Carbon neutrality maintenance needs to be considered as a state-of-art entity prioritizing the carbon capture, utilization and storage (CCUS) with green chemistry principles (Venkata Mohan et al., 2016; Venkata Mohan & Katakojwala, 2021). Integrated closed loop approach in the context of establishing waste biorefinery with multiple cohesive bioprocesses is ideal towards benefitted environmental and economic sustainability (Figure 1) (Venkata Mohan et al., 2016). Waste-fed biorefinery emphasizes on the thorough utilization of residual carbon savings of individual bioprocesses within a channelized network as incentives for additional products benefit with further reduction in footprints. Waste-fed biorefineries could be a techno-economically viable benchmark platform technologies with customized streamlining for integration with respect to the waste/wastewater/biomass characteristics. System thinking and life cycle considerations in the context of industry 4.0 and societal benefits need to accounted for cohesively inter-linking bioprocesses paving a futuristic way for establishing a circular economy concept.

METHANOGENESIS

Methanogenesis process involves conversion of CO_2/acetate/hydrogen/formate/pyruvate and other compounds from organic substrates to CH_4 depending on the microbial pathways involving metabolic/enzymatic variations (Borrel et al., 2016; Cotton et al., 2020; Van et al., 2020). Biologically methanogenesis could overcome the limitations by varied metabolite/electrochemical/enzyme/material interventions, in presence of diverse electron donors/shuttlers contributing to the overall process intensification (Baek et al., 2018; Zhao et al., 2020). These specified interventions could benefit in regulating the electron/carbon flux to impact on the overall waste valorization capabilities towards maximizing products/energy/renewable chemicals generation (Borrel et al., 2016). Hydrogenotrophic methanogenesis facilitates CO_2 conversion to CH_4 using hydrogen (H_2) as redox mediator or by the direct utilization of reducing equivalents (e^- and H^+) for the CO_2 reduction involving microbial interspecies electron transfer (Berghuis et al., 2019). Methylotrophic methanogenesis is the reversible process of hydrogenotrophic methanogenesis, where CH_4 is used as substrate towards CO_2 conversion with involvement of and redox mediators and enzymes (Sravan et al., 2020). Acetoclastic methanogenesis uses acetate as the precursor towards its conversion to CH_4 involving sequential steps with specified redox mediators and enzymes (Detman et al., 2018). Methanol is also a key product that can be generated during the methanogenesis pathways (Berghuis et al., 2019; Cotton et al., 2020; Sravan et al., 2020). It could also be prioritized and given emphasis under strict controlled process regulations to restrict CH_4 formation, where it essentially has customized applications while giving rise to methanol bioeconomy (Cotton et al., 2020). Regulation of these processes with different channelized strategies to result in an enhanced and specified products formation needs to be prioritized.

The residual/undesirable CO_2 component could be given substantial importance in biogas upgradation/other related processes to achieve a near zero emissions in line with the circularity context (Sravan et al., 2021;

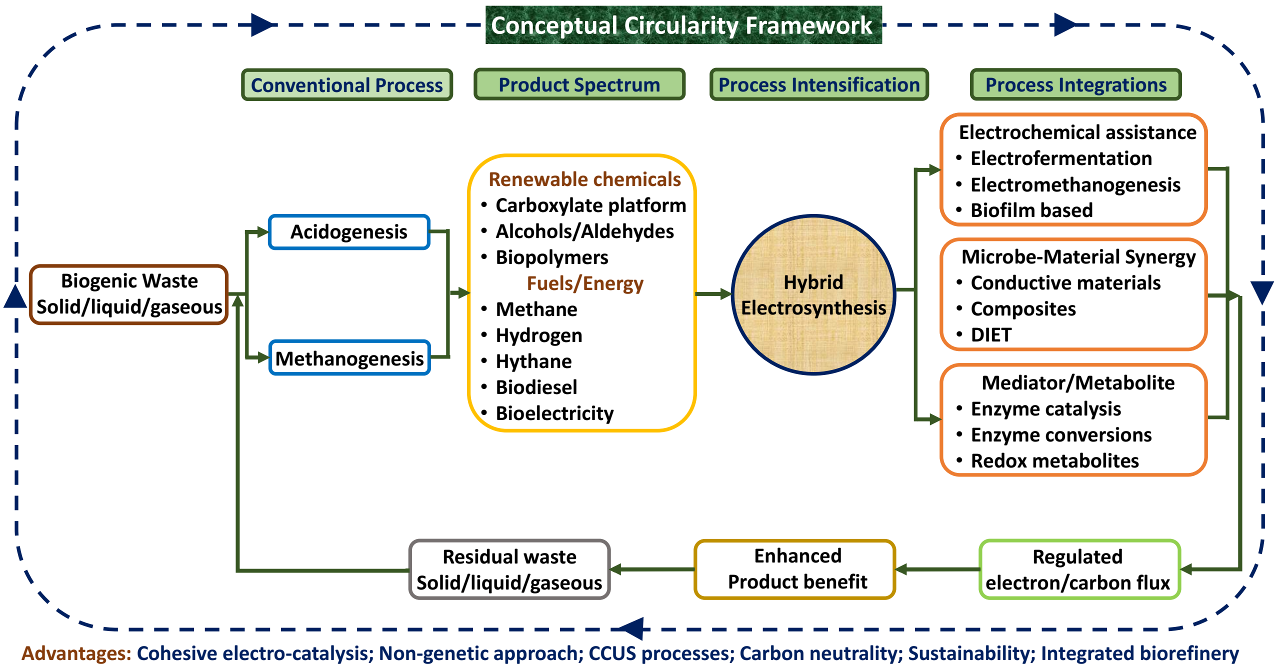

FIGURE 1 Conceptual circularity framework depicting possible integrations of biological and bioelectrochemical routes towards carbon neutrality.

Zhao et al., 2020). Microbe-microbe/microbe-material interactions need to be provisionally given importance with respect to the metabolite/electrochemical/enzyme/material interventions in biological/bioelectrochemical systems towards achieving higher CH_4/other bioproducts formation (Jiang et al., 2019; Nozhevnikova et al., 2020). Solid electrodes/conductive material and electrochemical supplementation (applied voltage/current) interventions during the biological processes (hydrogenotrophic/methylotrophic/acetoclastic) could additionally benefit in sinking/delocalization of the electrons, decreasing/neutralizing the system losses with higher CO_2/H_2 utilization towards enhancing CH_4/product formation along with the calorific value of bioenergy generated (Jiang et al., 2019; Martins et al., 2018). External energy provided impacts the microbial metabolism by creating a synergy between interspecies interactions by suppressing the intermediates formation towards increased CH_4 or specified products generation (Basile et al., 2020). Integration of syntrophic interspecies interactions with DIET (presence of pili or Omc complexes) is highly efficient in overcoming the intermediates formation as a result of direct transfer of reducing equivalents (e^- and H^+) between the microorganisms, thereby increasing the overall system performance (Nozhevnikova et al., 2020).

ACIDOGENESIS

Acidogenesis is key biological step in waste valorization (solid/liquid/gaseous) towards microbial conversion to predominantly carboxylate platform products (volatile fatty acids [VFA], that is, acetic, propionic, butyric, iso-butyric, valeric and iso-valeric acids), along with alcohols, aldehydes, H_2 and CO_2 production (Tharak & Venkata Mohan, 2022). The spectrum of products is of huge interest due to their commercial applications and economic viability garnered from the process (Venkata Mohan & Katakojwala, 2021). Hence, this biological process needs to be taken advantage of to establish integrative methods to maximize the production yields with focus on individual products output with purity (Chen et al., 2020). Integrations within the acidogenesis process could be established by regulating the metabolic/enzymatic reactions, while provision of solid electrodes/conductive materials/electrochemical supplementation could further benefit in process intensification towards enhanced products outcome (Jiang et al., 2019; Venkata Mohan et al., 2016). The VFAs could also act as electron donors in presence of H_2 and ethanol towards medium chain fatty acids (C_6 to C_8) or CH_4 formation via chain elongation within the biological microenvironment (Bertsch et al., 2016; Nozhevnikova et al., 2020). Acidogenesis process needs to be subjected to diverse pre-treatment strategies to avoid CH_4 formation, while enhancing the production of VFAs and H_2. Overall, the integrative approach of acidogenesis with carboxylate platform with methanogenesis could benefit in increasing the carbon/electron flux during the biological/bioelectrochemical/electrocatalysis processes to establish near carbon neutrality with maximized products outcome (Basile et al., 2020).

PROCESS REGULATIONS

Electro-catalysed interventions

Electro-catalysed strategies (electrofermentation/electromethanogenesis) could essentially help in process regulations by increasing the microbial metabolic capabilities and electron flux towards renewable chemicals and fuels production (Jiang et al., 2019; Sravan et al., 2021). Electrochemically steering the biological processes with the aid of applied current/voltage could benefit in sustained balance of electron flux, providing an additional reducing power to the biocatalyst towards higher microbial catabolic activity and electrometabolic conversion (Chen et al., 2020; Nozhevnikova et al., 2020). Electrochemical process regulation emphasizes on minimizing the process energetics, electron transfer potentials and system losses to maintain ideal process conditions towards improving products output (Sravan et al., 2021). The external/internal electrochemical factors influence to polarize the electrodes and increase the electron flux with efficient and interspecies compatibility to channelize the overall bacterial mechanisms, while regulating the redox intermediates involved in the electron transfer pathways (anode/cathode), that are crucial in driving the process specificity towards a single product synthesis (Singh et al., 2020).

Microbe-material-microbe synergy

Syntropic relations need to be maintained between two/more microbes towards establishing direct interspecies electron transfer (DIET) (Baek et al., 2018; Lovley & Holmes, 2022; Zhao et al., 2020). In this context, conductive materials/nanomaterials/hybrid synthesized nanomaterials/metals could be provided as additional intermediate electron acceptor and capacitative tool in biological/bioelectrochemical systems to address electron losses, thereby regulating electron flux of the overall system towards increased products output (Martins et al., 2018). These materials can be considered to have potential scope to replace the genetic manipulations required in the biological treatment systems to establish a streamlined bioprocess operation (Singh et al., 2020). Microbe-material interactions with the electrogenic interventions (in situ/ex situ current/voltage) in various specified bioprocesses provide the aspects of metabolic/mechanism regulation for improved resource valorization and waste/wastewater remediation (Martins et al., 2018). Contemporary research of material influence on electron transfer during biological processes with varied microenvironments and electrochemical interventions needs to be evaluated in relation to their mechanistic aspects towards maximizing specified products output.

Bioelectrochemical interventions with solid electrode placement or applied voltage/current involve enrichment of specific microbes, that is, exoelectrogens (Sravan et al., 2021). These microbes have the advantage of extracellularly transferring electrons, while electrotrophs can ably accept electrons from outer sources (electron shuttlers/solid electrodes/conductive materials) (Chen et al., 2020). Mostly, *Shewanella* and *Geobacter* sp., are well-studied exoelectrogenic microbes, while few methanogenic microbes such as *Methanosarcina* are studied as electrotrophs to reduce/convert substrate to methane (Detman et al., 2018; Nozhevnikova et al., 2020). Microbe-microbe interaction between the syntrophic partners depicted electron transfer by either indirectly through redox shuttler (H_2/formate) or directly by nanowires/outer membrane cytochromes (Omc) (Basile et al., 2020; Chen et al., 2020; Lovley & Holmes, 2022; Nozhevnikova et al., 2020), which are considered to be significant in novel products synthesis. Interspecies electron transfer (microbe–microbe interactions) integrated with solid electrode/conductive materials presence could benefit in regulating the biological processes with the materials establishing a microbe–electrode–microbe interactions and thereby acting as electron channelizing/delocalizing intermediates for efficient ORR rates and targeted products formation (Baek et al., 2018; Martins et al., 2018). Overall, the combination of exoelectrogens and electrotrophs in proportionality could mutually benefit in enhancing ORR to reduce system losses with respect to waste utilization/conversion towards value-added products (Lovley & Holmes, 2022). Applications-oriented synergistic microbial-material interactions with electrode/electrochemical manipulations needs to be critically evaluated for the benefit of overcoming electron losses and establishing the process as a non-genetic approach in modifying the microbial metabolic routes/pathways towards required product/energy/chemical generation.

Enzymes

Enzymes are biocatalysts with environmentally friendly nature and could be alternatives to chemical catalysts during biological processes (Detman et al., 2018). Enzymes are crucial in regulating the microbial metabolic pathways and significantly contribute to the choice of biobased products output with wide industrial applications (Singh et al., 2020). Oxido-reductases and transferases are key enzymes involved during the microbial metabolism, where the directive ability to increase the conversion efficiency of substrate utilization towards enhanced products formation is noticed (Detman et al., 2018). Specific enzymes, such as dehydrogenase, oxidase and catalase, with their cascadic availability during microbial metabolism drive the ORRs with determination of the fate of bioproducts formation (Bertsch et al., 2016; Detman et al., 2018). Hydrolytic enzymes (proteases, amylases, pectinases) are key in degradation of natural/complex polymers in the substrates and raw materials to result in simplified form of sugars for bioprocess feasibility in operation (Basile et al., 2020; Bertsch et al., 2016). Precursor specific enzymes, such as formate dehydrogenase (FDH), acetyl Co-A and pyruvate dehydrogenase (PDH), are crucial for preferred products outcome (Cotton et al., 2020). Process parameter regulations need to be strictly considered for enzymatic in situ/ex situ evaluation for both biological and bioelectrochemical processes (Basile et al., 2020; Singh et al., 2020). Recent integrative hybrid advancements like enzyme immobilization/nano-enzymes in a biological process or enzymatic electrocatalysis within a bioelectrochemical system needs specific emphasis with controlled electron/carbon flux conditions and operating parameters to align a specific network of required biobased products output under mechanistic routes regulation (Singh et al., 2020).

FUTURE PERSPECTIVE AND KEY CHALLENGES

Tuning the microbial biocatalysts with metabolic/electrochemical/enzymatic/material sources as non-genetic approaches to establish a profound grip on their mechanism/pathways is a futuristic priority in the context of circularity and requires coherent studies with process integrated perspective (Cotton et al., 2020; Sravan et al., 2021; Venkata Mohan et al., 2016). Biocatalyst-dependent mechanistic aspects along with strict process/mechanism regulations need to be considered for integrations with respect to using the end/intermittent products of one pathway and aligning it as a precursor, electron donor/acceptor or metabolites of another biocompatible pathway to increase the energy/chemical/products gain and process efficiency. Increasing the composition of precursor organic substrates (pyruvate/acetate/formate) is essential for increasing the product-directive research (Basile et al., 2020; Borrel et al., 2016; Cotton et al., 2020). Integrated studies with metabolic/electrochemical/enzymatic/material sources preferably help in increasing the precursors composition towards enhancing the energy/chemical/product value (Cotton et al., 2020; Sravan et al., 2021). Microbe ability to interact preferentially with various external electron acceptors is critical in determining the fate of specified product formation (Nozhevnikova et al., 2020). Electron transfer/flow depends on the redox changes and is directly related to the potential difference of concentrations of oxidized and reduced species (Basile et al., 2020). The overall energy gain during the biological process is the result of a cascading series of electron transfer reactions and is crucial for microbial growth and its related metabolic activity. Alterations of electron transfer with the function/choice of electron acceptors/donors in a biological system is essential for specific products generation. Terminal electron acceptors and several organic/inorganic compounds involve in critically altering the microbial metabolism, thereby influencing the overall process efficiency and required product synthesis (Sravan et al., 2021). Although the targeted product from fermentation routes might be specific, presence of intermediate electron acceptors/donors or metabolite/electrochemical/enzymatic/material alterations might drive the pathway to bypass the conventional pathway to produce a different targeted product of higher commercial value (Bertsch et al., 2016; Zhao et al., 2020). The induced sources create an optimized electron flux between the microbe–microbe/microbe–material interactions with syntrophic activation by tuning the intermediate electron acceptors/donors towards increased ORR rates in the biosystem (Nozhevnikova et al., 2020). Enzymatic electrosynthesis is useful in catalysing the microbial metabolism and related ORR towards specified products, but needs to be studied for economic aspects to evaluate the practical applicability of a biological/bioelectrochemical processes (Singh et al., 2020). In this integrated waste-fed biorefinery domain, unforeseen challenges are expected and could be suitably addressed by identification/development of efficient strains with catalytic/enzymatic properties suitable for carrying out the intended catabolic reactions towards targeted product/energy/chemicals generation. Basic challenges in the context of system losses (activation/ohmic/concentration) with operation of individual/conventional processes could be thoroughly benefitted by integrated mechanistic-oriented studies. Overall, the challenges with specific directive products-oriented mechanisms are pertained to be considered and researched towards establishing long-term stability of enzymes/co-factors/metabolites optimizing electrochemical/material requirements, along with considering their economic perspective. Waste-fed biorefinery aspects benefit in influencing the overall carbon/electron flux with circularity impact towards near zero carbon neutrality bioprocess.

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CONFLICT OF INTEREST

All the authors have declared no conflict of interest.

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