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Profitable biomethane production from delignified rice straw biomass: Effect of lignin, Energy and economic analysis

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Abstract

In this study, a cost and time saving strategy for the recovery of biomethane from rice straw using a novel phase-separation delignification process prior to a bacterial pretreatment (BP) has been reported. The rice straw was subjected to delignification using mechanical homogenization to remove the lignin content, which enhanced the mass transfer of cellulose to the second step, cellulase secreting bacterial pretreatment (CBP) process. The results showed that a higher lignin removal efficiency of 72% was achieved at an optimal biomass to water ratio of 0.04 (*w/v*) and a specific energy input of 114.3 kJ/kg TS. With a pretreatment time of 24 h, the delignified rice straw with the cellulase secreting *Bacillus sp.* pretreatment process resulted in the formation of higher soluble chemical oxygen demand (38.2%), cellulose (35.2%), and hemicellulose fraction (31.8%) than the CBP (22.92%, 21.2 %, and 19.1%) and control (3.43%, 3.16%, and 2.9%), respectively. The D-CBP (delignified cellulase secreting bacterially pretreated rice straw samples) achieved a maximum lignin content of 0.5 g/L that did not inhibit the methanogenesis process, resulting in a specific biomethane production of 165 mL/g VS. The results from large scale energy balance analysis revealed that D-CBP saved a maximum energy of 769.08 kWh/ton. The results from economic analysis for D-CBP indicated a net profit of 134.89 USD/ton and a cost-benefit ratio of 1.52 which was comparatively better than CBP (-86.07 USD/ton and 0.71).

Keywords **:** Rice straw; delignification; bacterial pretreatment; biomethane; energy analysis, economic analysis.

Introduction

In recent years, the need for bioenergy recovery from renewable biomass has been emphasised by researchers, national governments and different stakeholders. Primarily, this is attributed to the global demand for energy, pollution-induced environmental effects and fossil fuel depletion. The generation of biomethane from abundantly available lignocellulosic biomass could abbreviate the release of gaseous pollutants through fossil fuel replacement, resulting in the improvement of energy security and the economy of any country [1,2]. Among the various lignocellulosic biomass, agricultural residues are conceived as a valuable and renewable alternative for synthetic cellulose-rich substances [3–6]. Rice straw is one of the potential agricultural residues as they are inexpensive, renewable, easily available non-food resource, and possesses high bioenergy conversion potential [7–9]. Rice straw comprises of 30-35% cellulose, 18-25% hemicellulose, and 15-22% lignin [10–13]. The presence of cellulose, hemicellulose, and lignin in agricultural residues, i.e. the recalcitrant nature of biomass, hampers microbe and enzyme mediated biodegradation and bioconversion [14,15]. Therefore, pretreatment of biomass is essential prior to the bio-methanation process as the aforementioned components potentially resists biodegradation.

In the literature, various chemical, physical, and biological pretreatments have been recommended for disintegrating lignocellulosic biomass and to make avail the cellulose for subsequent biomethane production [16–18]. Every pretreatment strategy varies in their effectiveness and mode of action, which ultimately has an effect on the biomass conversion process [19,20]. Most of these pretreatments impact the total utilization of chemicals and energy, water necessity, loss of sugars, production of inhibitors, and generation of residues, except the biological methods. However, the limitation of biological pretreatment, i.e. enzymatic or microbe-mediated, for full scale applications, is its lesser effectiveness and insignificant lignin removal [21,22]. In the case of enzymatic pretreatment of rice straw, an elevated dose of the enzyme is usually needed owing to its firmness; however, the availability of commercial cellulase is still considered to be expensive at the industrial scale.

The application of mono or pure bacterial strains as a pretreatment strategy offers several advantages: less energy consumption, less capital cost, and mild operational/process conditions. However, bacterial pretreatment also has some limitations, such as insufficient mass transfer. For instance, it is recognized that the penetration of enzymes during biological pretreatment into the fibrously organized rice straw biomass is a cumbersome process. Besides, the presence of hydrophobic aromatic polymeric cross-linkage molecules such as lignin would adhere and twists with cellulose and hemicelluloses [23–25]. The accessibility of cellulosic fibers to the cellulase secreting bacteria is also restricted due to the presence of outer membrane of microfibrils [26,27]. Therefore, in addition to lignin removal, the microfibrils should also be disintegrated to make the fibers of cellulose amenable to the cellulase enzymes [26]. In this regard, previous reports have suggested that the existence of residual lignin in the pretreated biomass could undesirably impact the biological process as the cellulase enzyme secreted by the bacteria during the pretreatment step might get bound to lignin via higher hydrophobic and electrostatic forces. As an illustration, Li et al. [28] have reported that the existence of tangled lignin on disintegrated cellulosic polymers severely hinders the hydrolysis process induced by cellulase enzymes as the amenability to cellulose surface is decreased. In order to disintegrate the biomass and achieve high lignin removal, multiple steps of pretreatment have been recommended to delignify biomass although these steps could be energy intensive and expensive [29–31].

Delignification of biomass prior to the pretreatment process is important to facilitate better interaction between the active sites of the enzyme (cellulase) and the biomass and therefore, size reduction should be done to increase the contact surface area and to make access the cellulose for biological pretreatment [9,32–37]. However, most of these previous studies did not consider the extent of solubilization, enhanced methane production, i.e. the effect of lignin on methane production, and energy efficiency of the process. Besides, pretreatment using fungi (e.g. *Pleurotus ostreatus*, *Trichoderma reesei*) was done by most of the researchers in order to enhance the saccharification and bioethanol or biomethane production. Only limited studies discussed about methane production. For example, Mustafa et al. [37] discussed about fungal pretreatment of rice straw biomass using two fungal strains (*Pleurotus ostreatus* and *Trichoderma reesei*) and studied the efficiency of pretreated biomass on methane production. The authors reported that even though a strong correlation between methane production and selectivity value (ratio of selective degradation of lignin and cellulose) was achieved, the outcome of lignin removal efficiency was very low. They achieved only 33.4% and 23.6% of lignin removal efficiency from *P.ostratus* and *T.reesei* pretreated rice straw biomass, respectively. In addition, the authors suggested that though pretreatment of rice straw biomass by fungal pretreatment can considerably enhance the methane production, it is more essential to confirm superlative lignin removal during pretreatment as the presence of lignin may affect methanogenesis and even the biological pretreatment (in case of cellulase secreting bacteria or fungi). In a recent study, sequential pretreatment (milling for 30 min) followed by fungal pretreatment (for 30 d) was carried out to enhance delignification and subsequently increase the cellulose hydrolysis during saccharification [9]. As explained previously, the extended pretreatment time using physical and microbe-mediated approaches will make the process energy and cost intensive. From a practical view point, the extended pretreatment time can be reduced by selecting an appropriate biocatalyst, e.g. cellulase secreting bacteria to solubilize the rice straw. In this case, the bacteria that used for pretreatment was excellent cellulase secreting bacteria as it considerably solubilizes the rice straw biomass. Besides, the above-mentioned literature does not consider about solubilization extent, energy efficiency of the pretreatment, operational factors as a whole. It is thus apparent that while there is a handful of literature on biomethane production from rice straw biomass, no literature about energy efficient biomethane production from rice straw biomass using less energy demanding and effective phase separated pretreatments has been reported. Many literature have reported about various mechanical pretreatments (High pressure homogenization, cavitation effect and milling) on fractionation of lignocellulosic biomass – lignin removal and cellulose solubilization and their impacts on biofuel yield [38–40]. For instance, Jin et al [38] have achieved nearly 40% lignin removal through high pressure homogenization of lignocellulosic biomass. In this work, delignification of rice straw biomass was done by high shear homogenization. The process of delignification comprised of lignin removal and the disruption of microfibrils. During this process, the rice straw was constrained inside the equipment through a fast spinning rotor located inside a stationary duct (stator) having slits or openings. The rice straw was pushed by centrifugal forces to the outside region and the high velocity spin induced size reduction of the rice straw *via* the combined action of severe turbulence, cavitation, and blade-like tearing inside the thin slit between the rotor and the stator. As a result, the microfibrils in the rice straw got separated and the lignin was dispatched into the aqueous phase. Ghorbani et al. [9] reported that the delignification process improves the porous nature of rice straw biomass and provides more contact between the enzyme and the cellulose during bacterial pretreatment. In another study using rice straw, fungal treatment for ~ 30 d and milling showed synergistic impacts on lignin removal (>30%) and biomethane yield (263 L/kg VS) during anaerobic digestion [37].

Therefore, the main aim of this work was to test the feasibility of using phase-separated pretreatment to increase the extent of delignification, solubilization (soluble organics, cellulose, and hemicellulose), followed by bacterial pretreatment to achieve energy efficient biomethane production. Besides, an energy balance and cost analysis were done to compare the performance of phase-separated pretreatment with that of stand-alone bacterial pretreatment.

Experimental

Collection and processing of rice straw

Rice straw biomass was collected from a farm field located in Tirunelveli, Tamil Nadu (India). The collected rice straw was dried and cut manually into small pieces (< 1 cm) using a knife. The cut pieces were stored in an airtight container, at room temperature, for further use. The characteristics of the rice straw biomass were tabulated in Table 1. Fig S1 shows the methodology flow chart of the present study (*See supplementary file*).

Delignification of rice straw

The rice straw biomass was subjected to high shear homogenization (IKA T25 Ultra Turrax homogenizer, India, Model No - 3725001) by varying the time and rotation speed from 0 to 15 min and from 4,000 to 12,000 rpm, respectively. The samples collected at regular time intervals were analyzed for soluble components. After delignification, the slurry containing the solids and the liquid phase was subjected to filtration through a vacuum filtration unit. The obtained hydrolysates were removed and analyzed for soluble lignin content. The solids were washed with distilled water to remove the residual lignin and subjected to subsequent bacterial pretreatment.

Pretreatment using cellulase secreting bacteria

Delignified rice straw biomass (0.5 L) was taken in a 1 L conical flask. The cellulase secreting bacteria (0.5 g/L on dry basis; *Bacillus sp*. with accession number - KX373535), which was isolated and reported in a previous study [41] was also used in this study. The flasks containing the sample was subjected to a treatment time varying from (0 to 70 h), at a temperature of 40 ^{o}C (optimized in previous study) [41] and placed in an orbital shaker at 110 rpm to give mild aeration to the samples. Additional two flasks with the bacterially pretreated sample alone and untreated sample were subjected to the same operational condition to examine the impact of delignification due to bacterial pretreatment. All the experiments were done in triplicates, and significance of the data was checked using analysis of variance (ANOVA).

Biomethanation experiment

The biomethanation experiment was done as per the procedure described elsewhere [42,43], at a temperature of 35 ^{o}C and pH 7.0±0.2. This stable range of pH was maintained by adding a pinch of sodium bicarbonate at the start of the experiment. Bovine rumen fluid was used as the inoculum at an inoculum: substrate ratio of 0.5 g COD/g COD. The fraction of methane produced was quantified using a gas chromatograph (Model No G1311C, 1260, India). Assuming that the methane data resembles the exponential growth of methanogenic microbes, the values were modelled using the exponential Box Lucas fit, as described in Eq. (1):

$$y = a\times[1 - \exp(-b\times x)] \quad (1)$$

where, *y* – Specific methane production (mL/g VS); *a* - exponential methane production potential (mL/g VS); *b* - rate constant (day $^{-1}$) and *x* - digestion period (days).

Mass, energy and economic analysis

The mass, energy, and economic analysis of rice straw were done to assess the effect of delignification and bacterial pretreatment on biomethane recovery, but considering full scale applications. The energy and economic analysis were calculated according to the methodology described previously in the literature [31]. The amount of rice straw used as the basis for this analysis was 1000 kg. The parameters involved in the analysis are shown in Table 2.

Analytical methods

The solids content, the total COD and sCOD were determined as per the Standard protocol described elsewhere [44]. The lignin, cellulose, and hemicellulose content were determined as per the method described by Sluiter et al. [45]. All the experiments are done in triplicate and the average of three were taken as optimal values.

Result and discussion

Delignification of rice straw using shear homogenization

Impact of shearing time and rotation speed on delignification

The impact of shearing time and rotation speed on the total lignin removal and the release of soluble lignin from the rice straw is shown in Fig. 1. Evidently, the total lignin content reduced from 2280 to 1208.4, 1140, 912, 866.4 and 775.2 mg/L, respectively, at a shearing time of 9 min, and a rotation speed of 4000 to 10000 rpm. However, beyond a shearing time of 11 min, no significant improvement in the delignification was noticed. The shear forces induced *via* mechanical means disrupts the microfibrils and lignin of the rice straw thereby increasing the contact surface area. In this study, a shearing time of 9 min was considered to be optimal for effective delignification. Previous studies have reported a treatment time of 30 min as the optimum for rice straw biomass disintegration using milling process [46,47].

Similar to shearing time, rotation speed is also an essential factor to be optimized for effective delignification. The total lignin content decreased significantly when the rotation speed was increased from 4000 to 8000 rpm. For example, at a shearing time of 9 min, the total lignin content decreased from 1208.4 to 912 mg/L, while at rotation speed > 8000 rpm, no decrease in the total lignin content was observed. The soluble lignin content increased from 0 to 1368 mg/L under the optimal conditions and ~ 60% lignin solubilization was achieved.

Impact of specific energy on lignin solubilization at different power inputs

Power and specific energy input are important factors to be considered for any type of mechanical or physical pretreatment. Fig. 2a represents the impact of power and specific energy input on the lignin solubilization. Evidently, lignin solubilization has a direct correlation with the specific energy input of the medium. The lignin solubilization patterns can be organized into three trends, i.e. A1, A2, and A3. A1 designates a slow solubilization trend that was primarily due to low rotation speed and power input in the range of 4000 to 6000 rpm and 0.005 to 0.008 kW, respectively. A2 designates a raised solubilization trend at a rotation speed and power input value of 8000 rpm and 0.010 kW, respectively. A3 designates an insignificant lignin solubilization trend due to rotation speed and power input value in the range of 10000 to 12000 rpm and 0.013 to 0.016 kW, respectively. During low rotation speed and low power input (i.e. A1), very slow lignin solubilization (47-50%) at the optimal specific energy input of 293.8 to 440.6 kJ/kg TS was achieved. However, during A2, higher lignin solubilization of 60% was obtained at a specific energy input of 588.1 kJ/kg TS. Meanwhile, during A3, a very slight increase in lignin solubilization was noted, and at the spent energy was found to be nearly twice (1249-1469 kJ/kg TS) when compared to A2. For instance, to increase the lignin solubilization from 60-70%, a specific energy input of 1249 kJ/kg TS was required. Thus, it was confirmed that, an increment of rotation speed or shear time increased the lignin solubilization only marginally, but this could lead to wastage of energy during delignification.

Fig. 2b represents the effect of specific energy input on the total lignin content, the release of soluble lignin, cellulose and hemicellulose under the optimal conditions of power input and rotation speed (i.e. 0.010 kW and 8000 rpm). Cellulose and hemicellulose are considered to be indices for biomass disintegration as they are the major cell wall components of rice straw biomass. As evidenced in Fig 2b, a gradual decrease in total lignin content and an increase in the release of soluble lignin was noticed up to a specific energy input of 588.1 kJ/kg TS. At this particular energy, a higher total lignin reduction was obtained from 2280 to 912 mg/L. Similarly, the release of soluble lignin increased from 0 to 1368 mg/L. Further increasing the specific energy input to 718.7 kJ/kg TS, did not necessarily decrease the total lignin content (843.6 mg/L). On the other hand, the cellulose and hemicellulose content which are usually considered as the indices for biomass disruption was found to be zero up to a specific energy input value of 588.1 kJ/kg TS, indicating no biomass disruption. However, when the specific energy input was increased to 718.7 kJ/kg TS, the cellulose and hemicellulose content increased (i.e. from 25.4 and 36.2 mg/L), indicating the start of rice straw biomass disruption. These specific observations clearly confirmed that a specific energy input of 588.1 kJ/kg TS was sufficient for energy efficient delignification with no biomass disintegration.

Impact of biomass to water ratio on delignification

The biomass to water ratio is a critical factor that affects both delignification and its energy efficiency (Fig. 3). It can be observed that the lignin removal efficiency (%) increased with an increase in the biomass to water up to 0.04 *w/v*, reaching a value of 72%. The energy spent to achieve 72% lignin removal was calculated to be 114.3 kJ/kg TS. A further increase in the specific energy input and biomass to water ratio resulted in a decrease in lignin removal. This indicates the fact that the availability of higher amount of solids and lesser water content could disrupt the grinding impact of high shear homogenization. For instance, when the biomass to water ratio was increased to 0.045 (*w/v*), the lignin removal dropped to 60%; however, there was no significant decrease in the specific energy input (101.6 kJ/kg TS). Thus, an increased surface area for contact between the lignin and high shear homogenization treatment can be enhanced by decreasing the biomass to water ratio [9]. Therefore, the biomass to water ratio of 0.04 (*w/v*) was selected for further experiments.

Impact of delignification on the bacterial pretreatment

The delignified rice straw was subjected to bacterial pretreatment with cellulase secreting bacteria since the cellulose and hemicellulose were the predominant components. To study the effect of delignification on the bacterial pretreatment, experiments were performed with D-CBP (delignified cellulase secreting bacterially pretreated rice straw samples) and CBP (Cellulase secreting bacterially pretreated rice straw samples). The sCOD, cellulose and hemicellulose contents were employed as indices to evaluate the efficiency of pretreatment in control, CBP and D-CBP experiments, respectively (Fig. 4a). Fig. 4a clearly portrays the existence of two phases, i.e. an increasing phase (0 to 24 h) and a declining phase (30 to 72 h). The release of sCOD, cellulose, and hemicellulose contents during the increasing phase of D-CBP extends from 0 to 14516 mg/L, 5080.6 mg/L and 2903.2 mg/L, respectively. The sCOD values observed in this study was comparable with the results of Shetty et al. [48], where the authors achieved a similar rise in sCOD values during alkali pretreatment of rice straw. Likewise, for CBP and control experiments, the release of sCOD extends from 0 to 8710 mg/L, 3048.5 mg/L, and 1742 mg/L, respectively. The release of soluble components in CBP and D-CBP during cellulase secreting bacterial pretreatment could be due to the cellulolytic action of the biocatalyst. The cellulase enzyme secreted by the bacteria liquefies the cellulose and hemicellulose into the aqueous medium [9]. Comparatively, higher release of soluble components was noted with D-CBP than during CBP, implying the effect of delignification and the amenability of rice straw for cellulase secreting bacterial pretreatment.

Besides, a minor increase in the sCOD, cellulose and hemicellulose contents were noted in the control experiments, signaling the effect of homogenization (i.e. the stirring step for mixing the samples). During the declining phase, the release of sCOD, cellulose, and hemicellulose in D-CBP and CBP also decreased presumably due to starvation of cellulase secreting bacteria. A similar observation was also reported by previous researchers during the bacterial pretreatment of various organic biomass [49,50]. The sCOD, cellulose and hemicellulose solubilization (Fig. 4b) of D-CBP was found to be higher (38.2%, 35.2% and 31.8%) than CBP (22.92%, 21.2 % and 19.1%) and control (3.43%, 3.16% and 2.9%), respectively. The result obtained in the present study was consistent with other literature reported. For instance, Shi et al. [51] employed cellulase secreting bacteria, Cupriavidus basilensis B-8 which increased organic matter solubilization to 37.7% after 7 days of incubation. In another report, Zhong et al. [52] have disintegrated corn straw with bacterial, yeast and fungal consortia to get better solubilization. Among the yeast (*Saccharomyces* *cerevisiae sp., Coccidioides immitis sp.,* and *Hansenula anomala sp*.), cellulolytic bacteria (*Bacillus licheniformis sp., Bacillus subtilis sp., Pseudomonas sp.*), the fungus (*Pleurotus florida sp.,*) and the lactic acid bacteria (*Lactobacillus deiliehii sp.*), the cellulase secreting bacteria (*B. licheniformis sp., B. subtilis sp.,* and *Pseudomonas sp*) showed higher cellulolytic and hemicellulolytic activity and effectively solubilizes the cellulose.The observed differences in the values of sCOD, cellulose and hemicellulose during CBP and D-CBP were also statistically significant with *p* values of 0.0004, 0.0006 and 0.0001, respectively.

Box plot interpretation

To analyze and ascertain the distribution of the sCOD, cellulose and hemicellulose data obtained from CBP and D-CBP samples, a statistical analysis, in the form of box plot interpretation was performed (Fig. 5). In the control experiments, it was assumed that 25% of the data from sCOD, cellulose and hemicellulose (lower quartile) were less than 239 mg/L, 83.65 mg/L and 47.8 mg/L, respectively. Likewise, 75% of the data from sCOD, cellulose and hemicellulose (upper quartile) were less than 802 mg/L, 280.7 mg/L ,160.4 mg/L, respectively. In the case of CBP, 25 % of the data from sCOD, cellulose and hemicellulose (lower quartile) were less than 1528 mg/L, 534.8 mg/L and 305.6 mg/L, respectively, while 75% of the data were less than 5366 mg/L, 1878.1 mg/L and 1073.2 mg/L, respectively. Concerning D-CBP, 25% of the data from sCOD, cellulose and hemicellulose (lower quartile) were less than 2546 mg/L, 891.1 mg/L, and 509.2 mg/L, respectively, while 75% of the data (upper quartile) were less than 8930 mg/L, 3125.5 mg/L, and 1786 mg/L, respectively. From Fig. 5, it was reasserted that there were no outliers present as the data lies within the whiskers in the box plot. The skewness of the data was analyzed by the shape of the box plot. All the box plots were observed to be symmetrical in shape indicating that the data of sCOD, cellulose and hemicellulose for control, CBP and D-CBP samples, respectively, followed a normal type distribution. Therefore, from a statistical view point, it can be proved that the maximum sample value represents the optimal point and the data sets were not skewed and it followed a normal type distribution.

Specific methane production

The specific methane production and biodegradability of D-CBP, CBP and control rice straw samples are presented in Fig. 6a**.** The specific methane production during the start of the experiments were low due to the acclimation of inoculum to the substrate [31]. However, on the 5th day, Specific methane production in the control, CBP and D-CBP samples were 7.4 mL/g VS, 49.8 mL/g VS and 82.9 mL/g VS, respectively. The Specific methane production augmented with an increase in the digestion period and attained a higher value on the 15th day of digestion, i.e. 15 mL/g VS, 99 mL/g VS and 165 mL/VS, respectively, for the control, CBP and D-CBP samples. However, when these values were compared between CBP and D-CBP, D-CBP evidenced ~ 40% higher Specific methane yield. Chen et al. [11] reported that mechanical extrusion increased the methane yield by 32% when compared to milling pretreatment of rice straw biomass. Similarly, Bauer et al. [53] and Zhao et al. [54] achieved an increase in the methane yield by ~ 20% and 35% *via* steam explosion and mild acid pretreatment of rice straw biomass. In this study, during D-CBP, the disintegration of biomass increased the availability of sugar rich rice straw to the methane producing microbes.

The specific methane production data was fitted to the model (Eq. 1) and the kinetic parameters are shown in Table 3**.** As seen from the table, a higher specific methane production potential and rate constant value of 169.5 mL/ gVS and 0.2 day^{-1} was obtained for D-CBP when compared to CBP (p < 0.05; R^2 > 0.95). The effect of lignin inhibition on methane production is shown in Fig. 6b. The experimentally observed methane production values matched reasonably well (60-85%) with the theoretically predicted methane production values. It has been reported in the literature that lignin content > 1 g/L affects the hydrolysis and methanogenesis steps during anaerobic digestion [55]. In this study, the lignin content of the control, CBP and D-CBP rice straw samples were 2.09 g/L, 1.46 g/L and 0.585 g/L, respectively. Thus, it was confirmed that, in the case of D-CBP, most of the lignin were removed from the rice straw samples thereby enhancing the methane production as the remaining lignin content was only 0.585 g/L and it is not considered to be inhibitory. Fig. 6c represents the 95% confidence ellipse of biodegradability and the hydrolysis constant. D-CBP showed greater biodegradability and hydrolysis constant values (0.4 g COD/g COD and 0.2 h^{-1}), with narrow confidence ellipse revealing greater hydrolysis and better accuracy than CBP (0.23 g COD/g COD and 0.15 h^{-1}) that showed wider confidence with lesser accuracy. These results suggest that D-CBP showed better biodegradability when compared to the control and CBP.

Mass, energy balance and economic analysis

Fig. 7 represents the results from mass balance analysis of CBP and D-CBP by considering 1000 kg of solids (1 ton) as the basis. 1000 kg of solids was reduced to 800 kg and 640 kg during CBP and D-CBP, respectively, corresponding to 36% and 20% reduction during pretreatment. In the case of D-CBP, the enhanced delignification facilitated better bacterial pretreatment. During AD, the solids reduced further to 384 kg and 560 kg in D-CBP and CBP, respectively. From a process view point, due to the improved biomass solubilization that was achieved by high shear homogenization induced bacterial pretreatment, ~ 40% solids reduction was accomplished during AD in D-CBP. Comparatively, lesser solids reduction of ~ 30% was achieved during AD in CBP. The ultimate solids reduction was calculated to be 616 and 440 kg in D-CBP and CBP, respectively. The remaining waste solids that could be disposed in landfills from D-CBP and CBP was estimated to be 384 and 560 kgs, respectively.

Fig. 8 shows the results from energy balance and economic analysis of CBP and D-CBP for 1000 kg of rice straw (1 ton), respectively. In any energy balance assessment, the energy spent should be compensated by the energy output in order to obtain net the energy yield[56]. The input energy takes into account the energy consumed for high shear homogenization (i.e. for delignification), bacterial pretreatment, energy required for stirring, energy required to cultivate the biocatalyst, energy required to maintain the desired temperature, operation of the AD system heat loss and pumping energy. The total energy spent in D-CPB and CPB was estimated to be 265.80 kWh/ton and 234.05 kWh/ton, respectively. The slight increase in input energy during D-CBP could be due to the energy spent for delignification (i.e. the energy required for high shear homogenization). The two benefits of pretreatments were: energy obtained in the form of biomethane and reduction in solids content that requires ultimate disposal. The output energy obtained as biomethane during D-CBP and CBP was calculated to be 1034.88 and 425.04 kWh/ton, respectively. The obtained net energy of D-CBP and CBP were calculated to be 769.08 kWh/ton and 191.00 kWh/ton, respectively. Based on these real estimates, it can be assured that energy can be saved from D-CBP.

Similarly, the results from economic analysis showed that the total input cost for D-CBP and CBP were -257.133 USD/ton and -293.831 USD/ton, respectively. The total output cost of D-CBP and CBP were calculated to be 392.022 USD/ton and 207.76 USD/ton, respectively. The net profit obtained from D-CBP and CBP were 134.89 USD/ton and -86.07 USD/ton, respectively. Even though the energy cost for delignification was taken into account for D-CBP, it did not lead to any severe loss in the net profit of D-CBP. The higher net profit (obtained as methane energy and reduction in solids to be disposed) compensates the amount spent for meeting the energy requirements for delignification in D-CBP.

Cost-benefit ratio is an important parameter that decides the profitability of pretreatment. Cost-benefit ratio in excess of 1 indicates a net profit. In the present study, D-CBP achieved a cost-benefit ratio of 1.52, indicating net profit, whereas CBP achieved a cost-benefit ratio of only 0.71. Therefore, D-CBP can be conceived as a viable process for industrial scale applications. It was recently reported that, ~ 731 million tons of rice straw was produced globally, whereas 28.7% of its production was from India [57]. With the advent of improved rice residue management technologies, the collection of rice straw from agricultural lands could be improved in order to gain profit from rice production and reduce the environmental footprint. According to the statistics and the results of this study, if the annual production of rice straw in India is ~ 209.90 million tons, the methane production potential will be ~ 50 billion m^3. This estimate corresponds to the net annual energy of 21.35 Mtoe in India and it is expected to meet 10% of the annual energy needs of India.

Conclusions

From the present study, the following conclusions can be drawn which meet the goals of green chemistry and sustainability. To the best of our knowledge, this is the first study that demonstrates a phase separated pretreatment resulting in significant enhancement in delignification and solubilization under mild operational conditions that are devoid of severe and harsh operational conditions for profitable biomethane recovery.

- In the present study, the abundantly available, rice straw biomass, otherwise burnt in the field, has been utilized as a sustainable and renewable substrate for cleaner and profitable biochemical methane production.

- The mild biological pretreatment significantly enhances the solubilization of rice straw biomass without causing any drastic alteration in substrate environment (devoid of inhibitors formation)
- A higher solubilization of 38.2% was achieved through this effective phase separated pretreatment without using any harsh chemicals. Thus, it can be considered as a better alternative to harsh physiochemical pretreatments which are usually ends in recalcitrant formation.
- A higher delignification of 72% was achieved via mild dispersion treatment at very less specific energy input of 114.3 kJ/kg TS. The mild dispersion drastically reduces the energy cost associated with delignification.
- Lage scale energy and economic analysis implies that phase separation of renewable feedstocks (rice straw biomass) through mild dispersion (investing lesser treatment time) followed by biological pretreatment allows for benefits such as significant reduction in the overall cost with net profit of (134.89 USD/ton), easy and mild operational conditions without any recalcitrant formation.
- The results from commercial scale energy and economic analysis revealed that delignification prior to bacterial pretreatment saved significant amount of energy (cost-benefit ratio: 1.52) than bacterial pretreatment. Future research is still required to utilize the removed lignin as a resource/raw material for production of biopolymers, biochemicals and biofuels within an integrated biorefinery framework or eco-industrial park.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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Table 1 Characteristics of rice straw biomass

S. No	Parameters	Values
1	Total solids (g/L)	40 ± 1.22
2	Volatile solids (g/L)	31.2 ± 0.99
3	Total chemical oxygen demand (g/L)	38 ± 1.14
4	Soluble chemical oxygen demand (g/L)	0.05 ± 0.0015
5	Total cellulose (g/L)	14.4 ± 0.432
6	Total hemicellulose (g/L)	11.4 ± 0.342
7	Total lignin (g/L)	9.12 ± 0.274
8	Biomass: water ratio (w/v)	0.04

Table 2 Energy analysis parameters

Parameter	Unit	CBP	D-CBP	Reference
Initial Solids	Kg	1000	1000	This study
COD Solubilization	%	22.9	38.2	This Study
SS Reduction	%	20	36	This Study
Pretreatment time	h	24	24	This Study
Pretreatment temperature	^{o}C	40	40	This Study
Pump height	m	3	3	Assumed
Pump efficiency η	%	70	70	Assumed
Pumping Time	min	10	10	Assumed
Flow rate	m^3/s	0.0416	0.0416	Calculated
Electricity Consumption for AD Stirring (£)	kW/m^3	0.005	0.005	Kannah et al. [5]
Ambient temperature	^{o}C	25	25	This Study
Digestion temperature	^{o}C	35	35	This Study
Digestion time	days	15	15	This Study

Table 3 Derived kinetic parameters from exponential Box Lucas Model for methane production

Samples	Derived kinetic parameters						R^2 values
	a (mL/g VS)	Standard error	Probability (p value)	B (day^{-1})	Standard error	Probability (p value)	
Control	15.20	0.097	0.0003	0.13	0.007	0.0004	
CBP	101.7	0.648	0.0002	0.15	0.006	0.0003	0.95-0.99
D-CBP	169.50	1.08	0.0001	0.2	0.004	0.0001	

Control – Untreated rice straw; CBP- Cellulase secreting bacterial pretreatment; D-CBP- Delignified cellulase secreting bacterial pretreatment; a- exponential specific methane production; b- rate constant

Figure captions

Fig 1 Effect of high shear homogenization time and rotation speed (6000 to 120000 rpm) on total lignin reduction and soluble lignin release

Fig 2 : a) Effect of specific energy input on lignin solubilization at varying power input **b)** Effect of specific energy input on total, soluble lignin at optimal power input of(0.010 kW)

Fig 3 Effect of biomass to waster medium ratio on lignin removal efficiency with respect to specific energy input

Fig 4 Effect of cellulase secreting bacterial pretreatment a) soluble components concentration of rice straw biomass b) soluble components solublization at optimal operational conditions ((control - Untreated, CBP- Cellulase secreting bacterial pretreatment , D-CBP – Delignification followed by cellulase secreting bacterial pretreatment)

Fig 5 Statistical analysis of soluble components of rice straw biomass (control - Untreated, CBP- Cellulase secreting bacterial pretreatment , D-CBP – Delignification followed by cellulase secreting bacterial pretreatment) through box plot interpretation.

Fig 6 : a) Effect of cellulase secreting bacterial pretreatment cumulative methane production b) Effect of lignin on methane production c) Effect of cellulase secreting bacterial pretreatment on biodegradability and hydrolysis ((control - Untreated, CBP- Cellulase secreting bacterial pretreatment , D-CBP – Delignification followed by cellulase secreting bacterial pretreatment)

Fig 7 Mass balance analysis of D-CBP and CBP

Fig 8 Energy balance and economic analysis of D-CBP and CBP

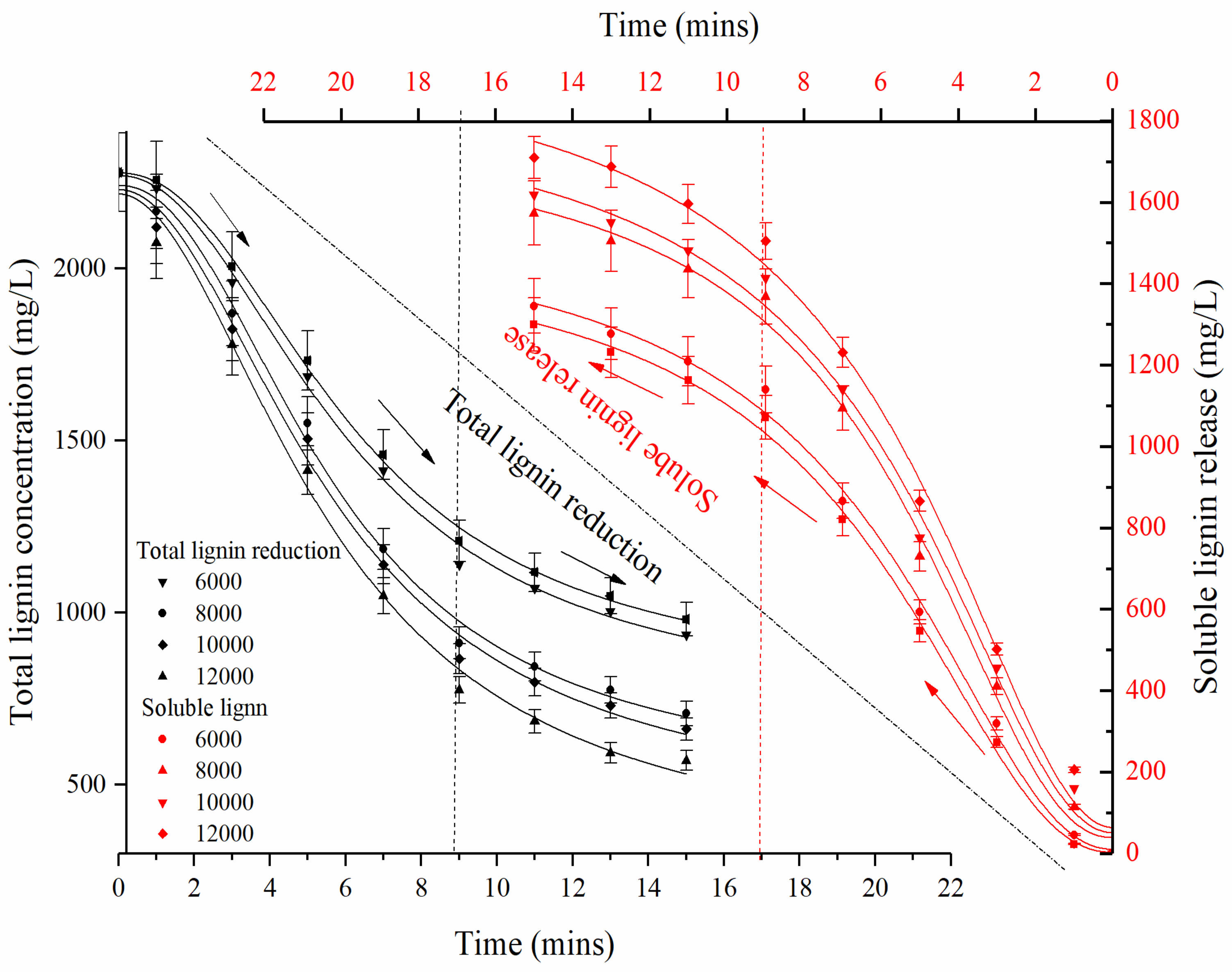


Fig 1 Effect of high shear homogenization time and rotation speed (6000 to 120000 rpm) on total lignin reduction and soluble lignin release

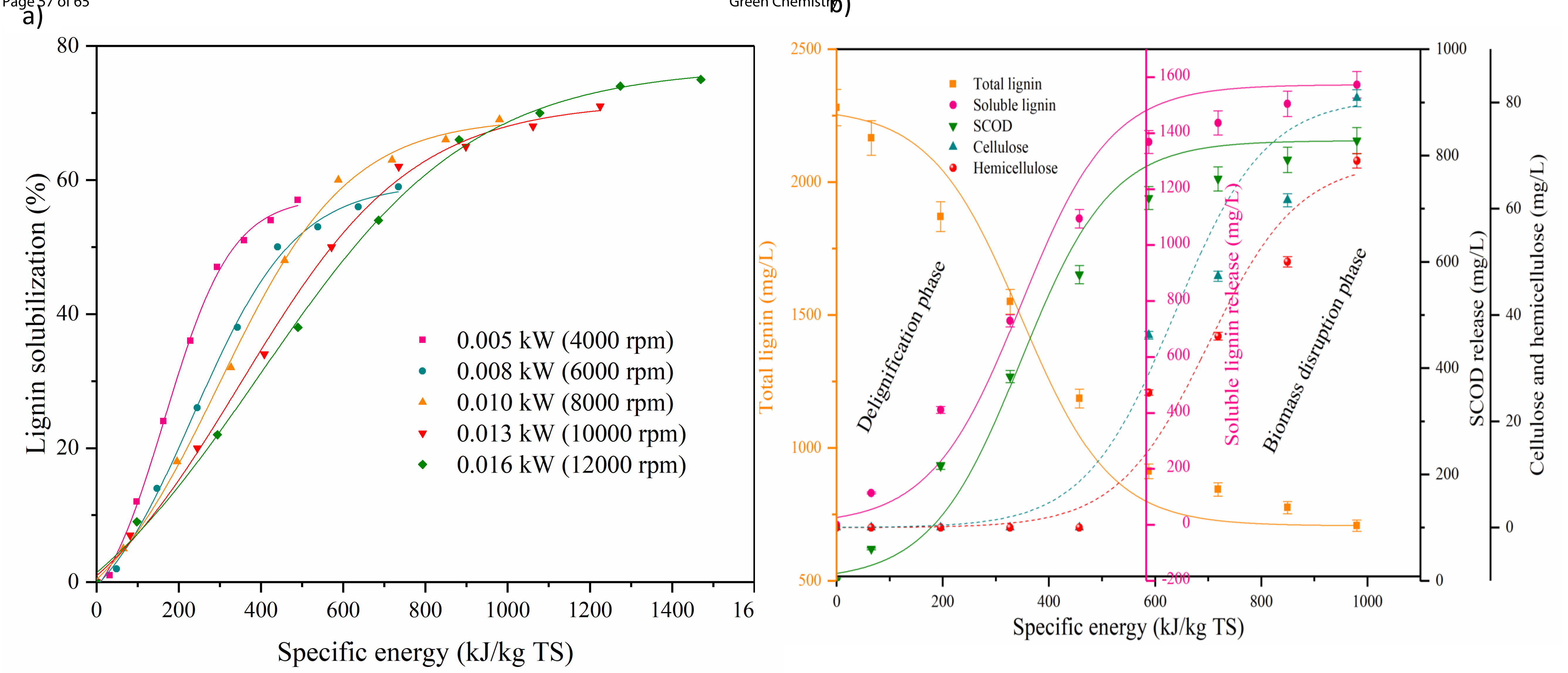


Fig 2 : a) Effect of specific energy input on lignin solubilization at varying power input **b)** Effect of specific energy input on total, soluble lignin at optimal power input of(0.010 kW)

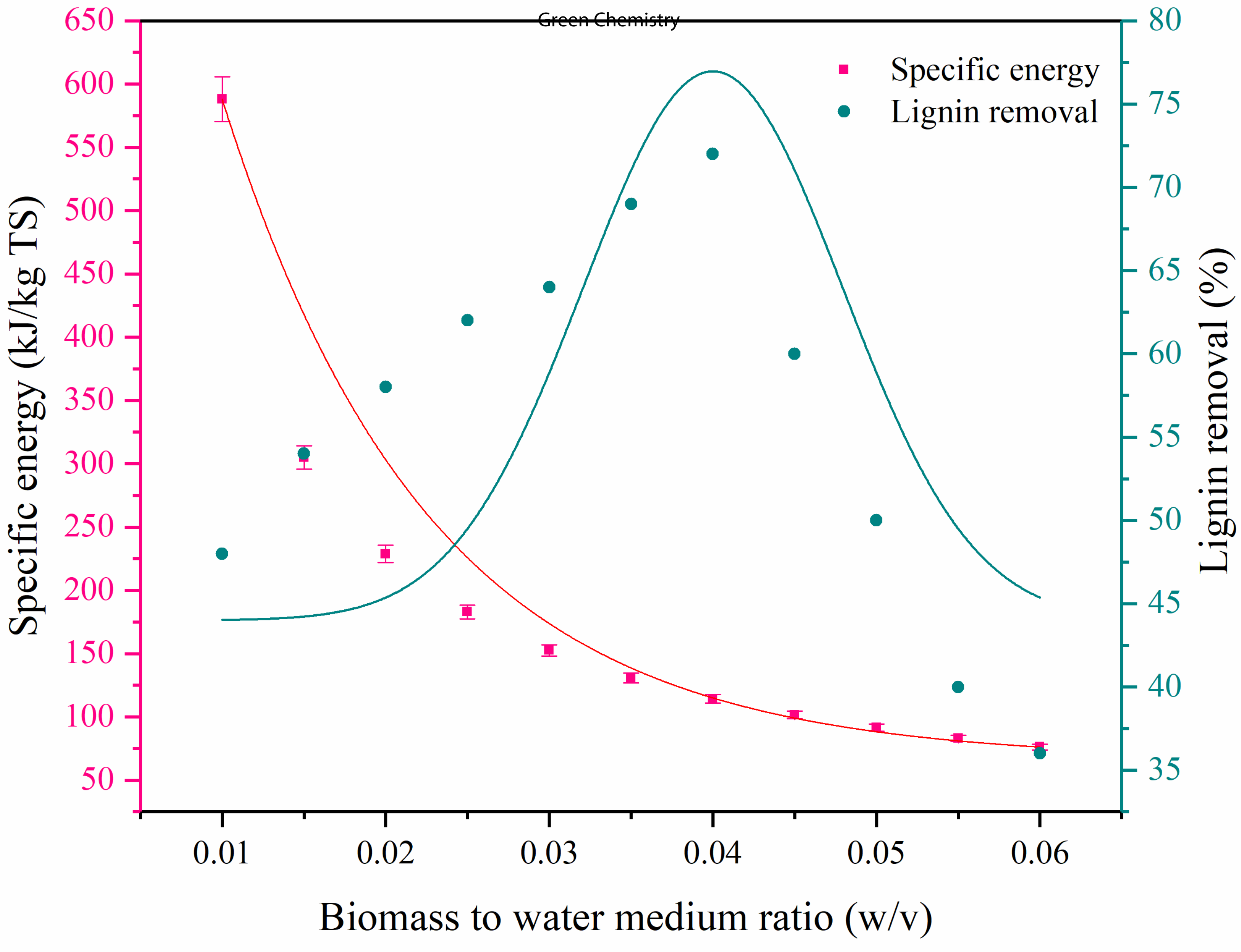


Fig 3 Effect of biomass to waster medium ratio on lignin removal efficiency with respect to specific energy input

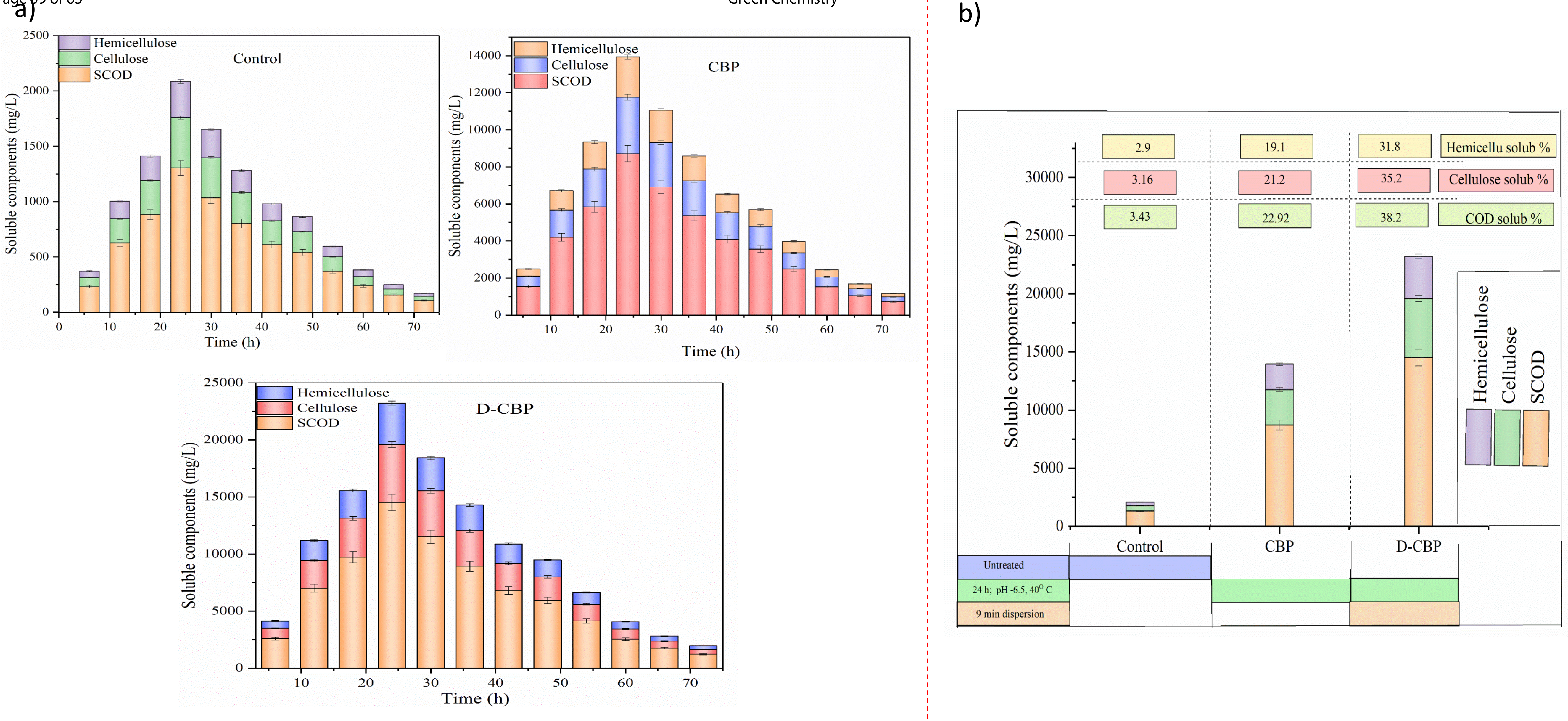


Fig 4 Effect of cellulase secreting bacterial pretreatment a) soluble components concentration of rice straw biomass b) soluble components solublization at optimal operational conditions ((control, CBP- Cellulase secreting bacterial pretreatment , D-CBP – Delignification followed by cellulase secreting bacterial pretreatment)

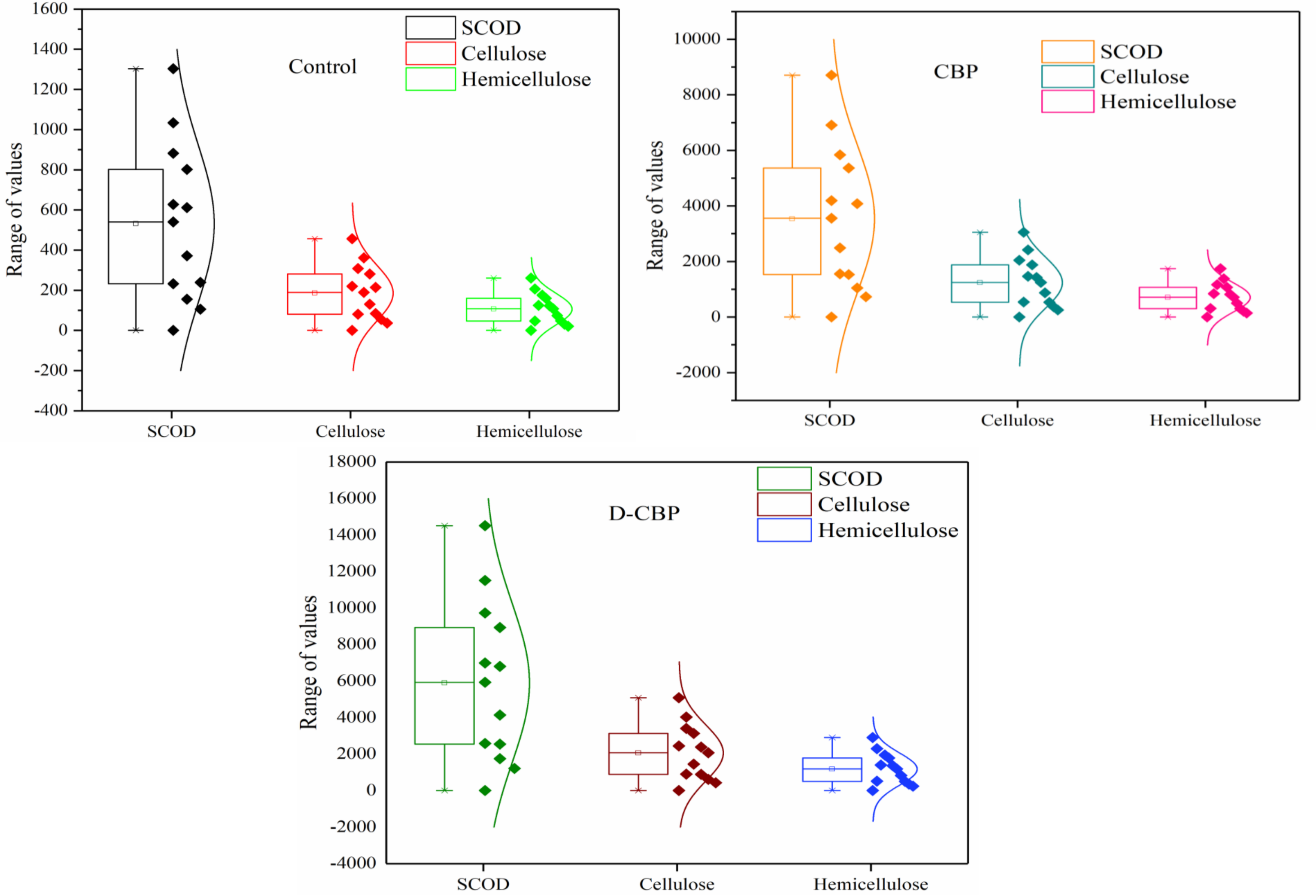


Fig 5 Statistical analysis of soluble components of rice straw biomass (control, CBP- Cellulase secreting bacterial pretreatment , D-CBP – Delignification followed by cellulase secreting bacterial pretreatment) through box plot interpretation.

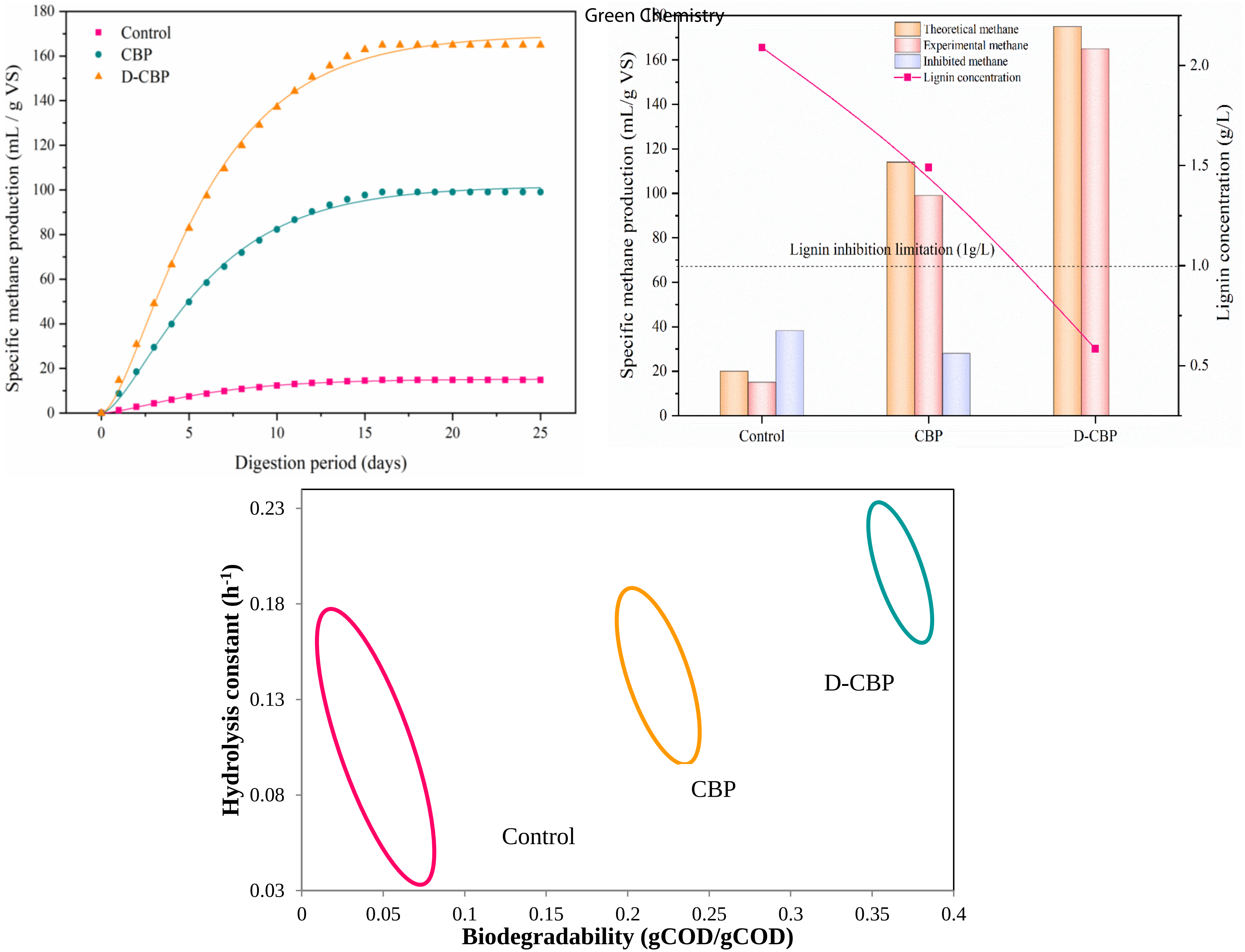


Fig 6 : a) Effect of cellulase secreting bacterial pretreatment specific methane production b) Effect of lignin on specific methane production c) Effect of cellulase secreting bacterial pretreatment on biodegradability and hydrolysis ((control, CBP- Cellulase secreting bacterial pretreatment , D-CBP – Delignification followed by cellulase secreting bacterial pretreatment)

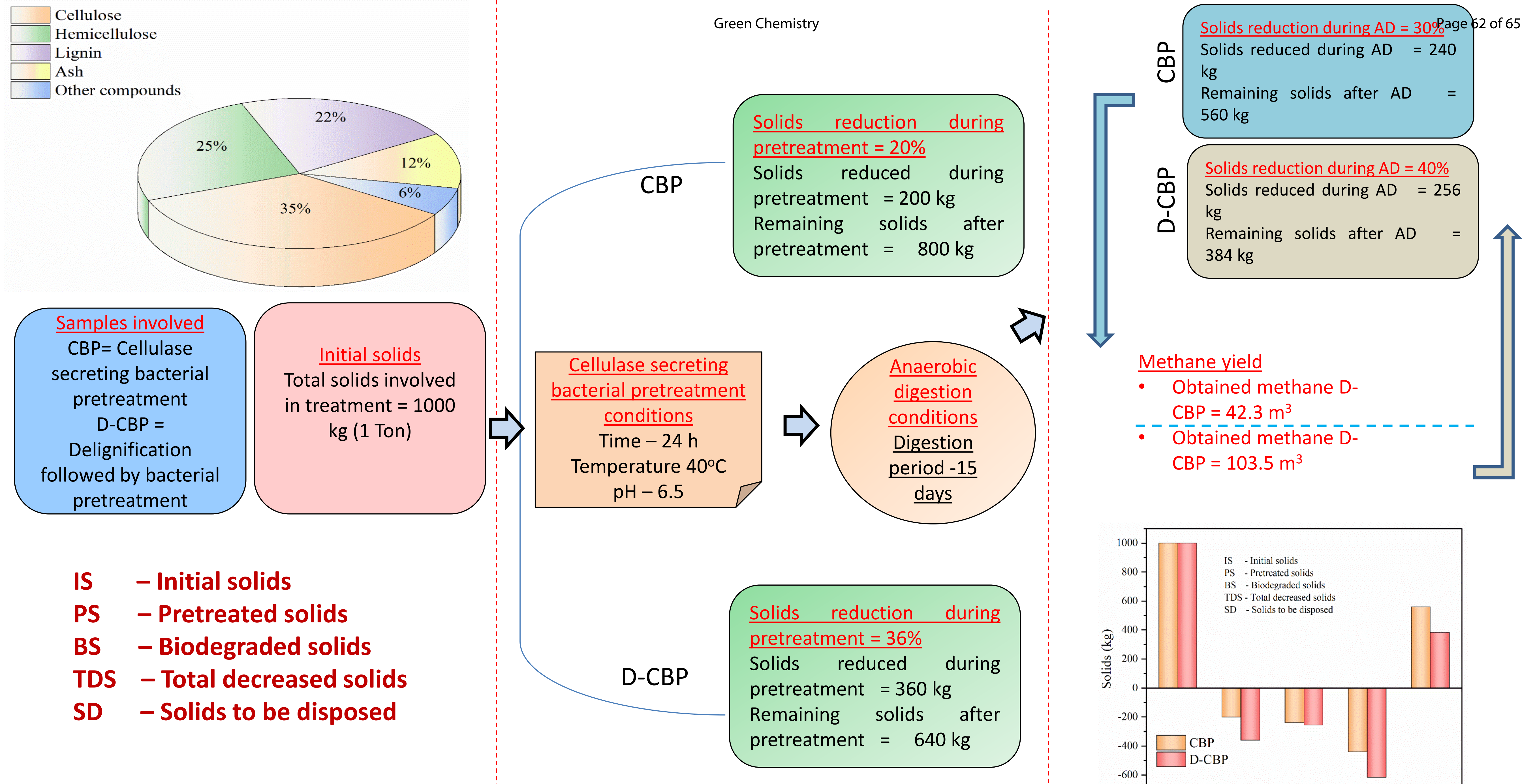


Fig 7 Mass balance analysis of CBP and D-CBP

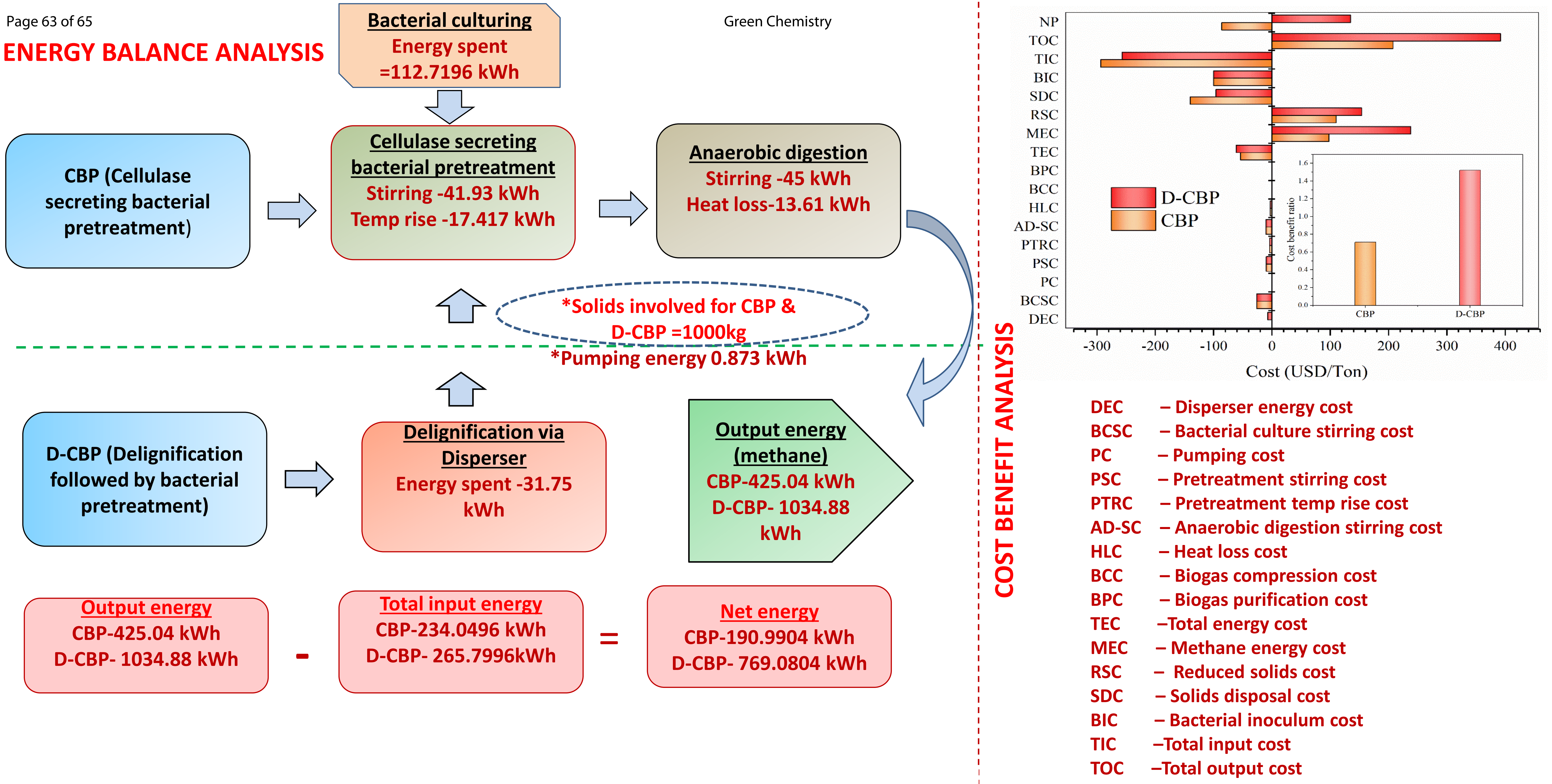
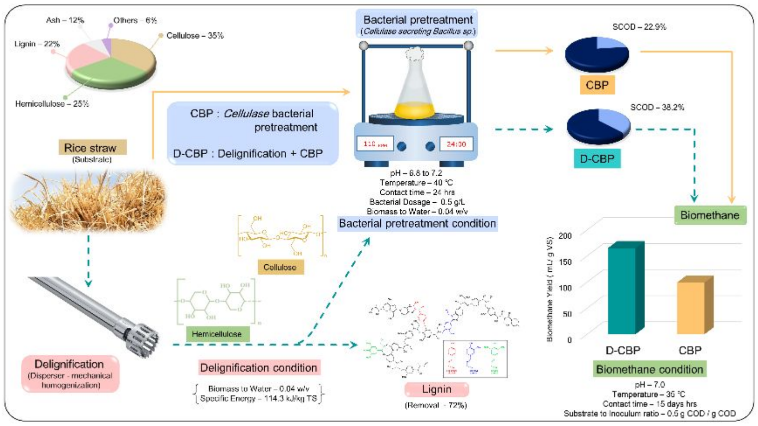


Fig 8 Energy balance and economic analysis of CBP and D-CBP

A table of contents entry



A cost and time saving strategy for the recovery of biomethane from rice straw using a novel phase-separated pretreatment

Electronic supplementary information

Profitable biomethane production from delignified rice straw biomass: Effect of lignin, Energy and economic analysis

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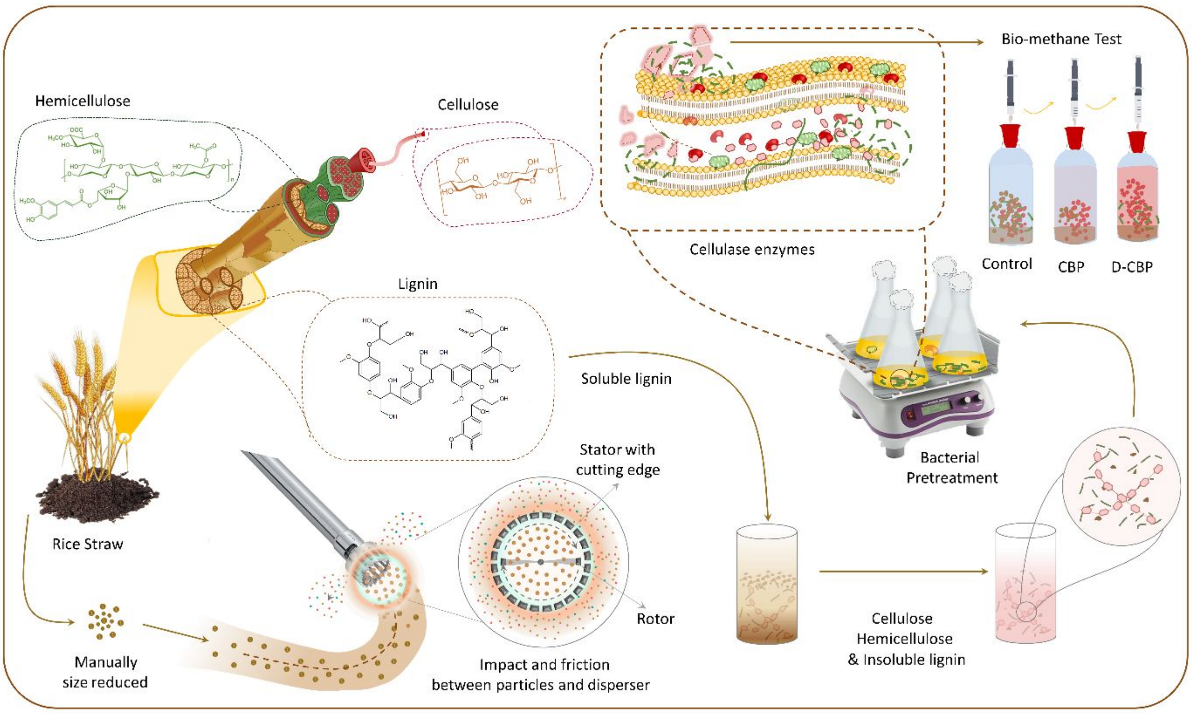

Fig s1 Methodology flow chart of the present study