INTRODUCTION

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Current scenario of declining fossil fuel reserves and environmental deterioration due to GHG emissions, soaring petroleum prices, concerns over the national energy security and dependence on oil-import have led researchers all over the world to search for alternative transportation fuels termed 'biofuels'. Biofuels, such as bioethanol, can be produced sustainably from biomass resources. Lower cost, and surplus availability of lignocellulosic biomass (LCB), has made it the most appropriate and sustainable feedstock for generating ethanol and other value-added materials via biochemical conversion route (Saini et al., 2015b). Some of the globally abundant LCBs are rice straw, wheat straw and sugarcane bagasse (SCB). LCB, the renewable resource for the production of cellulosic ethanol (Ragauskas et al., 2006), is mainly comprised of cellulose, hemicellulose, and lignin (Kuhad et al., 1997). The total LCB production in India alone exceeds 680 metric ton per annum which accounts for production of approximately 52 billion litres of bioethanol (Jain & Agrawal, 2018). In the year 2017-18, 1500 million litres of ethanol was produced, which could be used for only 5% blending in gasoline (E5). There is still a shortage of 1,100 million litres of ethanol for achieving the mandate of 10% blending (E10). Sugarcane is one of the highly produced crops in India, having annual production of 376 metric ton during 2019-2020 (http://www.fao.org/faostat). Sugar industries generates huge amount of bagasse as a by-product, which can be utilised to overcome the shortage in supply of bioethanol.

The biochemical conversion route involves four major unit operations, including pretreatement, hydrolysis, fermentation and ethanol recovery. The pretreatment process is vital for partial or complete removal of lignin and hemicellulose, reduction in cellulose crystallinity and increasing the porosity of the biomass. Pretreatment makes the cellulose amenable to cellulase enzymes during enzymatic hydrolysis. The resultant hydrolysate

containing monomeric sugars is fermented to ethanol by fermenting microbes. Hydrolysis is a critical step during which cellulolytic enzymes are used to convert complex carbohydrates of biomass into fermentable sugars. Sugars released during hydrolysis can be fermented into ethanol using various fermenting microorganisms via separate hydrolysis and fermentation (SHF) or simultaneous saccharification and fermentation (SSF). SHF is employed more commonly by using mesophilic yeast *Saccharomyces cerevisiae* operating at 28-30 °C. However, the major demerit of SHF process is accumulation of monomeric sugars which consequently inhibits cellulases and leads to poor yield of sugar. In contrast, SSF process uses the same reactor for biomass hydrolysis and ethanol production, which improves the process economics by decreasing overall process time. Moreover, obtaining high ethanol concentration is a major challenge at industrial level, as it reduces the energy consumption and associated costs during the recovery of ethanol by distillation. Higher ethanol yields require higher loading of biomass during fermentation. However, high solids cause mixing-problem due to high viscosity and reduce biomass conversion significantly. Therefore, a fed-batch approach for feeding of biomass during SSF is often employed. Fed-batch SSF not only eliminates the technical challenge of mixing and mass transfer, but also reduces end-product inhibition, increases dissolved oxygen and saccharification rate and overall ethanol productivity. The problem of contamination during the fermentation process can be tackled by using a thermotolerant fermenting microorganism capable to ferment at comparatively higher temperature, thereby, eliminating the growth of many mesophilic contaminating microbes.

Another major challenge during bioethanol production is the generation of degradation products during thermochemical pretreatment of biomass (Kang et al., 2014). The LCB-derived inhibitors majorly include furans and its derivatives, phenolics, and weak acids like acetic acid, carboxylic acid, etc. (Wang et al., 2018). Use of pretreatment slurry or the

unwashed biomass during fermentation inhibits fermenting microbes, thereby, reducing ethanol yield and productivity (Wang et al., 2018). Therefore, washing of the pretreated biomass is often needed to remove these inhibitors prior to fermentation, generating large amounts of waste-waters (Lyu et al., 2017). Chemical detoxification is also not a cost-effective method due sugar loss and increased operational time (Lin et al., 2020; López et al., 2004; Shibuya et al., 2017a). As an alternate approach, the fermenting microbes can be potentially improved to tolerate to inhibitors via strain improvement strategies. Genetic engineering based approaches of strain improvement require specific knowledge about underlying principles of tolerance and the target genes or their metabolic functions which makes its application difficult (Wang et al., 2018). Therefore, non-targeted and less complex strain improvement strategy is needed. One such approach for increasing the tolerance of fermenting microorganisms toward multiple inhibitors could be 'adaptive laboratory evolution (ALE)' which relies on accumulation of spontaneous mutations, generation after generation under constant selection pressure (Qin et al., 2016).

In order to address the above mentioned research gaps in cost-effective bioethanol production, the current study hypothesised the following points:

- Can we develop a robust strain of yeast which tolerates multiple stresses?
- Can we enhance production of cellulosic ethanol using the adapted yeast?
- Can we improve the efficiency of the ethanol production by adopting fed-batch fermentation?

Therefore, considering the need to develop a robust yeast cell factory for cost-effective production of cellulosic ethanol, the present study focused on the following objectives:

1. Screening and selection of thermo- and inhibitor tolerant yeast strain(s) for utilizing lignocellulosic wastes for bioethanol production.
2. Enhancing tolerance of potential yeast strain(s) against temperature and inhibitors stress through adaptive laboratory evolution.
3. Optimization of bio-process for maximum bioethanol production from lignocellulosic sugarcane bagasse at shake-flask level using developed yeast strain.
4. Improvisation and scale-up of bio-process for high gravity simultaneous saccharification and fermentation of sugarcane bagasse at bench-scale fermenter.