BCIL Biotech Consortium India Limited

Ethanol production from cellulosic hydrolysate as diluent to molasses using a robust yeast *Saccharomyces cerevisiae strain*

TECHNOLOGY AVAILABLE FOR TRANSFER

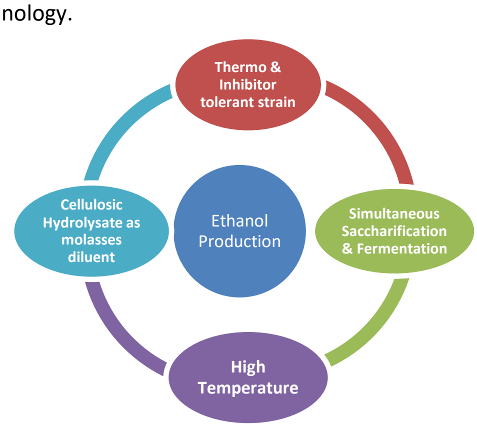
UNMET NEED AND OPPORTUNITY

The National Policy on Biofuels – 2018, provides an indicative target of 20% ethanol blending under the Ethanol Blended Petrol (EBP) Programme by 2030. Bioethanol is a preferred additive due to its remarkable characteristics, such as clean fuel, large-scale production from microbial sources, and compliance with existing engines.

The Bioethanol can be produced through molasses. However, Co-fermentation of molasses and biomass hydrolysate could be a potential strategy to boost ethanol yield and titre. It dilutes the concentration of inhibitors in hydrolysate (e.g. furfural, 5-hydroxymethyl-furfural and acetic acid) and increases the sugar content in the mixture. It also limits water use as a diluent to molasses and reduces distillation time.

In India, the climate in most regions is tropical, with temperatures between 40 and 45 °C during summers. Moreover, the heat generated during fermentation further contributes to keeping the fermenter's temperature high. In this context, yeast strains capable of producing high ethanol titre at elevated temperatures such as 35 °C and above are much needed.

This technology presents an opportunity to increase ethanol yield and productivity in the existing process or obtaining high yields by dilution of the molasses with Paddy straw hydrosylate (PSH) or Sugarcane bagasse hydrosylate (SBH) cellulose, reduce water usage for molasses dilution, save cooling costs during fermentation and increase ethanol yield and productivity compared to existing only molasses-based fermentation technology.



Factors optimized in this integrated technology for producing Bioethanol

UNIQUE FEATURES

- Thermotolerant and inhibitor tolerant yeast (*S. cerevisiae*) with superior fermentation performance in terms of yield and productivity at 35°C than commercially used strains Angel yeast (AY) and Ethanol red (ER).
- Cost-effective and integrative approach due to the following factors:
  - Fermentation at elevated temperatures with high sugar concentration saves costs.
  - More ethanol production is due to the utilization of cellulosic sugar along with molasses sugar.
  - Significant reduction in cost of molasses substrate due to its significant dilution with cellulosic hydrosylate.
  - Higher ethanol yield and titers save downstream processing cost.
- Numerous end uses in Beverage, Power generation, Transportation, Industrial and Medical sectors.

TECHNOLOGY

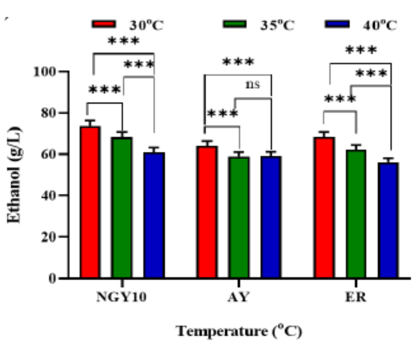
Fermentation by a robust yeast strain and using cellulosic hydrolysate as diluent to molasses leads to more ethanol production. This technology provides robust and efficient yeast S. cerevisiae strain for Bioethanol production which has many desirable properties like osmotolerance, thermotolerance, flocculation and high ethanol yield and productivity at 35°C. Along with the yeast strain this technology also provides optimized parameters for ethanol production from cellulosic hydrolysate as diluent to molasses for cost-effective and more bioethanol production in comparison with conventional ethanol production.

Table: Comparative fermentation profile of our yeast strain with well-known commercial yeast strains in batch fermentation using molasses diluted with water or paddy straw hydrolysate (PSH). Favorable molasses dilution is highlighted in red.

Strains	Parameters	Molasses dilutions with water			Molasses dilutions with PSH		
		1:1	1:2	1:3	1:1	1:2	1:3
S. cerevisiae	Ethanol (g/L)	68.66 ± 2.2*	67.59 ± 1.91	55.88 ± 2.23	70.66 ± 2.45	73.59 ± 2.29	63.88 ± 1.78
	Yield (%)	67.24	78.29	87.1	66.057	78.729	87.788
	Productivity (g/L.h)	1.43 ± 0.02	1.40 ± 0.03	1.16 ± 0.01	1.47 ± 0.01	1.45 ± 0.01	1.20 ± 0.03
	Glycerol (g/L)	12.52 ± 0.3	8.50 ± 0.23	5.41 ± 0.19	11.50 ± 0.5	9.58 ± 0.39	5.35 ± 0.21
	Acetic acid (g/L)	3.98 ± 0.16	2.41 ± 0.07	1.77 ± 0.03	3.39 ± 0.08	2.66 ± 0.07	1.72 ± 0.04
Angel Yeast	Ethanol (g/L)	61.61 ± 2.57	58.07 ± 2.22	52.07 ± 1.98	63.61 ± 2.78	63.57 ± 2.67	60.08 ± 2.36
	Yield (%)	64.68	67.88	80.86	63.537	68.579	82.288
	Productivity (g/L.h)	1.28 ± 0.03	1.2 ± 0.02	1.08 ± 0.01	1.32 ± 0.02	1.25 ± 0.04	1.13 ± 0.01
	Glycerol (g/L)	16.41 ± 1.0	10.2 ± 0.74	6.12 ± 0.05	13.7 ± 0.01	9.42 ± 0.52	4.45 ± 0.12
	Acetic acid (g/L)	3.40 ± 0.13	2.23 ± 0.04	1.99 ± 0.02	2.29 ± 0.002	2.36 ± 0.013	2.06 ± 0.004
Ethanol Red	Ethanol (g/L)	68.36 ± 2.19	61.43 ± 2.55	53.49 ± 1.98	70.36 ± 3.19	69.43 ± 2.83	65.36 ± 3.1
	Yield (%)	66.07	71.82	83.8	64.955	74.918	86.569
	Productivity (g/L.h)	1.42 ± 0.02	1.28 ± 0.01	1.11 ± 0.01	1.46 ± 0.03	1.32 ± 0.02	1.42 ± 0.01
	Glycerol (g/L)	15.86 ± 0.4	9.58 ± 0.67	6.44 ± 0.28	10.12 ± 0.3	9.33 ± 0.41	5.70 ± 0.22
	Acetic acid (g/L)	3.43 ± 0.13	2.19 ± 0.09	1.37 ± 0.02	2.74 ± 0.08	2.30 ± 0.07	1.57 ± 0.06

*Mean ± standard deviation, for n =3

Figure: Comparative fermentation profile of our yeast strain with well-known commercial yeast strains at 30°C, 35°C and 40°C using 1:2 molasses diluted with paddy straw hydrolysate (PSH) after 48 hours.



Fermentation profile of our yeast strain in 40% v/v molasses diluted with PSH at 35°C for 48 h in bioreactor.

Parameters	Data obtained
Ethanol (g/L)	104.63 ± 2.97
Yield (%)	92.62
Productivity (g/L/h)	4.35 ± 0.14
Glycerol (g/L)	8.15 ± 0.45
Acetic acid (g/L)	1.23 ± 0.08

*Mean ± Standard deviation, for n = 3

Table: Comparative Fermentation profile of our yeast strain with different substrate dilutions.

Parameters	Molasses with water as diluent	Molasses dilution with PSH	Molasses dilution with SBH
Theoretical Ethanol Yield %	12.3	14.4	15.3
Experimental Ethanol Yield %	8.6	13.26	13.8
Ethanol (g/L)	67.59	104.63	109.03
Productivity (g/L/h)	1.40	4.35	3.75

STAGE OF DEVELOPMENT

Technology is validated at laboratory scale and is ready for scale up.

INTELLECTUAL PROPERTY

- Indian and PCT application filed in 2022 and 2023
- Technology Know-How with the Institute.

LICENSING OPPORTUNITY

BCIL is looking for suitable company for licensing of this technology

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