Recent Developments on Bio Products Using Bioprocess Intensification in Biopharmaceutical Manufacturing

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Abstract

Bioprocessing for cell treatment is an order that scaffolds the cell treatment and bioprocessing (i.e. biopharmaceutical manufacturing) fields and is a sub-field of bioprocess construction. The aim of the present work is to develop optimum operating conditions for a sustainable, reproducible and robust manufacturing processes for the development of microbial feedstock. Bio-oil is produced by a pyrolysis process that involves the rapid thermal decomposition of organic materials such as wood and agricultural biomass, in the absence of oxygen. The quality of the bio-oil is dependent upon the type of biomass used. Process Intensification applies to replacing complex technologies with smaller, less expensive and more efficient integrated equipment and processes. It integrates, ideally, as many unit activities as possible into multifunctional ones for use in the chemical and biological industries. In comparison with traditional bio-processing technologies, emerging PI technologies such as membrane reactor, high-frequency magnetic impulse cavitation reactor and ultrasonic cavitation reactor tend to be cost-effective and environmentally friendly. Using different PI techniques, several bio-products were developed commercially, which lowered the cost of production and resulted in an increased yield of almost 87.5% as compared to the conventional bioprocess techniques. The present process for obtaining a blending or ageing additive from oak wood comprises of mixing red oak wood in subdivided or particulate form with water or aqueous alcoholic liquid containing up to, for example, 50% by weight of alcohol (e.g. wine or spirit).

***Key Words:** Bioprocessing, Intensification, Energy, Equipment Size, Production Capacity*

Introduction:

A bioprocess is a basic method that uses full living cells or their segments to produce desired things (e.g. microscopic organisms, chemicals, chloroplasts). For various organic and natural processes, transport of vitality and mass is necessary (Shular and Kargi, 2005). Regions need knowledge about how vitality and mass can be transferred through products, from food preparation (counting honey bee blending) to warm structure of buildings to biomedical devices to contamination control and an earth-wide temperature boost (force, heat move, and so forth). Bioprocessing or biotechnology is used with the direction of a biocatalyst, for example, a catalyst, microorganisms, plant cells, or creature cells in a bioreactor, to produce pharmaceuticals, nutrients, flavours, energies, and synthetic concoctions [1-3]. Bioprocessing for cell treatment is an order that scaffolds the cell treatment and bioprocessing (i.e. biopharmaceutical manufacturing) fields and is a sub-field of bioprocess construction. The aim of bioprocessing for cell treatment is to develop reproducible and robust manufacturing processes for the development of remedial cells (Karig *et al*., 2012).

Upstream Bioprocessing

For particular capacities and applications, bioprocessing hardware provides an expansive selection of gear. The hardware could be partitioned into three classifications in expansive terms and as for a procedure stream graph: upstream, downstream, and support. The creation of a host life form to deliver an object is handled by upstream gear. The product may simply be the living beings, it may be held inward to the creature, or it may be released into the medium of growth.

Downstream Bioprocessing

Downstream processing of biochemical products requires recovery from a complex mixture of molecules, impurities and contaminants by making use of dedicated downstream unit operations. Each unit operation will bring about a physical and chemical change that will alter the product concentration and/or degree of purity. Usually, several unit operations in series are necessary to affect the product specifications. The downstream concentration and purification process is an integral part of the production process and contributes very significantly to the overall process costs. The actual contribution of the downstream purification programme to the complexity and cost of the entire process depends on the nature of the product and its concentration in the reactor at harvest [4-6].

Industrial Biochemical and Biopharmaceuticals

A number of industrial chemicals traditionally produced from petroleum can be produced from plant sources. Vegetable oils from corn, soybean and canola can also be used as feedstocks for the manufacture of chemicals such as solvents, lubricants, waxes and adhesives. Biological or chemical processing of plant starches such as corn starch can produce organic chemicals such as acetic acid, succinic acid, glycerol and methanol, all of which are important feedstocks for the manufacture of high-value, bio-based materials and biochemical (Whistler *et al*., 1984; Sinha *et al*., 2011; Ostrander *et al*., 2015).

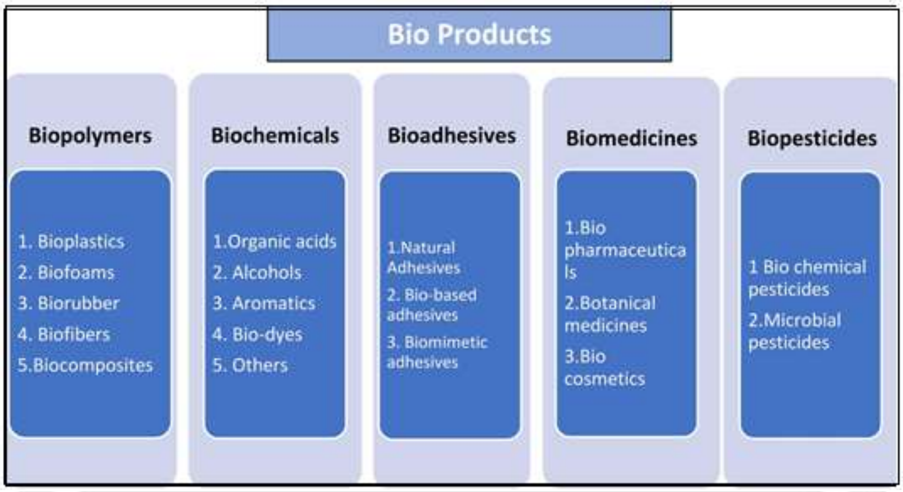


Fig: 1 Integral role of bio products in the growing bio economy.

Biomass and its Applications

Biomass is a versatile energy source. Organic matter that is used as a source of biomass energy includes trees, timber waste, wood chips, corn, rice hulls, peanut shells, sugar cane, grass cuttings, leaves, manure, sewage, and municipal solid waste [7].

Biomass systems range from small stoves used in homes for heating or cooking to large power plants used by centralized utilities to produce electricity. Industry and business use biomass for several purposes including space heating, hot water heating, and electricity generation. Many industrial facilities, such as lumber mills, naturally produce organic waste. Biomass could supply a large part of the world’s energy, through effective forest management, advance harvesting techniques and more efficient stoves and boilers.

Bioprocesses can be largely classified into three stages:

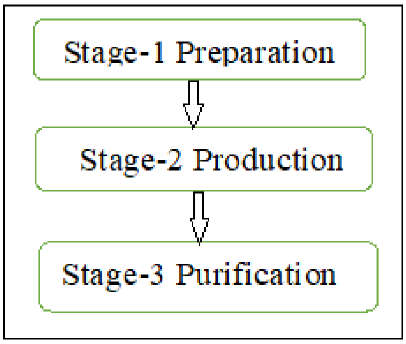


Fig 2: Bioprocess Stages

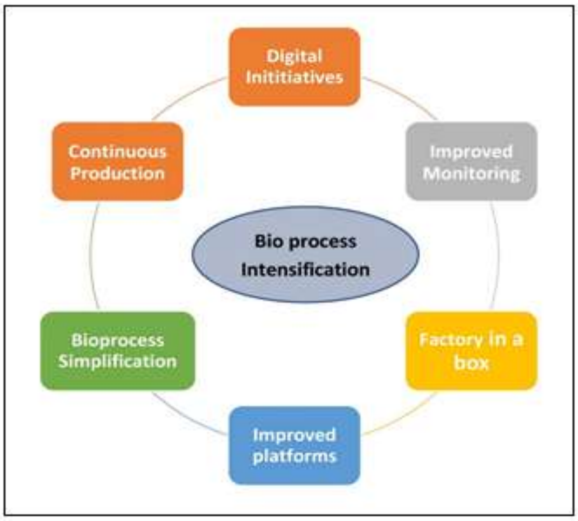


Fig 3: Recent technologies that can support bioprocess intensification.

Literature Review:

Our planet stores a huge amount of biomass available in different areas ranging from forests to oceans. Several reports estimate that the world's total biomass land and aquatic reserves are around 1.8 trillion tons and 4 billion tons, respectively. Moreover, the use of this resource does not show a homogenous distribution across the world. More specifically, in some developing countries biomass generates up to 50% of the total energy needs, through the combustion of wood, shrubs, as well as wastes of plant and animal origins while on the other hand, in the developed countries biomass energy production on average stands at approximately 11% of the total energy produced (Chum et al., 2011).

Process Intensification

Various journals and articles have been extensively reviewed based on the process intensification studies. The work various findings and fundamental research dated back from 1973, till recent advances in 2017 and development of novel intensification techniques and their applications to various fields have been discussed in this chapter.

Stankiewicz and Moulijn (2000) and Stankiewicz and Drinkenburg (2004) (Stankiewicz and Moulijn 2004) introduced a somewhat different definition of PI comprises novel equipment, processing techniques, and process development methods that, compared to conventional ones, offer substantial improvements in (bio) chemical manufacturing and processing [8].

Materials and Methods:

The materials used in the present research work include various chemicals, reagents, glassware, instruments and the biomass is obtained from red oak wood powder treated with different solvents. Bulk red oak wood shavings, chips, powder, BIOCELLULASE A20 and BIOCELLULASE TRI (Quest Bio products) and Chablis Blanc type wine, were procured from Thomas Baker, Mumbai, India. The chemicals, such as pyridine (AR), acetic anhydride (AR), chloroform (AR), methyl isobutyl ketone (MIBK, AR), hydrochloric acid (AR), and ethyl acetate (EtOAc, AR) were purchased from Tianjin Kermel Chemical Reagent Co., Ltd.

The standard LG and other chromatographic reagents (with purities > 99%) were purchased from Sigma Chemical Company (Beijing, China). High purity water was produced by Milli-Q system (Millipore, Bedford, MA, USA). Granular activated carbon (GAC) was made by our research group with a surface area of 716.6 m2/g. All solvents and samples were filtered through 0.22 m filter before injection [9-11].

Fig 4 : Analysis Lab for Qualitative analysis of bio products.

Red Oak and other oak wood selection criteria

The red oak chips and shavings were procured of best quality from Quest bio products and these or other types of oak wood are used in subdivided or particulate form, e.g. as chips, dust or the equivalent. It may also be possible to use other types of wood, e.g. beech wood, as a replacement for oak wood. This depends on the nature of the liquid to be blended with the blending additive of the invention.

Fig 5 a) Bulk red oak wood shavings (100%) b) Natural shavings/curls

Fig 6: Various Wood Samples Collected [From Various furniture stores- Telangana]

Fig 7: Finely ground red oak chips powder for further processing.

Fig 8: Red oak shavings for bulk processing into mechanical hopper.

Air-dried and medium toasted red oak wood samples were grinded and sieved into five particle size ranges: 300–350, 600–650, 900–950, 1200–1250, and 1500–1550 m. The samples were then dried in an oven at 80 ◦C until a constant weight was reached. All the samples were stored in sealed containers in a cool and dark indoor environment. The number of samples used for each pyrolysis experiment was around 15–30 g.

Experimental Work:
The experimental study of the present work proceeded through the following steps:

1. Preparation of red oak wood extract for distillation and further processing.
2. Production and purification of crystallized levoglucosan from pyrolysis of lignocellulosic biomass
3. Qualitative and quantitative analysis and description of experimental operating conditions.
4. Crystalized levoglucosan recovery
5. Analysis of the various factors that have contributed to the elevated bio product production and product enhancement and
6. Calculation of percentage yield of the Bio-oil and the associated pyrolysis bio products in both conventional and ATP-PI operational conditions.

Identification of Bioactive polyphenols in Red Oak Samples

Some high value polyphenols such as gallic and ellagic acids, catechin, quercetin and derivatives: naringenin and naringin were detected in Quercus infusions. Levoglucosan from the lignin cellulose biomass was a promising approach and the results of the study were compared with the conventional production and using Process Intensification. A distinctive HPLC profile was observed among the red and white oak samples. Comparatively, red oak wood chips have reported more of the anti-oxidant activity with additional properties [12].

Fig 9: (b): Particles in a Fixed Bed reactor.

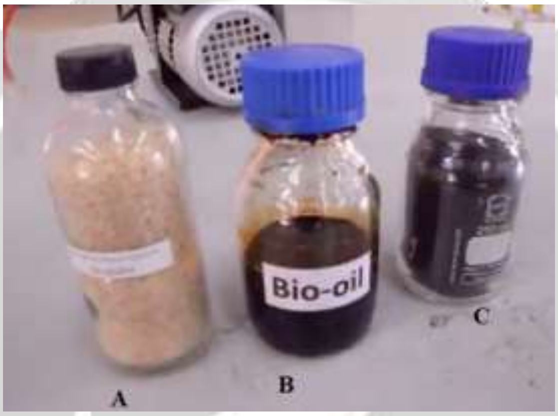


Fig 10: a): Wooden shavings of Dried Red oak fine quality; (b) Bio-oil after the recovery; (c) Bio-char of Pyrolysis of Lignin biomass.

Table 1: Temperature, pressure and mass flow rates for primary process streams

ID	**Name**	**Description**	**Temperature (°C)**	**Pressure (Pa)**	**Mass flow (tonne per day)**
0	BIOMASS	Biomass feedstock (50% moisture)	25	101 325	500
1	CRYSTALS	Crystalized levoglucosan product	25	101 325	6.6
2	H_2SO_4	Sulfuric acid reactant	25	101 325	8.0
3	NG	Natural gas for drying	40	101 325	0.0
4	PHENOLS	Phenolic oil product	25	101 325	80.9
5	PLCHAR1	Biochar product	25	101 325	32.7
6	PLCLGAS	Clean pyrolysis vapors without solids	500	101 325	239
7	PLFEED3M	Biomass feedstocks (3 mm-diameter) sized	101	101 325	272
8	PLNCG	Pyrolysis non-condensable gases	18	101 325	54.0
9	PLRECYCL	Pyrolysis gas recycle stream	200	101 325	317
10	PLSF12	Heavy and light ends	103	101 325	155
11	PLVAPORS	Pyrolysis vapors (with solids)	500	101 325	272
12	SF3–5	Pyrolysis tail gas	120	101 325	28.6
13	SYRUP	Spent mother liquor product	25	101 325	39.8

Biomass composition

The red oak biomass composition is studied in the present work. The red oak biomass contained 40% (dry matter biomass) of glucan (C6 sugar). The hemicellulose contributes toward C6 sugars in the form of glucan, mannan and galactan. The quantified C5 sugars from hemicellulose are arabinan and xylan. The major part of the lignin is Klason lignin (20.3% dry matter basis) while the acid soluble lignin is only 2.95% (dry matter basis). Other components include extractives and ash [13-15].

Table 2: Composition of red oak biomass

Red oak lignocellulosic component	**Dry matter (%)**
Total sugars	58.6 ± 0.43
Glucan	40.0 ± 0.22
Xylan	15.7 ± 0.23
Mannan	1.30 ± 0.34
Arabinan	0.34 ± 0.01
Galactan	0.92 ± 0.01
Klason lignin	20.3 ± 0.33
Acid soluble lignin	2.95 ± 0.03
Extractives	6.85 ± 0.07
Ash	0.40 ± 0.07
Total	89.1

Results and Discussions:

The present investigation deals with the conversion of red oak biomass into various industrially important bio-products at different temperatures. The various conditions which were optimized included the effect of pH, temperature, holding time, particle size, acid concentration and various conventional catalysts on the yield of levoglucosan and bio-oil.

Conversion Analysis of Biomass

The biomass samples (processed from red oak biomass) were converted to char, bio-oil, liquid, and gaseous products using pyrolysis process at different temperatures. The operating conditions of the conventional FBR were modified using ATP-PI (Auto Thermal Process Intensification mechanisms) and the % yield of the Levoglucosan was compared in both conventional and the ATP-PI reactor.

The amount of char from pyrolysis of the biomass samples decreases with increasing the pyrolysis temperature. The highest liquid (bio-oil) yields were obtained from the biomass samples between 600-650^{o}C. The yield of char and the chemical composition of char depends on the pyrolysis temperature. There have been numerous studies, which were created based on data from the physical or chemical compositions, proximate or ultimate analysis of the biomass fuels.

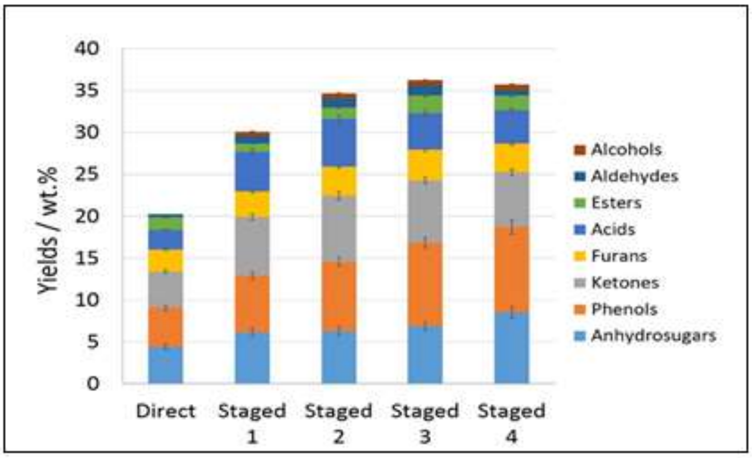


Fig 11: Chemical composition of red oak wood bio-oil from direct and staged pyrolysis (staged 1, 2, 3, and 4 were representative of pre-treatment temperature of 260, 280, 300, and 320°C, respectively, before pyrolysis in FBR at 520°C).

From the above figure, it could be seen that the low-temperature pre-treatment or low temperature torrefaction of biomass had a good enrichment for the light weight components of the bio-oils compared with the direct pyrolysis. The total yield of the chromatographic quantitative component was improved by 50–90%, i.e., reached to 31–38 wt. % based on the weight of the obtained bio-oils. The main categories of compounds such as phenols, ketones, furans, and anhydrosugars were all greatly improved.

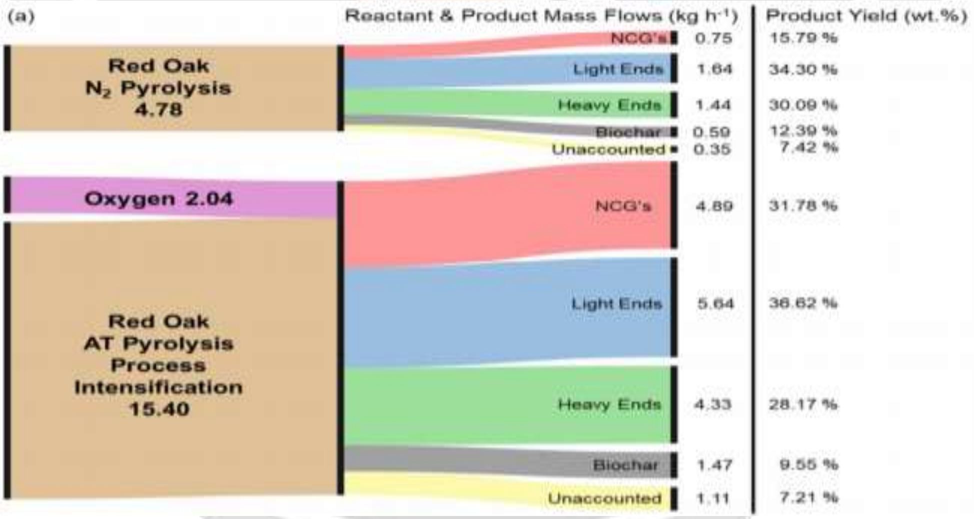


Fig 12 (a): Comparison of bioproducts processing throughput (kg hr-1) when operating the PPDU with conventional pyrolysis and autothermal pyrolysis with process intensification (ATP-PI). Product yields (wt.%) are calculated on a bioproducts basis.

Calculation of Yield (%) of the Bio-oil from Pyrolysis

Product energy yields (%) are calculated on a bioproducts input energy basis.

- The mass yield information was translated to carbon yields. Heavy-end carbon yield decreased by 3.0 wt. percent (a relative change of just -8.0 percent), while light-end carbon yield decreased by 5.1 wt. percent (a relative change of -21.3 percent). This means that carbon from bio-oil light ends is preferentially absorbed by ATP-PI relative to bio-oil heavy ends.
- AT-/PI lowered biochar's carbon yield by 4.9 wt. percent, reflecting a major relative shift of -25.0 percent. Since char is a commodity of relatively low value compared to the heavy ends, its position in autothermal pyrolysis is fortuitous as a major source of energy.
- The yield of non-condensable gases increased significantly by 10.8 wt. percent (a proportional improvement of +84.2 percent) as compared to the decrease in yields of char, heavy ends, and light ends. Considering that carbon oxides are formed by partial oxidation of charcoal and bio-oil, the increase in NCGs is not unexpected.

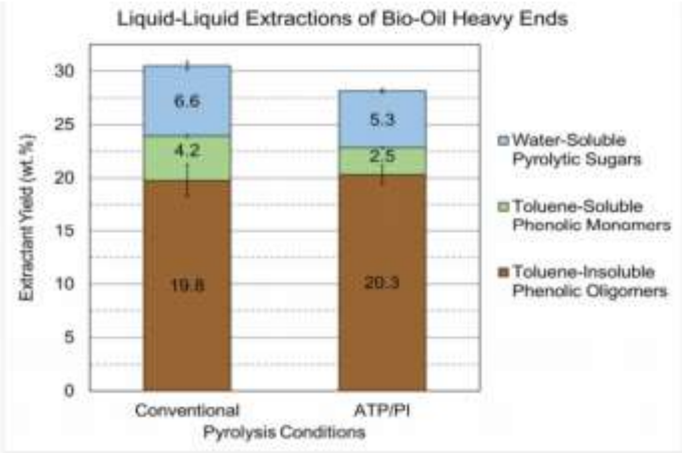
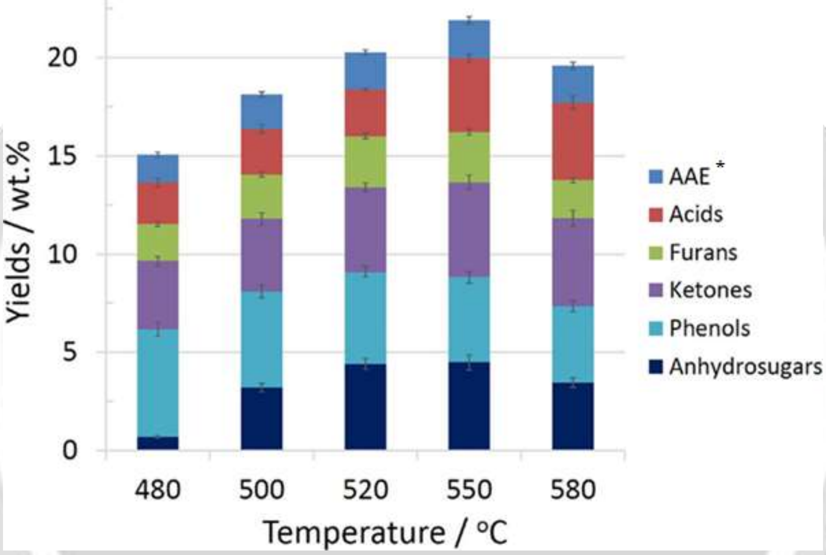


Fig 13: Comparison of liquid-liquid extractions of bio-oil heavy ends from conventional pyrolysis and autothermal pyrolysis with process intensification (ATP-PI). Extractant yields (wt.%) are calculated on a bioproducts basis.



*AAE (alcohols, aldehydes, and esters)

Fig 14: Influence of temperature on the lumped classes of red oak wood powder pyrolysis bio-oils.

As showed in the above figure, the yields of various chemical compounds quantified by GC–MS first increased and then decreased gradually with increasing temperature, with the maximum value emerged at 550°C, which correspond to 22.0 wt.% of the bio-oils. Among these, the most abundant species are hydroxypropanone (1.9–2.5 wt.%), acetic acid (2.1–3.3 wt.%), furfural (0.4–0.5 wt.%), 5-hydroxymethyl furfural (0.4–0.7 wt.%), 2-methoxy-4-methylphenol (0.2–0.8 wt.%), (E)-isoeugenol (0.2–1.1 wt.%), and levoglucosan (0.4–4.2 wt.%).

Conclusions:

To briefly summarize the process development, the process intensification Development Unit's overall function and efficiency increased as a result of achieving these particular objectives. Mass balances from conventional and autothermal process intensification studies on woody and herbaceous biomasses show that the modified process intensification generated significantly higher bio-oil yields than its previous configuration and other published literature. This study contributes to the advancement of biomass quick process intensification and helped to increase yields of industrially important bio products like bio-oil heavy ends from red oak and corn Stover biomass quick process intensification by reducing fouling problems with bio-oil heavy ends inside the process piping. This paved a promising path for the bio products production and purification on a industrial scale.

Conclusions

The following conclusions are drawn from the present research work.

- Through the application of various intensifying strategies, optimum operational conditions of a pilot-scale process intensification and fractional pyrolysis bio-oil collection device have been established through which a combination of fractionating bio-oil recovery, liquid-liquid extraction, and resin filtration which yielded a mother liquor containing 81.2 wt% db total sugars of which 44.7 wt% db was levoglucosan.
- One pass filtration with Sepabeads SP207 sufficiently removed phenolic compounds and other contaminants to readily crystallize sugars from the mother liquor.
- Solvent washing of the crystal mass allowed 24.7% recovery of levoglucosan from the mother liquor as crystals, which showed purity of 102.5% ± 3.109% wt% db at the 99% confidence level.
- This study offers a low-cost pathway for production of levoglucosan as a platform chemical in the production of biobased products as the techno-economic analysis results indicate that levoglucosan crystals could be produced *via* this process at a cost of $1333 per MT, which is ten times lower than the current market price range.
- Changing the conventional FBR parameters to run in either traditional or autothermal pyrolysis modes, was proven to be a crucial step in achieving maximum bio-oil from the red oak biomass.
- The amount of char from pyrolysis of the biomass samples decreases with increasing the pyrolysis temperature. The highest liquid (bio-oil) yields were obtained from the biomass samples between 600-650°C.
- Results of modification of the stirring speed showed that among different stirrer speeds in hydrogenation reaction, the bioproduct's content in biofuels increased considerably as the reaction was operated at 800 and 900 rpm.

Process intensification- a novel and promising approach for the production of desired bio products

To conclude, this research:

- This approach creates a novel methodology for analyzing the different processing strategies for the bio products chosen.

- Analyzes the bio products obtained using various analytical tools and techniques.

- Creates a new bioreactor to use phase intensification strategies.

- Investigates the impact of various process variables such as temperature, agitation intensity, pH, aeration, substrate selection, concentration, feeding rate, and bio product yield.

- Compares and contrast the yield and efficiency of the bio product using PI vs. traditional methodologies.

The overall results of this study reveal that, red oak biomass has the potential to produce pyrolysis oil by applying an auto-thermal process intensification strategy. An attempt has been made to increase the yields of bio-oil heavy ends from red oak biomass. Also this study reported the reduction of fouling problems with bio-oil heavy ends, by using an intensified auto-thermal process as compared to the conventional process.

Future Scope:

- The bio-products obtained from the processing of lignocellulosic biomass could be further converted to potentially high value-added products by recent advancements in chemical and enzymatic hydrolysis.
- The further investigation of purified bio-oil, which is energy-rich and easily transportable is a low-grade bio-diesel which needs further processing and purification to be used on a commercially and environmentally sustainable basis.
- Upscaling possibilities of the conventional FBR can be explored and further optimization studies can be performed to design an effective intensified reactor, to enhance the overall quality of the pyrolysis oil as a reliable microbial feedstock for a bio-based economy of the future.

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