Utilization of Carbon Sources for the Production of Bioplastic

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Abstract

Biodegradable plastics are the best "environmentally friendly" alternatives to petrochemical-based synthetic plastics for environmental sustainability and long-term development. Since it impacts the entire globe and all living beings, plastic pollution is a threat to climate change, but none more so than our food chain, which is drastically influenced by (micro) plastics being absorbed by marine organisms, endangering humanity's well-being. The synthesis of bioplastic Polyhydroxyalkanoates (PHA) by microorganisms is an alternative for this issue. Large-scale PHA production currently relies on discontinuous fed-batch culture in bioreactors. The raw material utilized here is also a primary waste concern to the environment. Like the thin stillage we used is from the waste of the distillery industry, but it is a good carbon source. The other raw material used is Corn steep liquor (CSL). It has high nitrogen content as well as an adequate quantity of carbon. The cost-effectiveness of administering PHAs and their derivatives for medical applications is incredibly high. The PHAs are a biopolymer and feasible alternative to synthetic plastics because of their biodegradability, many bacteria collect PHAs intra-cellularly, whereupon they function as a carbon and energy storage molecule. Polyesters are capable characteristics such as Temperatures of melting, glass transition, thermodynamic properties, Elastic modulus, and stress-to-break ratio.

Keywords- Polyhydroxyalkanoates, Thin stillage, Corn steep liquor.

1. Introduction

Plastic pollution is a dreadful issue that threatens our ecology. To achieve long-term development for the purpose of a sustainable environment, bioplastics must be adopted. PHAs are one of the potential options for these bioplastics. Due to their biodegradability, PHAs are a biopolymer that can successfully replace synthetic polymers. Many bacteria build up PHAs inside of their cells, where they act as a carbon and energy storage molecule. Polyesters can have characteristics including elastic modulus, stress-to-break ratio, melting and glass transition temperatures, and thermodynamic characteristics. PHAs are synthetic polymers that are being discarded since they are outstanding polymeric biocompatible and biodegradable that possessed characteristics similar to common polymers. (Reis et al., 2011).

PHAs have several beneficial properties, such as their biocompatibility, biodegradability, and thermoplasticity, they can be used in marine, agro-farming, and medicinal applications. (Philip et al., 2007).

Halophiles are regarded as a facility that produces higher consistency for a range of special characteristics. There are several distinguishing characteristics, including the need for high salt concentrations, which prevents microbial contamination, a stronger internal osmotic stress, which encourages PHA recovery through cell lysis, and the capacity to employ a variety of nutrients.

Most of the halophiles have the potential to naturally collect PHA intracellularly. Halophiles are a distinct and diversified family of microorganisms that, for instance, may live in salt marshes, salt pans, and hypersaline conditioned saltwater lakes. Mild, moderate, and intense halophiles are the three types of microorganisms that ingest salt. based on the salinity at which they will grow the best. Halophiles have various Due to their capacity for coping with severe environments, halophiles have many potentials and advantages for PHA generation. The main benefit of salt demand is that it considerably reduces the likelihood of contracting a microbial disease. Since cells can quickly break down in ordinary water owing to the high intracellular osmotic pressure, PHA recovery costs are reduced. Furthermore, halophiles can make PHA from a variety of inexpensive raw materials, which minimises the cost of fermentation, in contrast to non-halophilic bacteria. It appeared to be a feasible plan for producing PHA in response. (Quillaguamán et al., 2010).

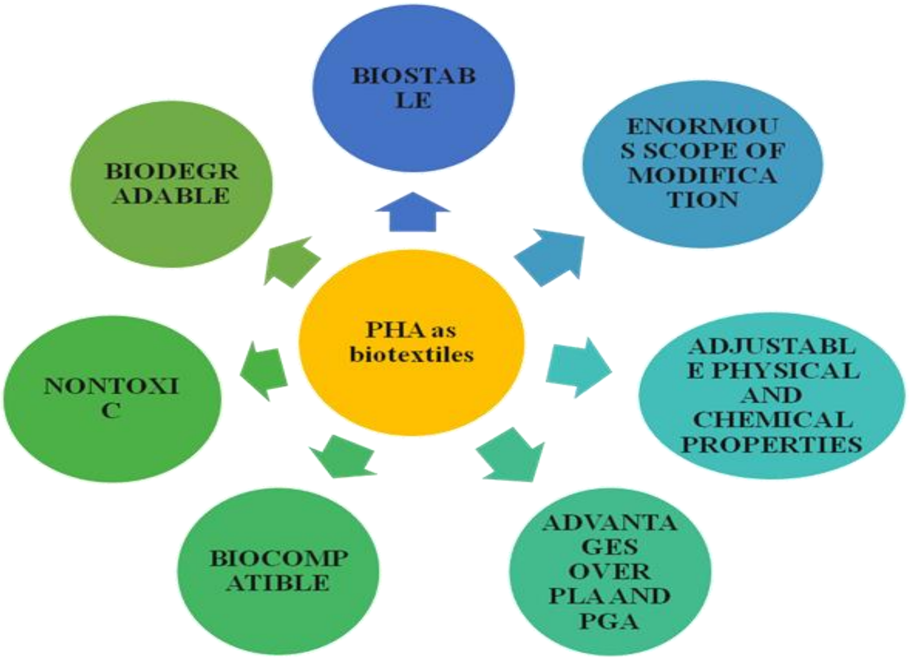


Fig. 1.1) Advantages of PHA as bio textiles.

However, there are still a lot of issues with using halophiles for large-scale PHA synthesis.

(a)For instance, treating saline fermentation effluent can be difficult.

(b) Fermentation equipment corrodes as a result of the extremely high salt concentration in the medium.

(c) Lack of genetic engineering tools and ill-defined systems may be further obstacles to large-scale PHA production with halophiles.

The result of continued research is the development of solutions to these issues.

(a) Salinized sewage can be cleaned up by marine microorganisms.

(b) Salt-resistant fermentation apparatus made of polymers, stainless steel, or ceramics is being used to grow halophiles.

(c) For instance, it is believed that the development of halophile strains through genetic engineering will enable them to produce PHA on a large scale.

Researchers used fermentation in a fed-batch environment and an escalating dosage technique to get the highest levels of PHA concentration and output. Another strategy for raising PHA synthesis is to optimize fermentation conditions. As a result of the fermentation process' unbalanced fermenter production and buildup of PHA granules, the microbes' survival strategy encourages PHA synthesis. Process variables and substrate feeding tactics have a big impact on product synthesis. Several feeding sources were used with a finite resource to maximize cell growth and PHA output. The PHA content may be increased using a variety of fermentation techniques, primarily a two-step fermentation: first in batch mode to adapt the microbe to the environment, then in fed-batch mode to quickly increase cell density. The use of resource-limited sources was made. Many fermentation techniques, primarily a two-step fermentation in which the microbe is first adjusted to the environment in batch mode, and then the cell density is rapidly increased in fed-batch mode, may help to improve PHA concentration. Furthermore, the aerobic dynamic feeding by pulses of the carbon supply enables the growth in PHA content.

PHA market expansion is anticipated to be aided by expanding renewable resource availability, increasing competition for biodegradable polymer use in biomedical, packaging, and food components, as well as attractive green procurement rules. PHAs are used to manufacture the majority of packaging materials for food and disposable items. As an alternative, mcl-PHAs are thermoplastic materials that can be used in high-value applications such implantable medical devices, surgical sutures, and biodegradable drug delivery matrices. In polymer-based devices for controlled medication delivery and hormone release, for instance, biodegradable polymers are essential. Retrievable scaffolds 3D printed for tissue regeneration, such that breakdown products are not toxic to the body. Many PHAs address these challenges because there in-vivo degrading monomeric and oligomeric enzymes have no detrimental effects on living cells or tissues.

Production of PHA has been said to be constrained by the expense of making the compound, which can be generated utilizing a variety of accessible carbon sources that have proven to be effective. It has been demonstrated that high Carbon-Nitrogen ratios promote PHA deposition. Consequently, there is a high demand for carbon sources that are efficient, long-lasting, and easy to obtain. High-purity substrates, such as glucose, continuous batch and fed-batch production scales, and enormous amounts of solvents and/or labour in downstream processing are the main causes of their high price.

Therefore, current research has focused on identifying the bacterial strains that can be used to produce PHA. Yet affordable, ecologically friendly, and readily accessible sustainable sources of carbon are also offered, like rice bran, dates, and soy molasses.

The three key factors influencing the price of PHA synthesis are substrate use, fermentation method, and PHA recovery. Large-scale PHA synthesis helps the fermentation industries grow. When compared to petroleum-based polymers, PHA manufacturing has a substantial economic disadvantage.

Halophilic bacteria are producing PHA’s in an open, non-sterile fermentation process, which lowers costs over an aseptic operation because it:

(a) does not need to be sterilized.

(b)Has an adaptive capacity connected to microbial variety; and

(c) Has facilitate to minimize production costs.

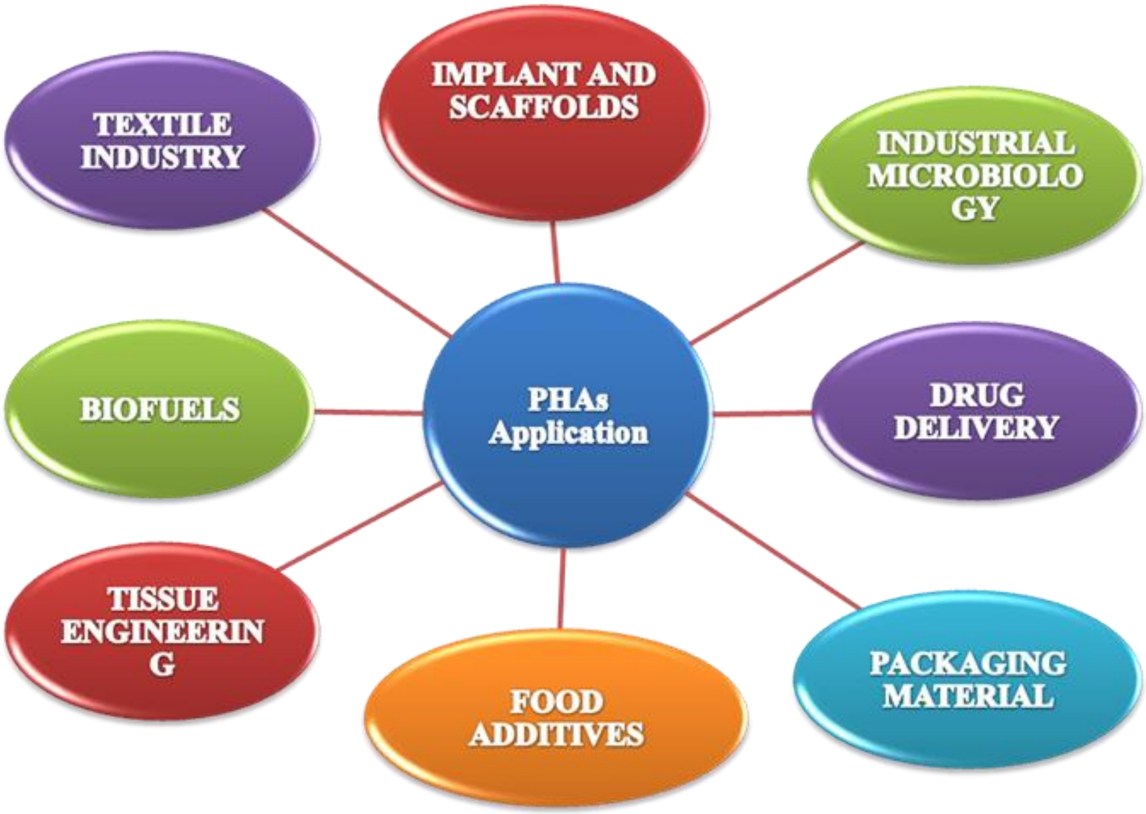


Fig. 1.2) Applications of PHA

The most extensively studied PHA, polyhydroxybutyrate (PHB), is said to have physical properties similar to those of polyethylene and might be used, among other things, as a reusable substrate surface in packing films, containers, or paper coverings (Lee, 1996; Reddy et al., 2003).

Only a very small number of microbial strains are assumed to be effective substitutes for extensive PHB production. Some well-known organisms include *Escherichia coli*, *Azotobacter vinelandii*, and *Alcaligenes latus*, which are not require carbohydrate restriction for PHB. These microbes include *Wautersia eutropha* and *Pseudomonas*, which exhausts PHB in the presence of an excess carbon source and a dietary component. The polymer can be produced by the first two strains when it is in its active growth stage. (Lee et al., 1994).

Because the carbon component, fermentation method, and downstream sorting of the composite material evaluate the efficacy and economic history of the PHB production system, developing cultivation situations for microbes that support higher PHA concentration and efficiency from cheap and ecofriendly carbon materials is critical(Choi & Lee, 1999). Hence, less expensive substrates like raw sugar can also be used because *A. vinelandii* and *A. latus* may use glucose as a carbon resource to generate PHB. These cultures can produce some of the highest cell densities ever seen and can store 75–85 weight percent store enormous amounts of PHA (65 weight percent) from carbs, but it also produces an extracellular glycoprotein.

It could also obstruct the purification of the polymer and requires high salt concentrations (at least 30% w/v) for optimal PHA production. *Halomonas boliviensis,* a halophilic microbe, has been shown to produce PHB using a range of carbohydrates, including volatile fatty acids, mono- and disaccharides, and hydrolyzed starches, which may include a combination of malto-oligosaccharides (Quillaguamán et al., 2006). This study outlines the modification of cultivation settings to increase the proportion of organic matter produced by *H. boliviensis* during PHB synthesis(Page & Cornish, 1993; F. Wang & Lee, 1997).

3. May use substrates that are renewable.

To reduce overall production costs, there are still a lot of obstacles to overcome.

The goal of this experiment was to produce PHA, increase the cellular biomass of PHA-producing bacteria in a 3 L fermenter, and characterize PHA while also maximizing PHA production.

In order to analyze the raw sample, numerous tests were run, including the following:

(a). Total Organic Carbon (TOC)

(b). Total Nitrogen (TKN)

Tests were run in order to characterize the samples, including:

(a) Crotonic Acid Analysis

2. Polyhydroxyalkanoates

To solve these issues, there are other possibilities, like synthetic and biodegradable polymers. PHAs are polyesters that work with various microorganisms toand a carbon reservoir within the cell. PHA is significant as a possible material for composites and biobased composites because of its combination of biodegradability, thermoplasticity, and biocompatibility. (Page & Cornish, 1993).

PHAs are a type of linear polyester made of hydroxy acid monomers (HAs) connected by ester bonds. This connection is created by joining the carboxylic group of one monomer to the hydroxyl group of the monomers nearby.

PHAs are divided into two classes according on how many carbon atoms are present in the monomers:

(a) scl-PHAs (short chain length PHAs), and

(b) mcl-PHAs (mid chain length PHAs) (medium chain length PHAs).

SCL-PHAs, which contain 3-5 carbon atoms, are produced by a wide variety of bacteria, including *Cupriavidus necator*. species produce mcl-PHAs, which are composed of monomers with 6–14 carbon atoms(Chae & An, 2018).

Additionally, when mixed substrates are employed, bacteria can generate copolymers. The microbes transform carbon sources into scl-copolymers like poly (3-hydroxyhexanoate-co-3-hydroxyoctanoate) (P(3-hydroxyhexanoate-co-3hydroxyoctanoate) and scl-copolymers like poly(3-hydroxybutyrate-co-3 hydroxy valerate) (P(3HB-co-3HV) or poly(3-hydroxybutyrate A scl-mcl-copolymer comprised of scl and mcl-monomers is poly(3-hydroxybutyrate-co-3-hydroxyhexanoate)(Han et al., 2017; Khanna & Srivastava, 2005). PHAs block copolymers are produced by microorganisms as a result of the different modifications that carbon sources go through during the bacterial fermentation process. The bacterial strain used and the carbon molecule given as a growth substrate regulate the structural composition of PHA polymers. Monomers with branched, aromatic, halogenated, and even epoxidized structures have been found to exist on the side chain, which can be saturated or unsaturated. *Pseudomonas putida* produced PHAs polymers, such as those with a bromide and an aromatic group. in addition to Chemically manipulating the PHA side chains allows for the integration of the desired functional group into natural PHAs, significantly altering the material properties of the polymer (Philip et al., 2007). Moreover, Pseudomonas putida allowed researchers to more precisely make and separate functional PHAs, random and block co-polymers, and PHA homopolymers.

2.1 Characteristic features of PHA

Above conventional polymers, polyhydroxyalkanoates offer a number of important advantages. A. Biodegradability – This property allows microorganisms to break down the polymer into simple constituents and utilize the resulting products in a fair length of time. Particularly PHAs totally degrade into water and carbon dioxide as their byproducts. While being biodegradable, PHAs do not supply to the growth of landfills since, unlike ordinary plastics, they can be composted after use (Pederson et al., 2006). This has major implications for the agriculture sector because frequently used agricultural films can naturally deteriorate after usage. Biodegradable polymers are appealing in the medical field because they can be utilized to promote cell proliferation in vitro, facilitate tissue growth in vivo, and regenerate cartilage.

2.1.1 Biocompatibility

It is one of the additional distinctive benefits of PHA-based biodegradable polymers. PHA-based compounds are non-toxic and widely distributed in biological cells, showing up in organisms from nearly every stratum. PHA molecules appear to be present in the cytoplasm, internal fluids, membranes, and lipoproteins because they can penetrate the hydrophobic and watery environments of the cell membrane. As a result, many other uses are possible, including temporary stents, patches, bone plates, nails, screws, and implant materials in general. (Fritzsche et al., 1990).

2.1.2 Closed carbon cycle

Unlike petroleum-based polymers, PHAs do not contribute to global warming. This is because the carbon dioxide produced by the microbial breakdown of PHA biopolymers originates from a sustainable source and was initially produced by green plants during the photosynthetic fixation of CO2 (Hartmann et al., 2006). This logical assumption leads to the conclusion that the carbon flux during the creation and breakdown of biopolymers is balanced. However, the need for energy to power the manufacturing process may cause CO2 to build up in the atmosphere.

2.1.3 Halophiles

Extremophiles are organisms that have adapted to harsh environments and are more resistant to microbial contamination. Halophilic bacteria, for instance, can develop swiftly on media with high salt and pH levels, making them more contaminant resistant than most other microorganisms can(Koller et al., 2010; H. H. Wang et al., 2011).

Their name is a translation of a Greek word that means "salt-loving."

Some of the first scientists to identify PHA accumulation by halophilic archaea was Kirk and Ginzburg in 1972. *Haloferax mediterranei*, the best PHA halophilic archaeon producer to date, was found in seawater evaporation ponds close to Alicante, Spain.

2.2 Conditions for PHA synthesis

Most bacteria produce PHAs when their growth is challenged by the depletion of nutrients like nitrogen, magnesium, phosphorus, or oxygen. The availability of too much carbon is required for PHA synthesis, which is the discrepancy between production and consumption (Snell et al., 2009).

2.3 Criteria for High PHA Productivity

The fermentation medium must be tuned since it affects how cells develop and how desired metabolites are expressed, which alters productivity as a whole. (Zhao et al., 2003). To maximize production, fed batch and continuous fermentations ought to be used. Additional factors that must be considered when choosing practical microorganisms include the cell's capacity to utilize a low-cost carbon source, growth rate, rate of polymer synthesis, and maximal amount of polymer accumulation (Chen & Wu, 2005). Additionally, it's essential to provide the bacteria with the correct amount of carbon and nutrients in order to prevent low cell concentration and insufficient PHA synthesis.

2.4 Applications

The capacity to synthesize PHAs from renewable carbon sources and the utilization of specialized carbon sources for tailored polymer biosynthesis promise a long-term "green chemistry" strategy that might also generate polymers with unique features. (Koller et al., 2010)

(a) PHAs are biodegradable, oxygen- and hydrophobic-impermeable packing materials. Compost bags and food packaging can be made of polymers.

(b)To create biodegradable personal hygiene products, the films can be utilized as laminates.

(c) PHAs can be used to create products like nonwoven textiles by being woven into fibres.

(d) Using an electron beam source and cross-linkers like sulphur or peroxides, unsaturated PHAs are capable of being used to make biodegradable rubber.

(e) PHAs may also be used to replace or create ion conducting polymers.

(f) They might be used as a alternate for latex in paper covers, as a flavorings component in food, and as a dairy cream alternative.

(g) Synthetic plastic-based latex coatings on cheese can be replaced with PHAs to provide functional protection, such as ripening control, mechanical protection, and bacterial protection.

(h) It could be utilized with bottles and containers, as well as cups, plates, and trays. These compounds are simple to manufacture due to their biodegradability in both aerobic and anaerobic conditions.

(i) As a result, they can help reduce trash sent to landfills and boost composting efficiency. Biodegradable film offers the potential to reduce maintenance and disposal costs in agriculture.

(j) In addition to numerous other biomedical applications, PHAs are being researched for usage in vascular systems, orthopaedic applications, drug delivery, urological stents, barrier materials for guided tissue regeneration in periodontitis, computer assisted tomography, and ultrasound imaging. (Yue et al., 2014).

2.4.1 Biomedical Applications

PHAs are currently known as biodegradable polymers with a biological (microbial) origin and a non-toxic nature, in contrast to ordinary plastics, which can be dangerous. PHAs have been found to be essential for certain medical procedures, such as the engineering of biological tissues.

2.4.1.1 In Drug Delivery

The requirement for a much larger range of biodegradable and biocompatible polymers to be employed as drug carriers in the emerging field of drug delivery resulted in an increase in interest in employing PHA for medical applications(Rodriguez-Valera et al., 1980). Biodegradable polymers that contain abandoned medications can be implanted in the body and used for targeted drug delivery with controlled drug release over several days. Because of their adaptability and variety of properties, biodegradable PHAs are being used as alternatives to conventional drug delivery systems. Particularly PHA-based drug carriers have enormous potential for cancer treatment and controlled delivery of drugs like steroids, vaccines, and other biological molecules. They may be made to deliver medicines to particular organs or tumors(Brophy & Voigt, 2014).

3) Effect of Different Sources on Fermentation Process

Some other way to maximize PHA generation is to optimize fermentation conditions. Finally, the sections that follow provide a quantitative and qualitative review of how physio-chemical characteristics and fermentation types affect PHA synthesis in haloarchaea (Sudesh et al., 2000).

a) Salinity Effect

At a 15% salt concentration, PHA accumulation was greater than at a 30% salt concentration. Additionally, the accumulation of PHA in *Halomonas boliviensis* was assessed at varied salt concentrations of 15, 20, 25, and 30%. It was shown that PHA accumulation worked best at a total salt content of 25% (which included NaCl, CaCl2, MgSO4, MgCl2, KCl, NaHCO3, and NaBr). A 22% salt content was shown to be preferable to 30% for PHA deposition in *Halomonas boliviensis* (Lee, 2000).

Recent research has revealed that salinity has a role in EPS and PHA buildup in *Halomonas boliviensis*. When activated sludge was utilized as the inoculum and extremely high salt concentrations were applied, it was shown that PHA was destroyed more quickly than it was produced in response to high osmotic stress. (Winnacker, 2019).

b) Carbon And Nitrogen Ratio

A sufficient carbon supply and a sufficient nitrogen source are important elements in amassing significant levels of PHA. Extracellular polymeric substance (EPS) and PHA accumulation are significantly influenced by the C/N ratio (Lenz & Marchessault, 2005). An increase in EPS production was caused by greater nitrogen concentrations, whereas an increase in PHA buildup was caused by lower nitrogen concentrations. The majority of the carbon substrate was converted into PHA synthesis during nitrogen constraint.

c) Oxygen Effect

Another factor that influences cellular metabolism is the amount of oxygen available to the cell during fermentation (Babel & Steinbüchel, 2001)

The primary goal of aeration is to supply adequate oxygen to microorganisms growing in submerged cultures to meet their metabolic needs (Zinn et al., 2001). Agitation, on the other hand, tries to assure that the microorganisms and nutrients in the broth are distributed evenly. It is possible to compare the effects of agitation and static conditions on bacterial growth and PHA generation by inoculating the culture in one set of production media kept static and the other set in an orbital shaker at 37°C for 200 rpm shaking. (Koller et al., 2017).

d) Feeding Approach

A favourable outcome was achieved by giving glycerol to culture bacteria in a fed batch fermentation. agitation and stillness's effects

The upgrading of this technology to an industrial scale requires additional optimization and R&D. Only at that point can the production be profitable.

4. Material and Method

4.1 Media Preparation:

For growth media preparation we requiredTryptone-5 g; NaCl-200 g; $MgSO_4$.7H2O-20 g, Yeast extract-4 g; Agar-17 g, KCl-5 g and $CaCl_2.6H_2O$-0.2 g etc. We maintain media pH between7.0-7.2.

4.2 Raw Material Used in The Fermentation

a) Thin stillage (TS): It is often known as distillery wastewater, is a type of distillery stillage that results from subtly fermenting grain-based feedstock (Chen & Jiang, 2017; Poltronieri & Kumar, 2018). Its volume is often fairly considerable, being roughly 10 times greater than that of ethanol produced. For the most part, thin stillage from rice wine distilleries is undoubtedly rich in carbon sources and organic acids. However, they believed that TS was an inconvenience to the distillery industries because to its low pH and generally high biological oxygen requirement.

b) Corn Steep Liquor (CSL): The process of steeping is the first step in the production of starch. It does wonders for softening the maize kernel and making it simpler to separate the starch granules from the gluten, hull, fibre and germ. As a result of this process, maize steep liquor is produced, which is widely used as a source of nitrogen for fermentation. (Singh Saharan et al., 2014).

4.3 Fermenter Processing

Halomonas boliviensis was considered for PHA productivity research at the fermenter level due to its quick growth and accumulation of PHA, which saves energy during the scale-up process. Using the optimum medium, the 4 days developed culture of *H. boliviensis* was inoculated in a 3 Litre fermenter for batch fermentation with a working capacity of 1.2 Litre Observe the fermenter for 4 days and take O.D. by spectrophotometer.

4.4 PHA Extraction from Production Biomass

Although they are the most commercially relevant, downstream processes (PHA recovery and purification) are one of the least studied parts of the entire PHA manufacturing process. The two methods of choice in the downstream phase are the use of sufficient organic solvents to remove PHA granules from inside bacterial cells or additives/chemical agents to disrupt the cellular matrix (dissolution of non-PHA cell mass, NPCM) and release intracellular PHA. These two approaches are frequently combined with pre-treatments (such as with an oxidant, or thermally assisted) to increase cellular membrane permeability. (Braunegg et al., 1998). Solvent-based processes have high energy requirements for solvent evaporation and partial/total water removal from bacterial biomass in order to improve the interaction between hydrophobic solvents and PHA granules stored within the cells, as well as a high requirement for the number of solvents needed (up to 20 times the PHA-rich biomass), which results in a high cost of operation. The creation of novel techniques and methodologies for PHA recovery is one of the key factors impacting the viability of a PHA manufacturing bio-refinery utilising microbial mixed fermentation. By using a modified sodium hypochlorite-chloroform dispersion process, the polymer from the bacterium was recovered (Srivastava et al., 2018). According to reports, all solvents containing chlorine are excellent for extracting PHA polymers because they are chlorinated. When sodium hypochlorite (pH-12.0) was added, the bacterial cell wall was completely disintegrated and lysed. The lower chloroform phase is where the PHA granules that are produced as a result of cell lysis are collected. On evaporation, the viscous polymer solution containing chloroform created a thin, transparent layer.

4.5 Quantification of Produced PHA

A percentage of the dry weight of the cells was used to quantify the amount of PHA that was extracted (% PHA). PHA was calculated using the ratio of the total dry weight of the extracted PHA to the total dry weight of the CDW. Content (Khanna & Srivastava, 2005).

4.6Crotonic Acid Assay

The conversion of PHAs into crotonic acid by sulphuric acid treatment facilitated this experiment and allowed for the quantification of PHA concentration in a sample using a spectrophotometric assay. Crotonic acid standard curves were created using solutions with different acid concentrations. between 100 and 800 micrograms.

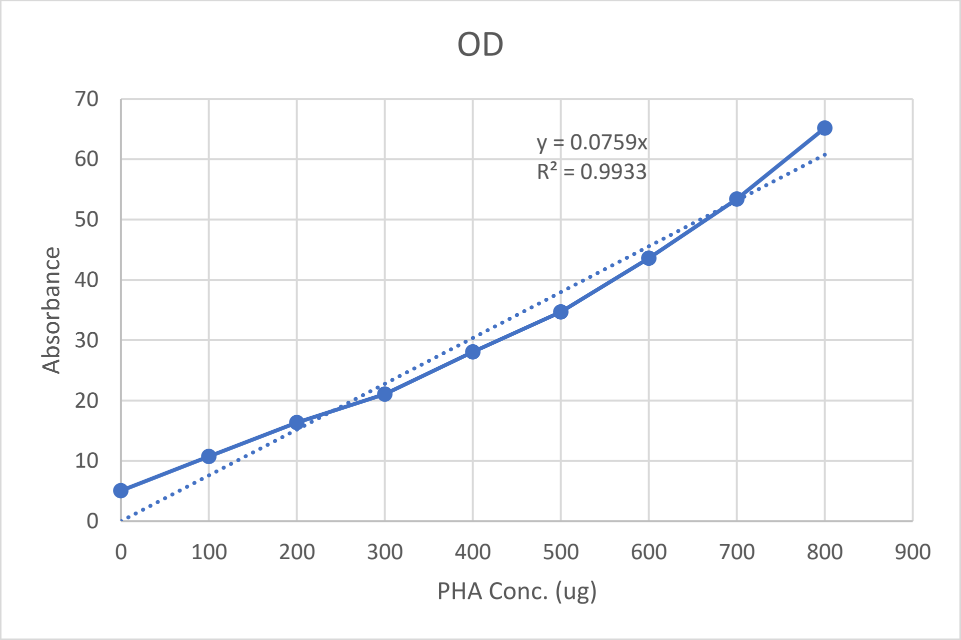


Fig.4) Standard Curve for Crotonic acid

5. Results

As a carbon source for the initial manufacture of the media, the raw materials thin stillage and Corn steep liquor were utilised, together with the two concentrations.

Standard carbon sources like glucose and sucrose (table sugar) were utilised in the tests so that the results could be compared to the raw materials.

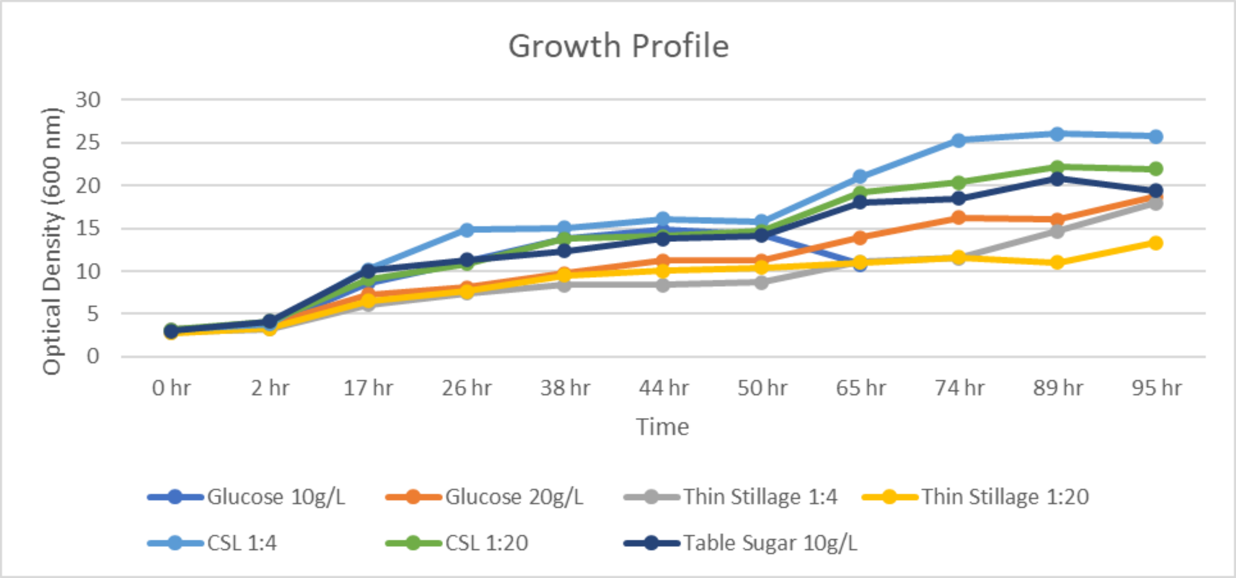


Fig. 5.1) Growth profile of *H. boliviensis* obtained utilising different carbon sources.

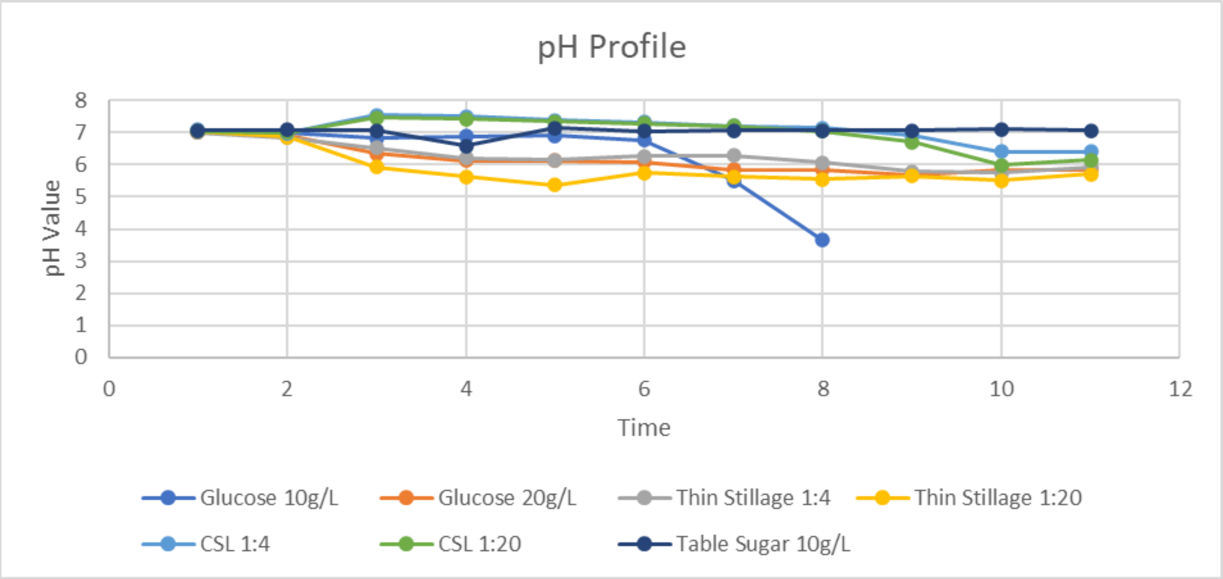


Fig. 5.2) pH profile of *H. boliviensis* obtained utilising different carbon sources.

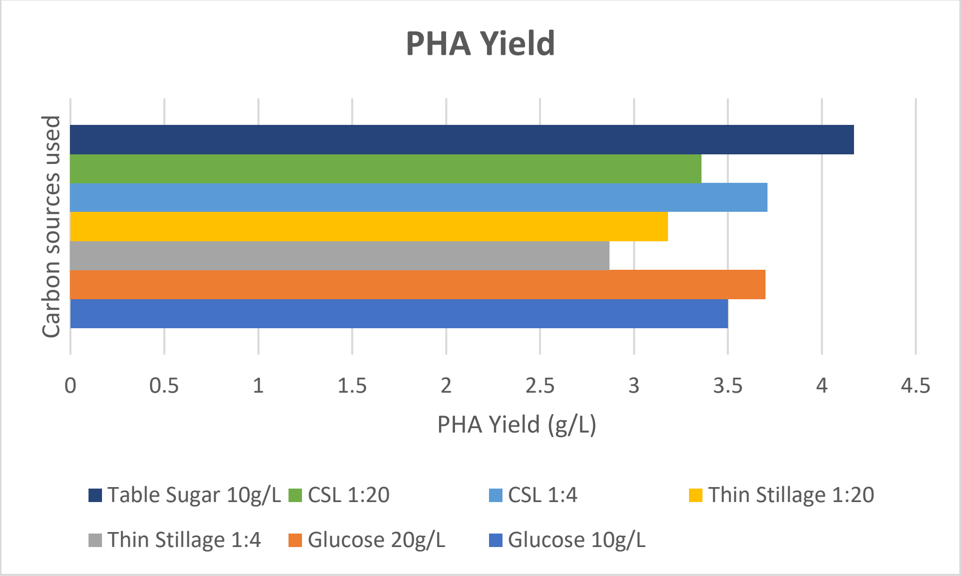


Fig 5.3) PHA yield produced from *H. boliviensis* utilising different carbon sources

Fig. 5.4) PHA Biopolymer film produced using thin stillage (TS)

Fig.5.5) PHA Biopolymer film produced using corn steep liquor (CSL)

Results show that growth from Thin Stillage (TS) is less than growth from a conventional carbon source. However, it is clear that the growth has been somewhat slowed down as a result of the presence of impurities. But compared to other carbon sources, the CSL has performed better.

Standard Sucrose has done excellently when it comes to the buildup of PHA in the cells. The CSL is superior to the Thin Stillage in terms of the raw materials needed.

6) Crotonic Acid Assay Result

The absorbance of the sample was taken at 208nm for the PHA conversion to crotonic acid.

OD- 1.032

Standard Equation y=0.0759x

Calculation

Put the value of absorbance in the standard equation-

1.032=0.0759x

13.6ug PHA

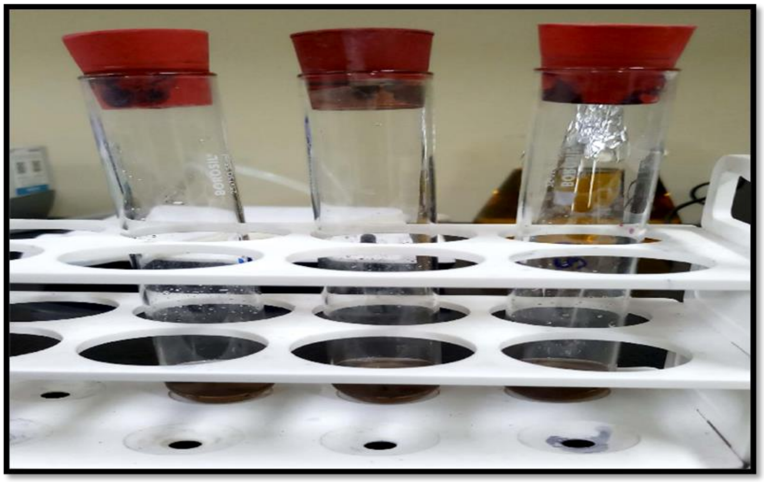


Fig. 6.1) Crotonic acid assay sample preparation

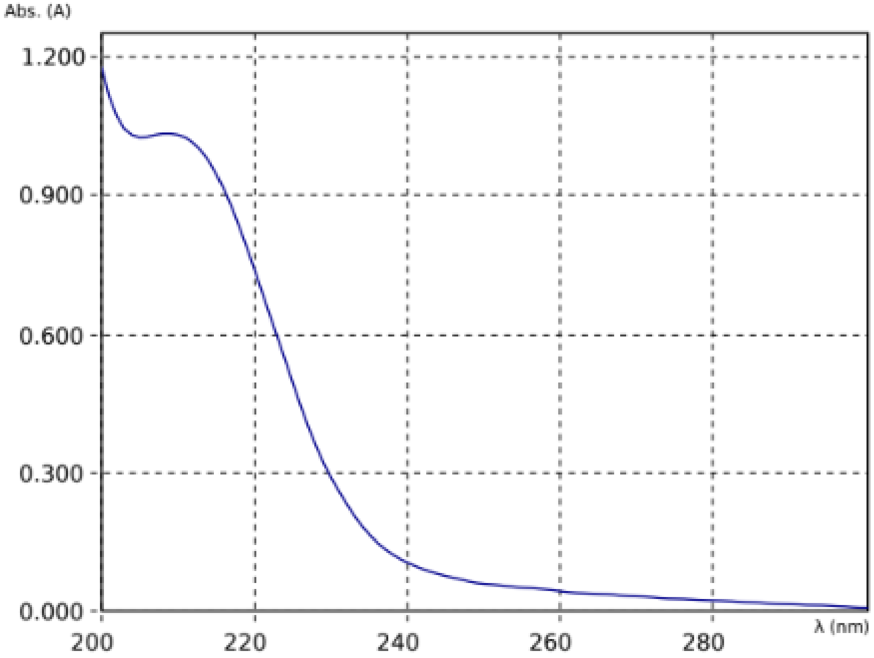
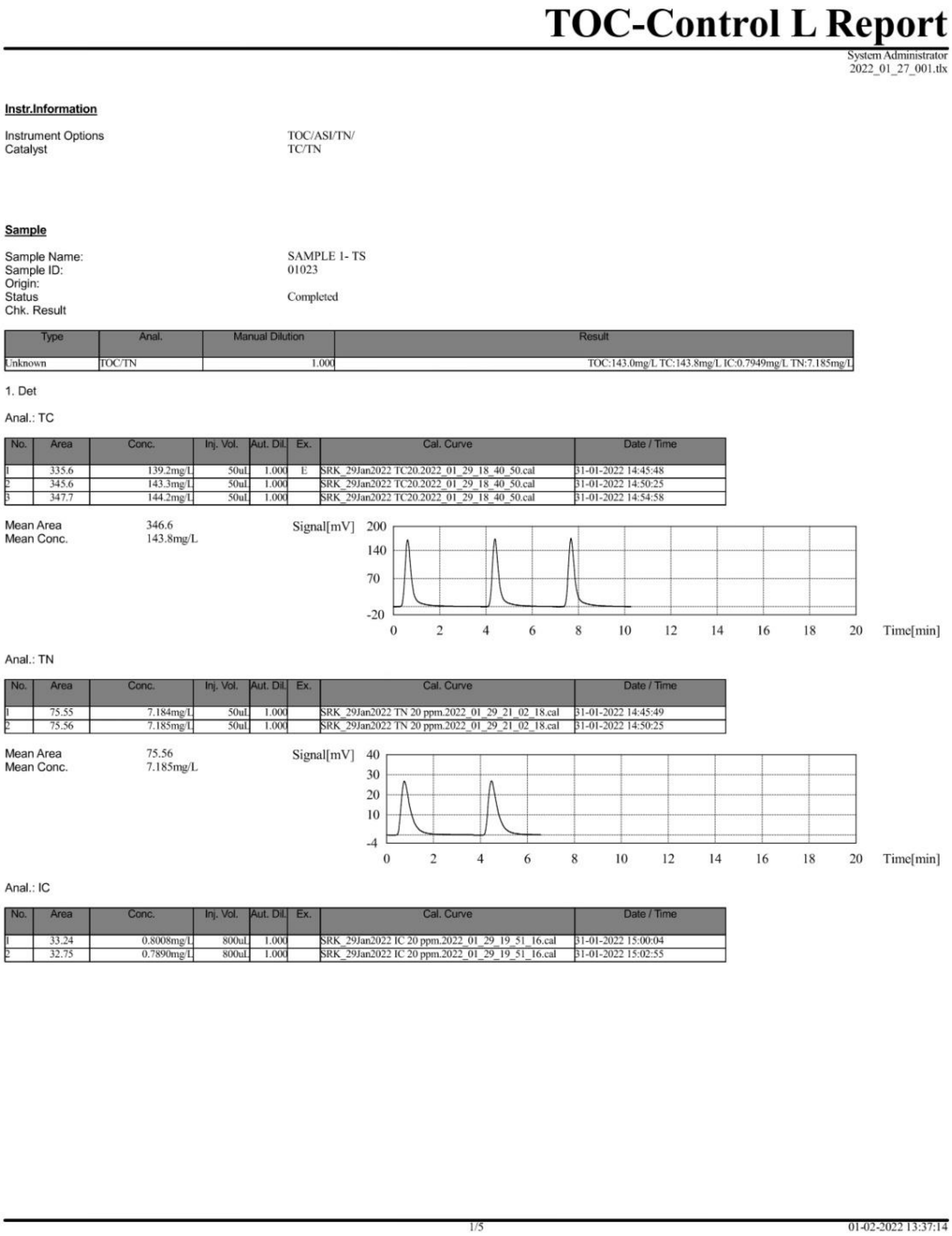


Fig. 6.2) Crotonic acid measurement in spectrophotometer

The purity of PHA is confirmed by the crotonic acid assay as the PHA is completely converted into the crotonic acid by conc. Sulphuric acid while the impurities do not. As the measured sample taken for this test is 15ug and in assay result says that it is 13.6ug.so accordingly it is 90.6 percentage pure.



TOC-Control L Report

System Administrator
2022_01_27_001.tlx

Instr.Information

Instrument Options	TOC/ASI/TN/
Catalyst	TC/TN

Sample

Sample Name:	SAMPLE 1- TS
Sample ID:	01023
Origin:	
Status	Completed
Chk. Result	

Type	Anal.	Manual Dilution	Result
Unknown	TOC/TN	1.000	TOC:143.0mg/L TC:143.8mg/L IC:0.7949mg/L TN:7.185mg/L

1. Det

Anal.: TC

No.	Area	Conc.	Inj. Vol.	Aut. Dil.	Ex.	Cal. Curve	Date / Time
1	335.6	139.2mg/L	50uL	1.000	E	SRK_29Jan2022 TC20.2022_01_29_18_40_50.cal	31-01-2022 14:45:48
2	345.6	143.3mg/L	50uL	1.000		SRK_29Jan2022 TC20.2022_01_29_18_40_50.cal	31-01-2022 14:50:25
3	347.7	144.2mg/L	50uL	1.000		SRK_29Jan2022 TC20.2022_01_29_18_40_50.cal	31-01-2022 14:54:58

Mean Area	346.6
Mean Conc.	143.8mg/L

Anal.: TN

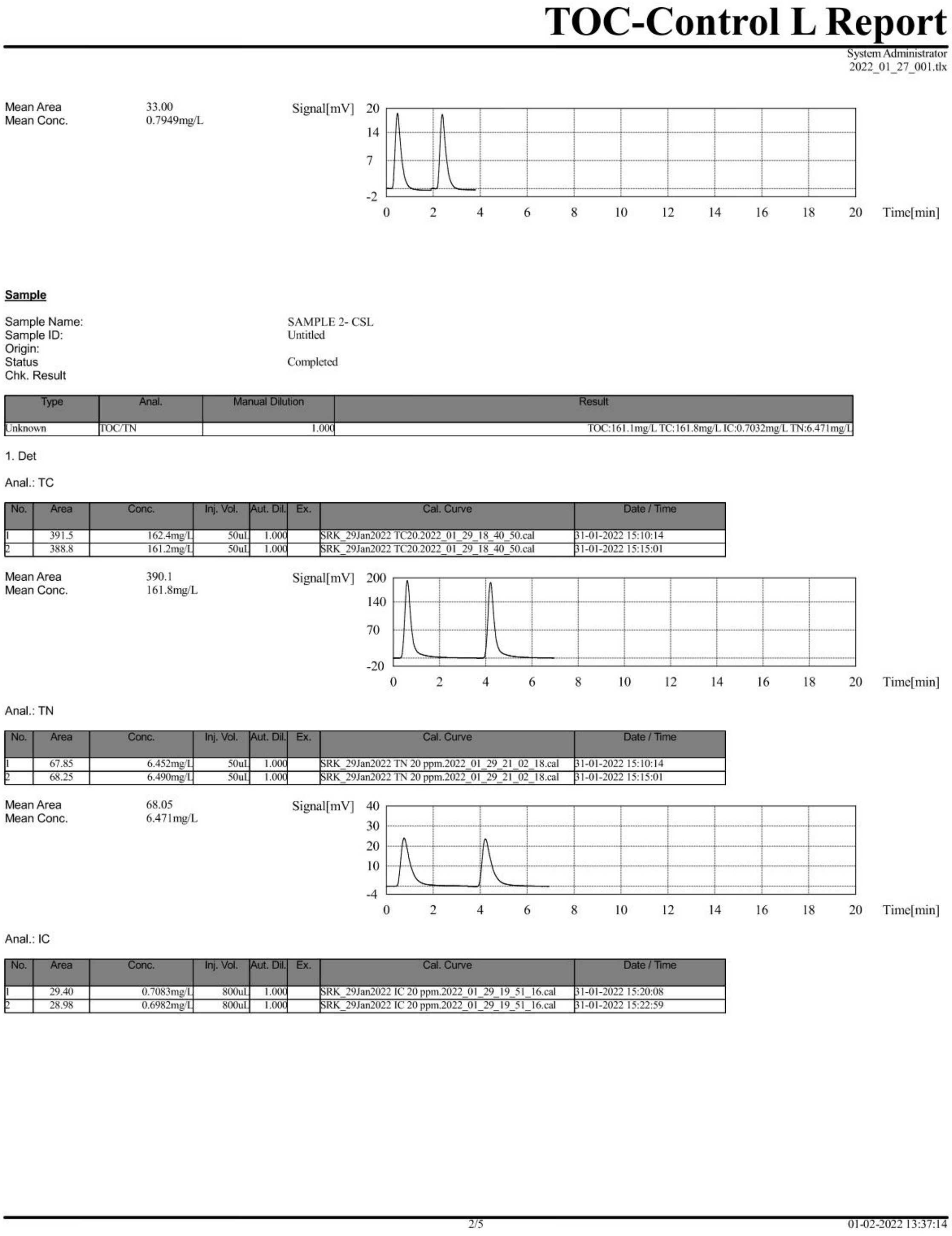
No.	Area	Conc.	Inj. Vol.	Aut. Dil.	Ex.	Cal. Curve	Date / Time
1	75.55	7.184mg/L	50uL	1.000		SRK_29Jan2022 TN 20 ppm.2022_01_29_21_02_18.cal	31-01-2022 14:45:49
2	75.56	7.185mg/L	50uL	1.000		SRK_29Jan2022 TN 20 ppm.2022_01_29_21_02_18.cal	31-01-2022 14:50:25

Mean Area	75.56
Mean Conc.	7.185mg/L

Anal.: IC

No.	Area	Conc.	Inj. Vol.	Aut. Dil.	Ex.	Cal. Curve	Date / Time
1	33.24	0.8008mg/L	800uL	1.000		SRK_29Jan2022 IC 20 ppm.2022_01_29_19_51_16.cal	31-01-2022 15:00:04
2	32.75	0.7890mg/L	800uL	1.000		SRK_29Jan2022 IC 20 ppm.2022_01_29_19_51_16.cal	31-01-2022 15:02:55

1/5 01-02-2022 13:37:14



TOC-Control L Report

System Administrator
2022_01_27_001.tlx

Mean Area 33.00
Mean Conc. 0.7949mg/L

Sample

Sample Name: SAMPLE 2- CSL
Sample ID: Untitled
Origin:
Status Completed
Chk. Result

Type	Anal.	Manual Dilution	Result
Unknown	TOC/TN	1.000	TOC:161.1mg/L TC:161.8mg/L IC:0.7032mg/L TN:6.471mg/L

1. Det

Anal.: TC

No.	Area	Conc.	Inj. Vol.	Aut. Dil.	Ex.	Cal. Curve	Date / Time
1	391.5	162.4mg/L	50uL	1.000		SRK_29Jan2022 TC20.2022_01_29_18_40_50.cal	31-01-2022 15:10:14
2	388.8	161.2mg/L	50uL	1.000		SRK_29Jan2022 TC20.2022_01_29_18_40_50.cal	31-01-2022 15:15:01

Mean Area 390.1
Mean Conc. 161.8mg/L

Anal.: TN

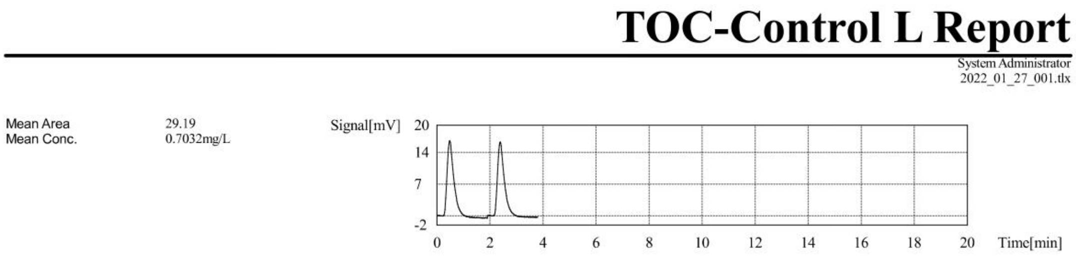
No.	Area	Conc.	Inj. Vol.	Aut. Dil.	Ex.	Cal. Curve	Date / Time
1	67.85	6.452mg/L	50uL	1.000		SRK_29Jan2022 TN 20 ppm.2022_01_29_21_02_18.cal	31-01-2022 15:10:14
2	68.25	6.490mg/L	50uL	1.000		SRK_29Jan2022 TN 20 ppm.2022_01_29_21_02_18.cal	31-01-2022 15:15:01

Mean Area 68.05
Mean Conc. 6.471mg/L

Anal.: IC

No.	Area	Conc.	Inj. Vol.	Aut. Dil.	Ex.	Cal. Curve	Date / Time
1	29.40	0.7083mg/L	800uL	1.000		SRK_29Jan2022 IC 20 ppm.2022_01_29_19_51_16.cal	31-01-2022 15:20:08
2	28.98	0.6982mg/L	800uL	1.000		SRK_29Jan2022 IC 20 ppm.2022_01_29_19_51_16.cal	31-01-2022 15:22:59

2/5 01-02-2022 13:37:14



TOC-Control L Report

System Administrator
2022_01_27_001.tlx

Mean Area	29.19
Mean Conc.	0.7032mg/L

Fig. 6.3) TOC & TN Analysis Report

7. Conclusions:

Plastic pollution is a threat to our environment that needs to be combated globally. Plastic pollution is right up there with climate change in terms of how it affects the entire world and all living things, but none more so than our food chain, which is significantly impacted by (micro)plastics being ingested by marine species, compromising humanity's well-being. The microbial production of PHA bioplastic and its use as a remedy for this issue are particularly encouraging.Williams and Martin (2005); Shrivastav and colleagues (2013).

Large-scale PHA production currently relies on discontinuous fed-batch culture in bioreactors. These methods have a variety of limitations, including low productivity, variable output quality, poor availability of specific carbon substrates, and needless time spent redesigning reactors. The findings of the research mentioned above are very encouraging and show that halophiles have a lot of promise for producing PHA through biotechnology. PHAs have been demonstrated to be significant in a number of industries. PHAs and their derivatives have extremely high cost-effectiveness when used for medical applications.

According to Novikov et al. (2002), PHAs can be utilised to treat conditions including cancer, malnutrition, neurological and metabolic disorders, control blood sugar levels, and environmental health monitoring.

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