# Bioprocessing Technology, Process Safety Hazards

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## Introduction

We are in the transition of a new industrial revolution- a revolution emerging from advancements in biotechnology. In the future, some believe biotechnology will provide us with the ability to produce the chemicals and chemicals we use daily, engineer microbes to break down plastics in landfills, our own cells can be programmed to cure diseases and much more. Bioprocessing is the production of a value-added material from a living source. The living source(cells) responds to its environment to give the desired product. Bioprocess involves bacteria, mammalian cells, fungi, plants, insects etc. The products that are manufactured from bioprocessing are the substances that the cell has naturally, or genetically altered and created from the cell (introduced by recombinant DNA technology) or the waste produced from the growth. We need to isolate the cell from a heterogeneous population to use in bioprocessing. The common products produced from Bioprocessing are biopharmaceuticals, food products, amino acids, enzymes, vitamins, dyes, steroids etc. Companies continue to shift to biological processes or develop novel bioproducts. The increase in bioprocess facilities also brings a variety of new hazards related to the process and production. There are many hazards and risks related to commercial bioprocessing. Process Safety incidents in these industries result in fatalities, injuries, facility damage, environmental impact, production loss, as well as a company's reputation impact. This report focuses on processing technology, major applications of bioprocessing technology, collection and analysis of incidents that happened in the bioprocessing industry for root causes and lessons learned.

## Bioprocessing Technology

The major processes that are involved in the production flow of bioprocessing are Fermentation, Recovery, and Purification. The scaling up of seed stock and the fermentation process is

considered an upstream process, which occurs early in the process, while the rest is a downstream process.

**Fermentation**

Many of today's biotechnology companies rely on fermentation to produce products. This process takes place in a bioreactor, providing the optimum environment for the reaction. The conditions are designed so that the yield of the product is optimized. Based on the properties of the cell and the desired product, a cell is selected for the fermentation process. A cell needs nutritional products to grow, and they are provided by the media. Some cells are aerobic, and some cells are anaerobic. Aerobic cells need oxygen to grow, and Anaerobic cells do not need oxygen. Some of the industrial aerobic processes include citric acid production, production of Baker's Yeast, production of Penicillin, and production of High Fructose Corn Syrup. Some of the industrial anaerobic bioprocesses include ethanol production, lactic acid production, and acetone-butanol production.

A seed stock of selected cells is taken and put in a small quantity of media. When the cells consume most of the nutrients and the population is grown, they are moved into a larger vessel with more growth media and this process repeats until the quantity of cells is sufficien and they can be transferred into a production vessel. This production vessel is known as Bioreactor or Fermenter.

Under a controlled environment and a sufficient amount of fresh media available, the cells grow to produce the desired product. If the cells are aerobic, the fermenter mixes the cells throughout the media evenly and supplies the oxygen required for growth.

Key factors that need to be monitored include pH, temperature, dissolved oxygen, pressure, and nutrient levels.

| Input | Output |
| --- | --- |

| | |
|---|---|
| Oxygen<br>Carbon and energy source<br>Nitrogen source<br>Other Requirements (P, S, Na, K, Mg etc.) | Carbon dioxide<br>Biomass<br>Metabolites<br>Water<br>Heat |

*Table 1 Substrate Input and Output*[1]

Almost all bioprocesses follow the same growth pattern. The four phases of the growth pattern are Lag Phase, Transient Acceleration Phase, Log or Exponential Phase, Deceleration Phase, Stationary Phase, and Death Phase. The lag phase is the initial period in the cell population life in a bioreactor where the cells need time to adjust to the new environment. When the cells adapt to the new environment, they begin to grow slowly in the transient acceleration phase and then the cells divide by binary fission, and it creates exponential growth by doubling each generation. The deceleration phase follows the exponential phase. When the cells almost consume one or more essential nutrients in the media, toxic metabolic waste products build up, the growth declines, and the cells start dying. This phase is known as the deceleration phase. When the number of cells that are produced is equal to the number of cells that die, this phase is known as the stationary phase. The death phase is when the number of cells that die exceeds the number of cells produced.

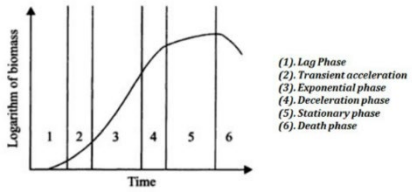


*Figure 1 Typical growth curve of a cell population*[4]

The different types of bioreactor processes are Batch process, Fed-Batch process, and Continuous process. In the batch process, the cells are inoculated into a fixed volume of media. The cells grow and accumulate in the fermenter as the nutrients are consumed. The fresh media is neither added nor the used media removed from the bioreactor. Hence the concentration of the nutrients decreases continuously. The batch process is mainly used for small quantities of production.

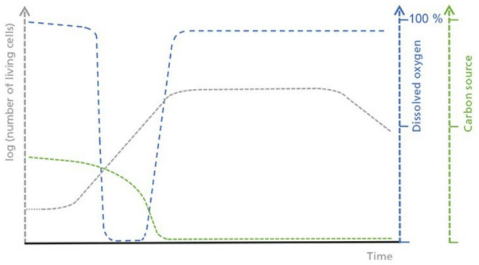


*Figure 2 Schematic representation of correlations between living cell concentration, dissolved oxygen, and limiting carbon source in batch operation.*[5]

In the Fed-Batch process, nutrient media is prepared and inoculated with the culture (cells) and then incubated for a particular time. During the course of incubation, a particular nutrient is added at intervals without removing the used-up media. Hence the volume of the culture increases continuously. Some nutrients are essential for the process but when these nutrients are provided in higher concentrations in the culture, they inhibit the growth of bacteria and ultimately stop the fermentation. Therefore, such nutrients are initially kept in lower concentrations and are slowly and continuously added during fermentation. Almost all the media's nutrients are consumed at the stationary phase, the fermentation is stopped, and the broth is harvested.

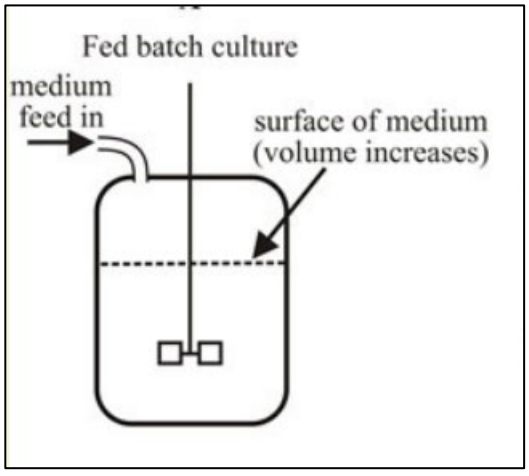


*Figure 3a Fed-batch culture sketch*[6]

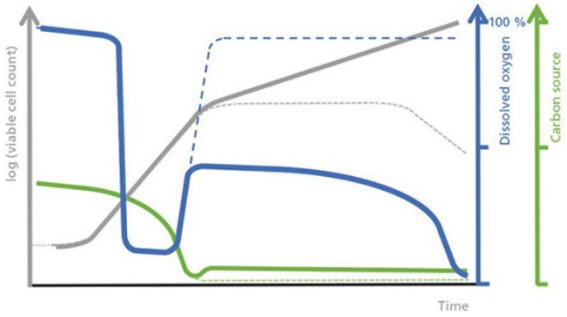


*Figure 3b Schematic representation of correlations between living cell concentration, dissolved oxygen, and limiting carbon source in Fed-batch operation.*[5]

The continuous process is also known as an open system of cultivation. Fresh sterile medium is continuously added to the vessel and the used-up media with cell culture is removed continuously at the same rate. The volume of bacterial density remains the same in the bioreactor. In this technique, bacteria grow continuously on their log phase and this type of growth is known as steady-state growth. The cell density in the continuous culture remains constant and it is achieved by maintaining constant dilution and flow rate.

| Characteristics | Batch Process | Fed-Batch Process | Continuous Process |
|---|---|---|---|
| Cultivation system | Closed | Semi-closed | Open |
| Addition of Fresh Nutrition | No | Yes | Yes |
| Volume of culture | Constant | Increases | Constant |
| Removal of Waste | No | No | Yes |
| Chance of contamination | Minimum | Intermediate | Maximum |
| Growth Phase | Lag, log, stationary and decline phase | Lag, log, stationary and decline phase | Lag and log phase |
| Log Phase | Shorter | Longer | Longest and continuous |
| Density of Bacteria | Change with time | Change with time | Remain constant |
| Product Yield | Low | Medium | High |

*Table 2 Comparison of Batch, Fed-batch, and Continuous Processes*

**Recovery**

The final product of fermentation is called broth. Broth contains the molecule of interest. The two ways in which a product of interest is produced by the cells are Extracellular and Intracellular. Extracellular production is when the cell secretes the product of interest. Certain amino acids, enzymes, and monoclonal antibodies are produced by extracellular production. In this case, the cells are discarded at the beginning of separation and the medium is kept for further processing.

Intracellular production is when the product of interest is produced by the cell and kept inside the cell. During the early stages of separation, the biomass is collected and then disrupted to release the product. The molecule is locked inside the host cell and millions of these host cells are

suspended in the pool of depleted media and metabolic waste products. Recovery processes commonly use a variety of operations, such as centrifuge, cell disrupters, and microfiltration to isolate the product. At this point, the process stream is referred to as “Clarified Lysate”.

**Purification**

A series of chromatographic steps with distinct modes of separation is used in the purification of a product. A column is a cylinder filled with glass, ceramic or polymeric beads (often called resins) which are engineered to interact with or bind with molecules based on one or more physical properties. Chromatography relies on differences. Each molecule has a unique set of physical characteristics such as size, charge, and extent of interaction with water. Chromatography uses these differences to separate the target protein from other proteins and chemicals. The most used chromatographers include:

- Adsorption Chromatography which is based on the adsorption of solute molecules onto solid molecules.
- Size exclusion Chromatography in which some beads have small holes in them and can temporarily trap or at least slow down smaller molecules as they travel through the column of resin beads, while molecules too large to enter the pores move around the beads and exit the column first.
- Ion-exchange Chromatography where opposites attract. So, a negatively charged chromatography bead will attract and bind to positively charged components in the process stream. Likewise, a positively charged resin bead will attract negatively charged components in the process stream.
- Hydrophobic interaction Chromatography that separates molecules based on their hydrophobicity. Molecules that readily interact with and dissolve in water are called

hydrophilic(water-loving), while those that don't are called hydrophobic(water-hating). Proteins contain regions that are hydrophobic and regions that are hydrophilic. Because water tends to form a shield around the hydrophobic patches within the proteins, they are exposed to interact with resin beads. By adding salt to the protein solution, we remove the water shield, exposing those hydrophobic patches on the protein and the resin so they can interact.

- High-Pressure Liquid Chromatography which is based on general chromatographic principles where high pressure is applied to the packed column.

**Some Applications with Process Flow Diagrams**

Biopharmaceuticals:

The medical drugs produced by the biotechnological process using molecular biology methods are called biopharmaceuticals. The protein is produced by the living organism in the upstream process whereas a series of purification steps are performed to meet certain purity specifications in the downstream process.

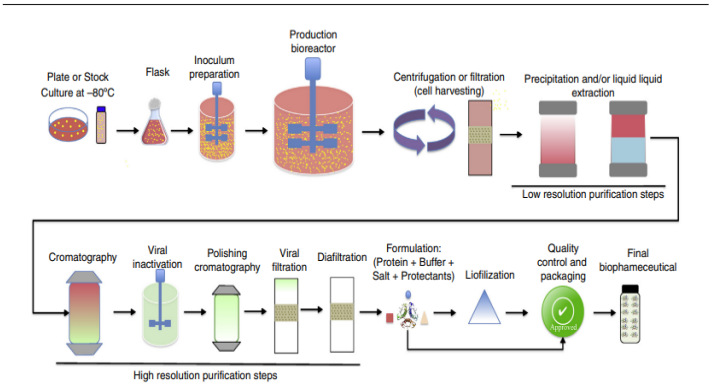


*Figure 4 General flowchart for biopharmaceuticals manufacturing[10]*

Biodiesel:

Vegetable oils, used cooking oils, yellow grease, or animal fats are used to produce biodiesel. Transesterification is used to convert fats and oils into

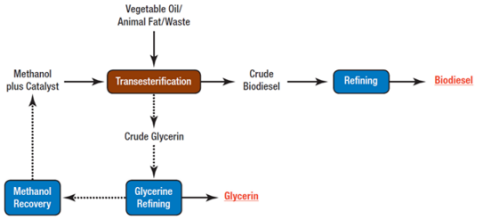


*Figure 5 Schematic of Biodiesel production[7]*

biodiesel and glycerin (coproduct). In the presence of a catalyst, oil or fat is reacted with short-chain alcohol to produce biodiesel and glycerin/ glycerol.

Brewing:

Beer is produced by steeping starch sources such as cereal grains, barley, millet, cassava, and sorghum in water and fermenting the resulting liquid with yeast. The processes involved are milling, mashing, lautering, boiling, whirlpooling, cooling, fermenting, maturing, filtering, and packing.

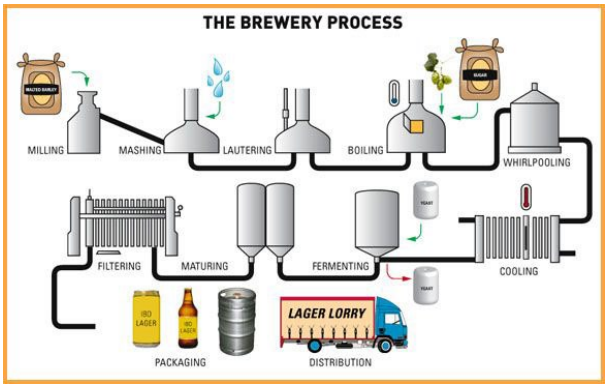


*Figure 6 Flowchart of brewing process*[11]

Biosurfactant:

Biological Surface-Active Agents are a diverse group of molecules. They contain a polar head and a non-polar head. Their structure makes them surface tension reducers. They are produced from microorganisms during biodegradation. They are capable of solubilizing hydrophobic substrates such as oils, antibiotics, hydrocarbons etc.

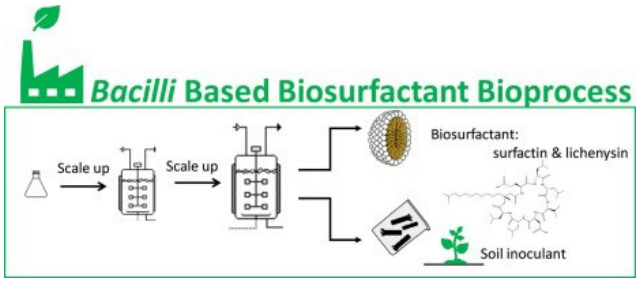


*Figure 7 Flowchart of Bacilli based biosurfactant bioprocess.*[12]

Bioethanol:

Bioethanol is a potential substitute for gasoline and can be used directly in vehicles or with a blend of gasoline. The first-generation (1G) bioethanol is produced from corn or sugarcane, and the second-generation (2G) bioethanol is produced from lignocellulose. Some examples of

lignocellulose biomass are cornstalks, switchgrass, wood, agricultural and forestry residues, herbaceous crops, wastepaper and paper products, food industry waste and municipal solid waste. Third-generation (3G) bioethanol is produced when algae are used as feedstock. The processes that are involved in the generation of biofuel include pretreatment, hydrolysis (except for sugar cane) and sugar conversion to bioethanol.

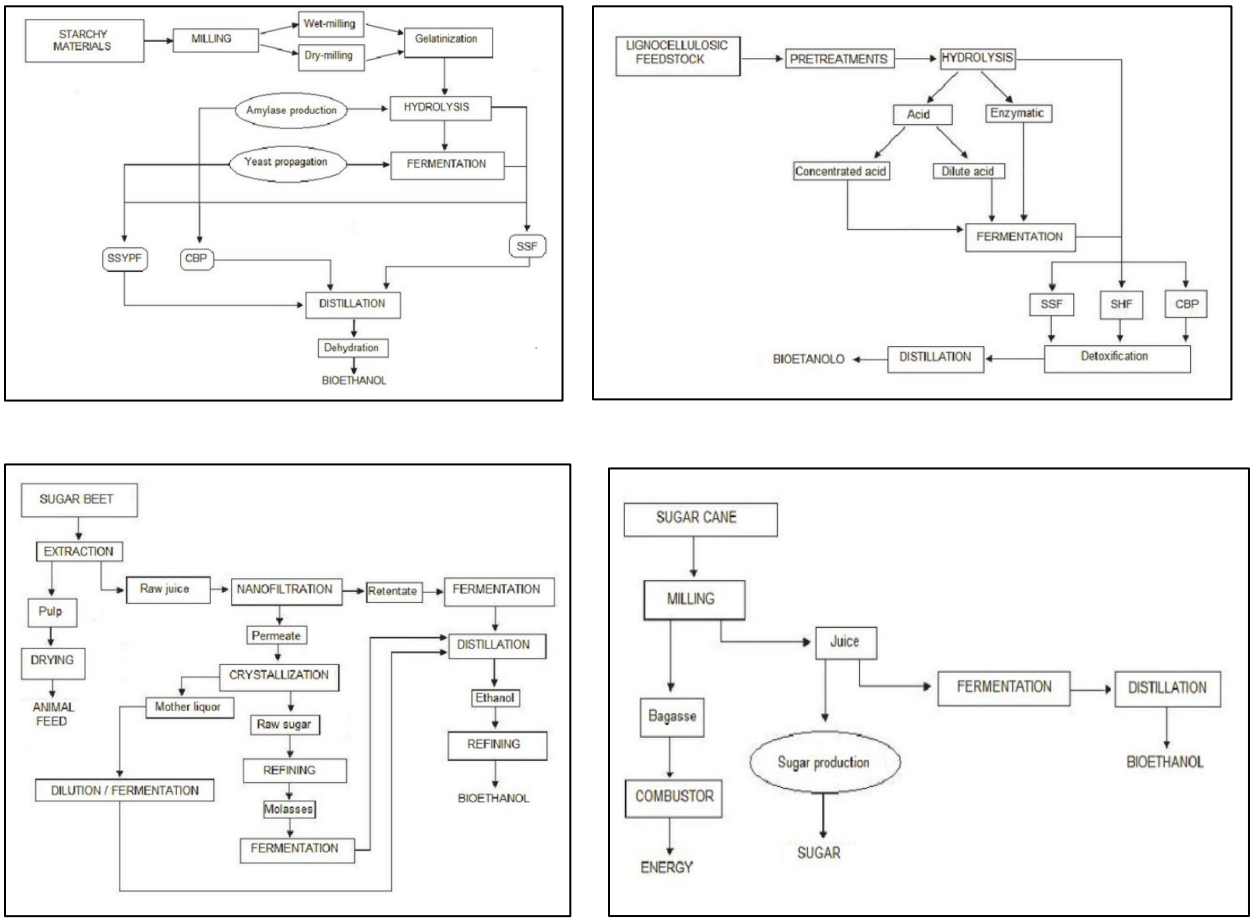


*Figure 8 Flowchart of bioethanol from materials containing a) starch b) lignocellulose c) sugar beet d) sugar cane.*[9]

**Database**

The first step in the analysis was the data collection of incidents that happened in the bioprocessing industry. The incidents were collected from the following sources.

-Specific accident databases and sources such as the US CSB (US Chemical Safety and Hazard Investigation Board)[14], eMARS (Major Accident Reporting System)[15], ASM (Abnormal Situation Management)[16], and OSHA (Occupational Safety and Health Administration)[17].

-Information that was reported on newspaper websites and on the web. The database is clearly impacted by the reporting of incidents, likely only those that are newsworthy, resulted in fatalities or serious injuries, or significant property loss.

The keywords bioprocess/bioindustry/bio was combined with one of the following keywords: "incident", "explosion", "fire", and "accident". The search was carried out by translating European languages. One shortcoming of this analysis and the related process safety research is the absence of a database of incidents. While the above-mentioned databases exist, they all have several shortcomings. None of the resources contains all the information that is required for the analysis.

Identified 54 incidents based on this search. The collected incidents were segregated based on the type of industry, root cause, location etc. for the analysis. All the 54 collected incidents with details such as date of the incident, location where the incident occurred, root cause, fatalities and injuries are included in Appendix 1.

Analysis:

The distribution of incidents with time seemed important and is shown in Figure 10. The highest number of incidents was recorded in 2019 and 2022 with 6 incidents, followed by 2013, 2004, and 2009 with 5, 4, and 4 incidents respectively. The incidents remained constant from 1990 to 2000 with 1 incident each year. The bioprocess industries are growing and hence the incidents in recent times are more when compared to the 1990s.

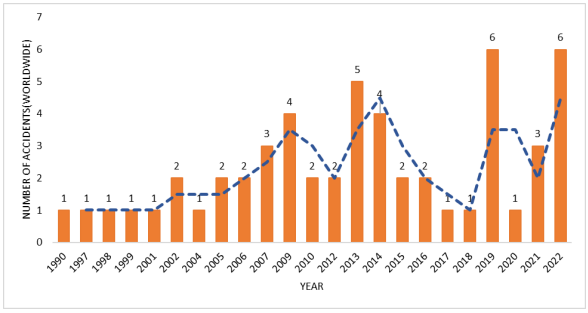


*Figure 10 Distribution of incidents with time*

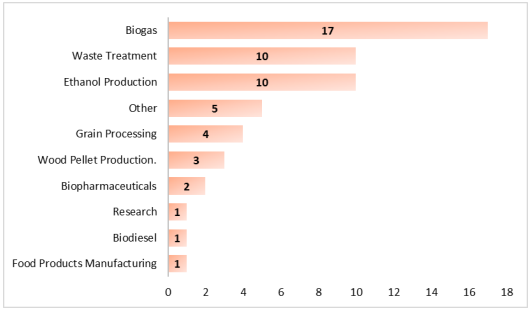


*Figure 9 Distribution of incidents with type of industry*

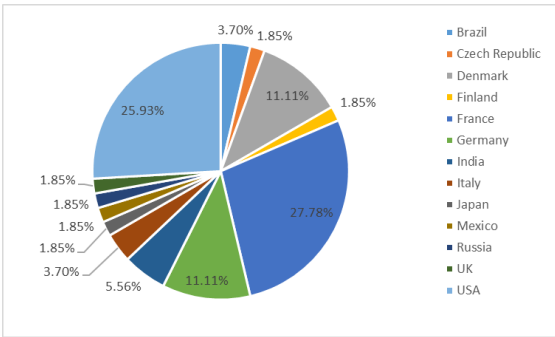


*Figure 12 Distribution of incidents with location*

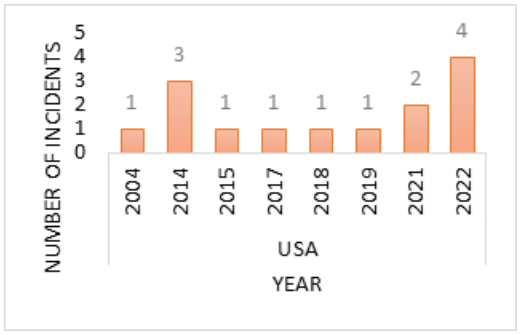


*Figure 11 Distribution of incidents in the USA with time*

Distribution of incidents with type of industry is shown in Figure 9. Among the 54 incidents collected, the Biogas industry, Waste Treatment, and Ethanol Production industries have the highest number of incidents whereas the Research, Biodiesel and Food Products manufacturing have the lowest number of incidents. Per Figure 12, most of the incidents occurred in the United States and France while around 35% of the incidents occurred in Denmark, Germany, India, Italy and Brazil. Figure 11 shows the detailed study of incidents that occurred in the United States with time. The incidents were constant from 2015 to 2019 and increased gradually from 2019 to 2021, but remain relatively low at a couple a year.

The incidents were segregated based on the factors that caused the incident. Most often, there might be many factors that contribute to an incident where some initiate the incident and others increase the severity of the incident. The 18 contributing factors are listed below:

1. Safety Culture
2. Hazard Awareness and Identification
3. Process Hazard Analysis (PHA)
4. Operating Procedures
5. Work Permit System
6. Personnel Training
7. Mechanical Integrity
8. Safeguards, Controls and Layers of Protection
9. Preventive Maintenance
10. Management of Change
11. Contract Management
12. Design

13. Human factors
14. Facility Siting
15. Pre-Startup Safety Review
16. Regulations and Regulatory Oversight
17. Natural Disaster
18. Emergency Preparedness and response

A detailed list with the definitions is available in the Journal of Loss Prevention in the Process Industries[13]. There are around 17 incidents that resulted in Dust Explosions, with few additional details as to the true root cause(s). Hence Dust Explosion is also considered one of the factors responsible for the incidents along with the other factors leading to a list of 19 factors.

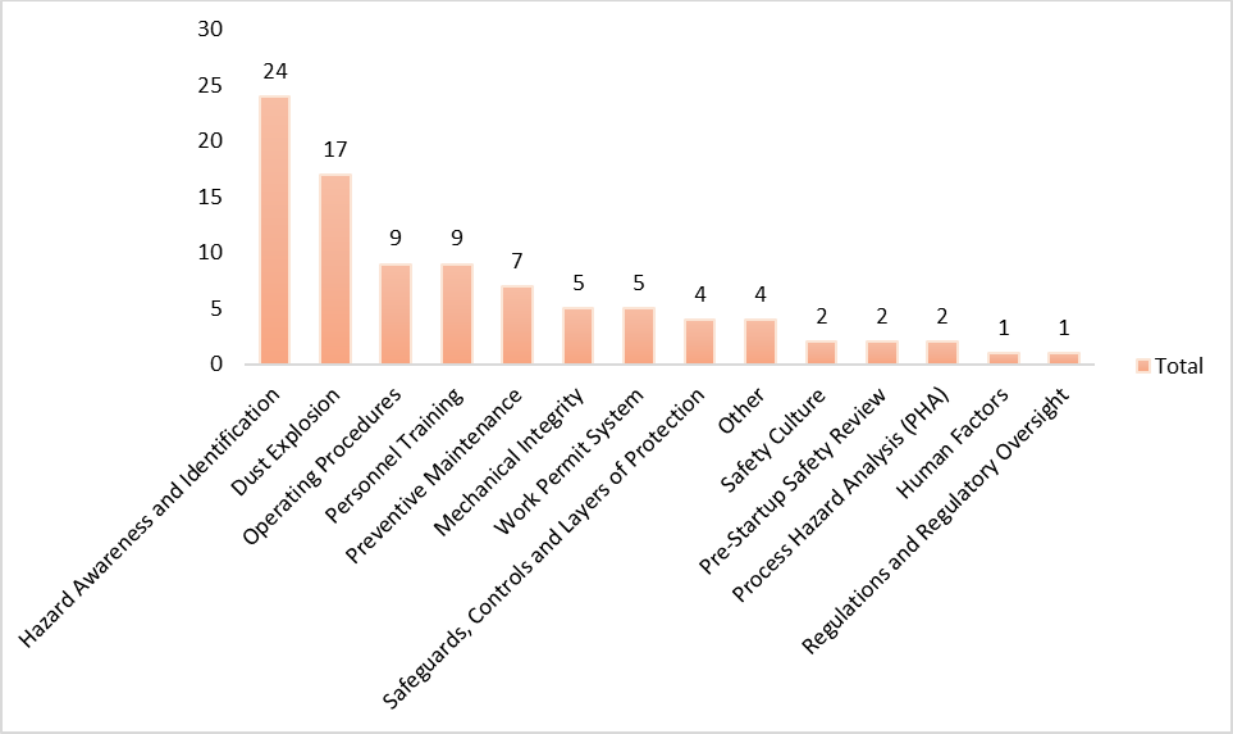


*Figure 13 Distribution of incidents with factors causing the incident.*

Aa ahown in Figure 13, among all the categories, most of the incidents were caused by Hazard Awareness and Identification, and Dust Explosion. The root causes of the incidents that were not identified among the 18 categories and the Dust Explosion are mentioned in “Other” category.

The analysis of the number of fatalities and injuries with location, type of industry, root cause and time are shown in Figures 14, 15, 16 and 17 respectively. The Biogas industry has the highest number of fatalities and Ethanol Production has the highest number of injuries. India, the USA, Mexico, and Germany have the highest number of fatalities, whereas the USA and Germany have the highest number of injuries. Hazard Awareness and Identification has contributed to the highest number of fatalities as well as injuries and most of the incidents in the dataset are caused by this same root cause. 2009 has the highest number of fatalities and 2017 and 2005 has the highest number of injuries.

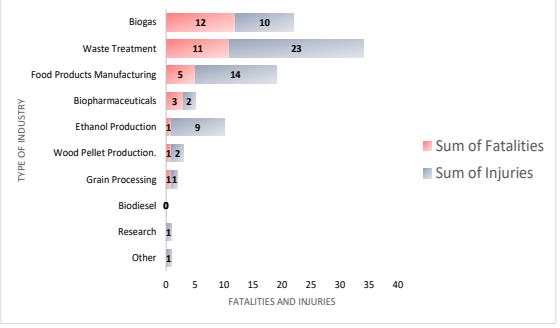


*Figure 14 Distribution of fatalities and injuries with type of industry*

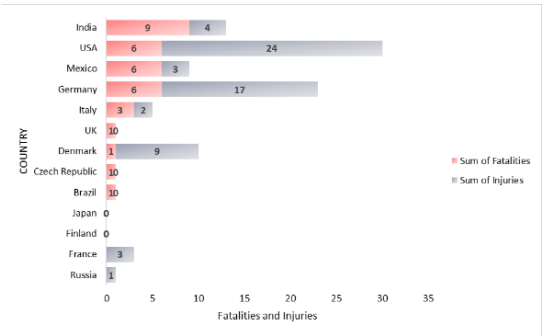


*Figure 15 Distribution of fatalities and injuries with location*

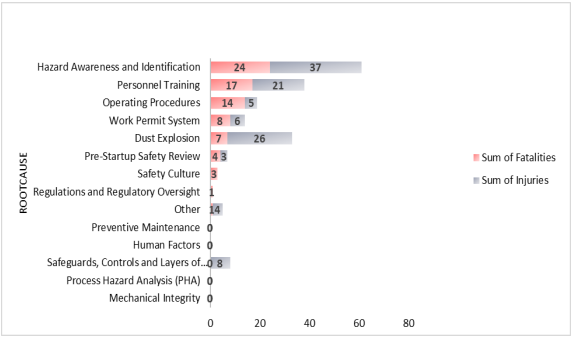


*Figure 16 Distribution of fatalities and injuries with root cause*

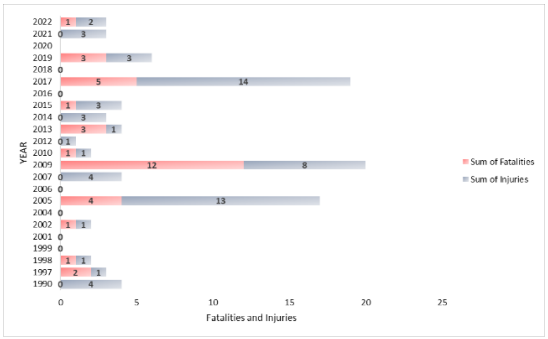


*Figure 17 Distribution of fatalities and injuries with time*

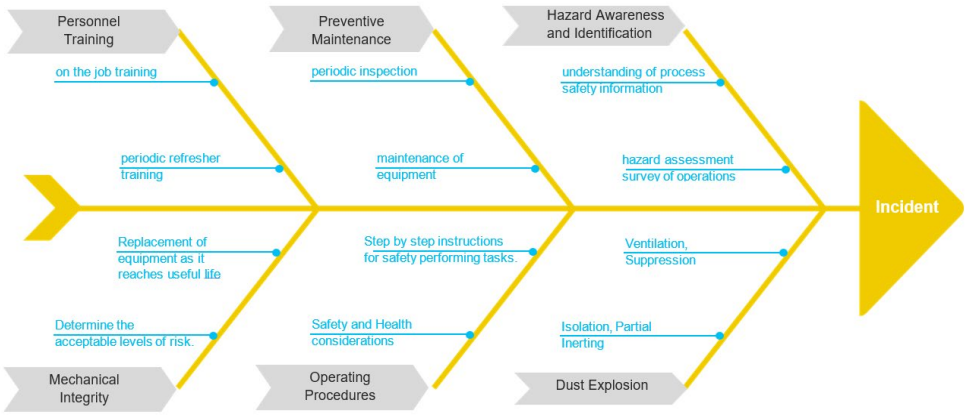


Figure 18 Fishbone diagram of major accident causes in bioprocessing industries.

## Bioprocess Safety Management Practices

The exposure to risks and hazards present in bioprocessing industry operations establishes the rationale for enhancing bioprocess safety. A facility must be well maintained and operated despite being well-designed to prevent accidents. To ensure all safety aspects, receive proper priority, a commitment to safety from all management levels is essential. Various bioprocess safety issues need to be studied during research, development, and manufacturing. Periodic inspections are to be done to ensure industry standards are followed. Emergency plans need to be developed to handle any incident. There are various tools available for identifying bioprocess safety hazards such as Material Safety Data Sheets (MSDS), incident learning, development reports, literature surveys, and laboratory data.

Facility-specific risks should be analyzed based on identifying and understanding bioprocess hazards and the existing facility design. The risks need to be analyzed to have knowledge of bioprocess safety hazards and loss scenarios in the facility these hazard properties might lead to

and to determine if the existing safeguards are sufficient. This assessment should be done at each stage of development, facility design, alteration, or operation.

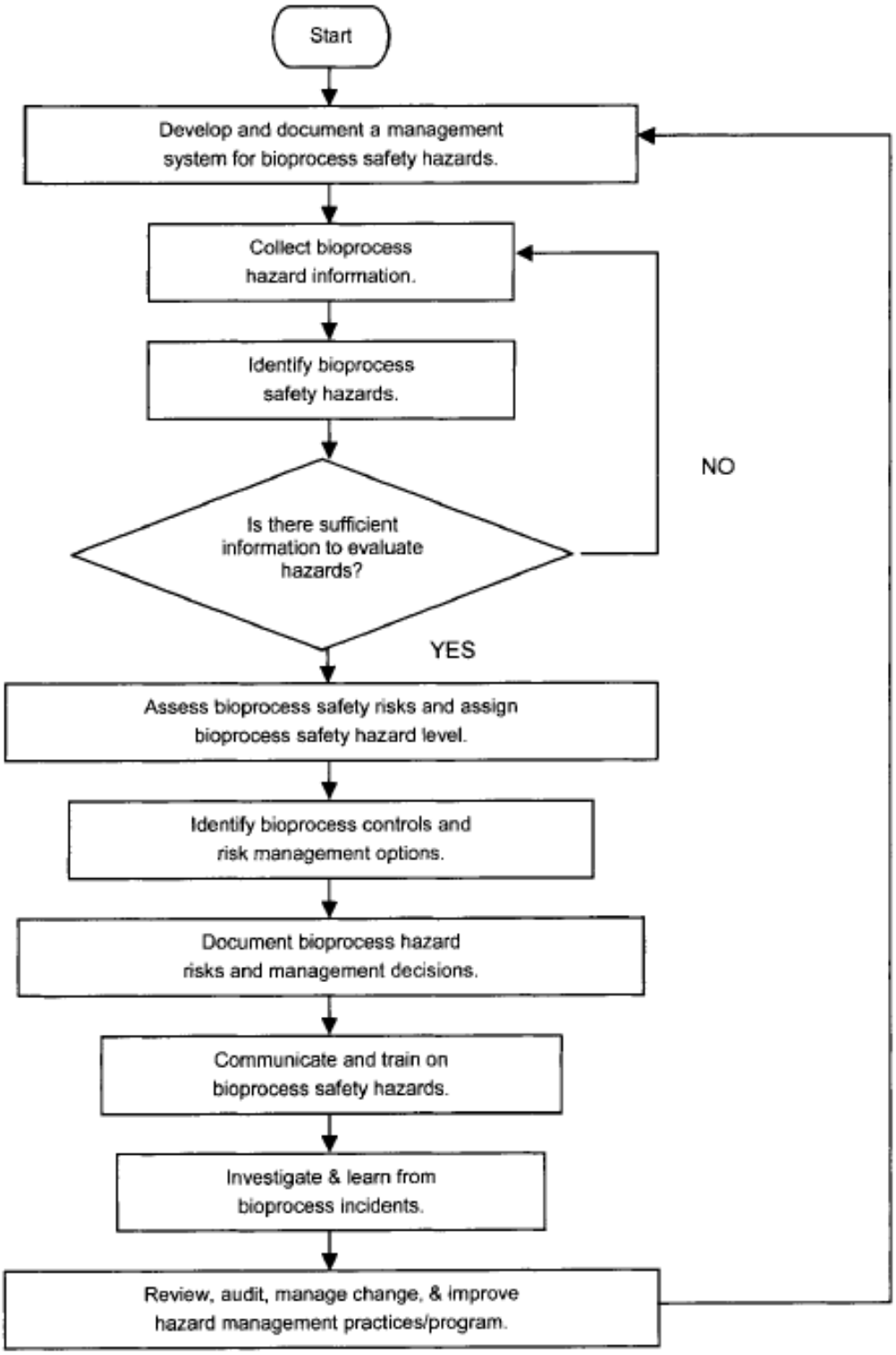


*Figure 19 Bioprocess Hazard Management Implementation Flowchart*[18]

All the documentation needs to be retained or kept up to date during the lifetime of the entire facility. Communication and training are the two most important aspects in the management of hazards. All the employees and the contract workers must receive safety training. All the operating personnel should have a complete idea of what might happen if the process is operated in the wrong way or if certain materials are mixed. The established instructions should be followed by all the personnel operating in the bioprocessing site.

All the near misses and incidents involving bioprocess safety need to be reported and investigated. The root cause should be documented, and corrective action should be taken and documented. All the lessons learnt should be documented and communicated to avoid future accidents. The essential means of improving the process safety management system continuously is to make use of all the below elements:

- Employee input,
- Active monitoring,
- Periodic reviews,
- Management of change,
- Audits or assessments of several types, and
- Keeping abreast of new technology.

A risk-based strategy recognizes that all hazards and risks in a facility are not equal. Using the same type of practice to manage every hazard is inefficient. Allocating resources that focus on higher risks and greater hazards allows a facility to prevent allocating more resources to lower risks, thereby freeing resources with tasks with higher risk exposure. While developing or revising

any process, the best approach to establish bioprocess safety is to involve the stakeholders in each step.

## Conclusion

The bioprocessing industry is experiencing fast growth worldwide. Companies continue to shift to biological processes or develop novel bioproducts. The increase in bioprocess facilities also brings a variety of new hazards related to the process and production. The number of accidents occurring in this industry is also growing fast. Several lessons should be learnt from these accidents. A proper accident reporting system should be maintained. Most of the accidents are caused by Hazard Awareness and Identification, and Dust Explosions. This should be improved by incorporating process safety measures followed in other industries and by following a proper process safety management system.

## Steps Forward

References:


1. Webinar replay - bioprocessing: Applications and recent advances, YouTube. (2020). https://www.youtube.com/watch?v=r89JRbMEHec (accessed February 15, 2023).
2. M.L. Shuler, F. Kargi, Bioprocess Engineering Basic Concepts., Addison Wesley Longman Ltd, 2013 (accessed February 15, 2023)..
3. Author links open overlay panelD. Cossar, AbstractThe inherent variability of living organisms is a particular challenge for bioprocessing where the native phenotype of a microorganism is subverted to manufacture value-added products. The elements of a generalized production process are presented, Bioprocessing techniques, Comprehensive Biotechnology (Second Edition). (2011) (accessed February 15, 2023).. https://www.sciencedirect.com/science/article/pii/B9780080885049000787
4. Bhawya, Sowntharya, Batch and continuous fermentation process, EasyBiologyClass. (2023). https://www.easybiologyclass.com/industrial-fermentation-process-batch-fed-batch-and-continuous-fermentation/ (accessed February 15, 2023).
5. T. Allman, The difference between batch, fed-batch and continuous processes, Blog. (2021). https://www.infors-ht.com/en/blog/the-difference-between-batch-fed-batch-and-continuous-processes/ (accessed February 15, 2023).
6. G.D.R.and A.P.J.T. David Moore, 21st century guidebook to Fungi, second edition, by David Moore, Geoffrey D. Robson and Anthony P. J. Trinci, 17.10 Beyond the Batch Culture. (2019). http://www.davidmoore.org.uk/21st_century_guidebook_to_fungi_platinum/Ch17_10.htm (accessed February 15, 2023).
7. Biodiesel production and distribution, Alternative Fuels Data Center: Biodiesel Production and Distribution. (n.d.). https://afdc.energy.gov/fuels/biodiesel_production.html#:~:text=Biodiesel%20is%20produced%20from%20vegetable,and%20glycerin%20(a%20coproduct). (accessed March 1, 2023). (accessed February 15, 2023).
8. I. Edeh, Bioethanol production: An overview, IntechOpen. (2020). https://www.intechopen.com/chapters/74319 (accessed March 1, 2023).
9. (PDF) advances in the development of Bioethanol: A review - researchgate, (n.d.). https://www.researchgate.net/publication/221914252_Advances_in_the_Development_of_Bioethanol_A_Review (accessed March 1, 2023).
10. Biopharmaceuticals from microorganisms: From production to purification, (n.d.). https://www.researchgate.net/publication/309470086_Biopharmaceuticals_from_microorganisms_from_production_to_purification (accessed April 8, 2023).
11. The brewery process - from milling -> distribution all about microbrewery: Follow microbrewery is easy on...: Beer Brewing process, beer making process, Beer Brewing, Pinterest. (2017). https://in.pinterest.com/pin/409405422362805740/ (accessed April 8, 2023).
12. Author links open overlay panelRéka Czinkóczky, Highlights•Fermentation data for the two leading biosurfactant fermentations of Bacilli.•Techno-economic assessment to overcome the problem of high capital requirements.•The comparison of 4 scenarios.•The usefulness of equipment sharing is demonstrated.•S, AbstractThe useful products like enzymes and biosurfactants render Bacilli good candidates for bioprocesses. Despite the

lack of the few reports on Bacilli based technologies, Techno-economic assessment of bacillus fermentation to produce surfactin and lichenysin, Biochemical Engineering Journal. (2020). https://www.sciencedirect.com/science/article/pii/S1369703X20302734 (accessed April 8, 2023).
13. Author links open overlay panelYichen Wang a, a, b, c, AbstractProcess safety incidents can result in injuries, M.S. Mannan, et al., Factors contributing to US chemical plant process safety incidents from 2010 to 2020, Journal of Loss Prevention in the Process Industries. (2021). https://www.sciencedirect.com/science/article/abs/pii/S0950423021001212 (accessed April 8, 2023).
14. About the CSB, CSB. (n.d.). https://www.csb.gov/ (accessed April 8, 2023).
15. M.A.H.B.I.C.T. Team, 2-factor authentication, EUROPA. (n.d.). https://emars.jrc.ec.europa.eu/en/emars/content (accessed April 8, 2023).
16. Abnormal Situation Management® (ASM) Consortium. (n.d.). https://www.eemua.org/Glossary/A/Abnormal-SMC.aspx (accessed April 8, 2023).
17. Department of Labor Logo United Statesdepartment of Labor, Home | Occupational Safety and Health Administration. (n.d.). https://www.osha.gov/ (accessed April 8, 2023).
18. Guidelines for process safety in Bioprocess Manufacturing Facilities, Wiley, Hoboken, 2011.
19. Author links open overlay panelValeria Casson Moreno, Highlights•A database of 169 accidents involving biogas was built and analysed.•Gas releases, AbstractIn the last 10 years biogas production tripled, Analysis of accidents in biogas production and upgrading, Renewable Energy. (2015). https://www.sciencedirect.com/science/article/abs/pii/S0960148115303700?via%3Dihub (accessed April 8, 2023).
20. I.T.V.N. Desk, Two persons killed in Maharashtra biogas explosion, English. (2013). https://www.indiatvnews.com/news/india/two-persons-killed-in-maharashtra-biogas-explosion-19667.html (accessed April 8, 2023).
21. Case Details > Spontaneous Ignition of Meat and Bone Meal under Storage Caused Due to Fermentation with Rain Water. (n.d.). https://www.shippai.org/fkd/en/cfen/CC1200015.html (accessed April 8, 2023).
22. Vaurio- ja Onnettomuusrekisteri Varo, VARO. (n.d.). https://varo.tukes.fi/ExtranetHome/Incident/4146 (accessed April 8, 2023).
23. Vahid.ebadat, Explosions associated with ethanol production, Stonehouse Process Safety. (2022). https://stonehousesafety.com/explosions-associated-with-ethanol-production/ (accessed April 8, 2023).
24. R. Keefe, Two employees injured when fire ignites in grain dryer at ethanol plant, Dust Safety Science. (2022). https://dustsafetyscience.com/dryer-fire-south-bend-indiana/ (accessed April 8, 2023).
25. C. Cloney, Dryer fire reported at ethanol plant in South Bend indiana, Dust Safety Science. (2018). https://dustsafetyscience.com/grain-dryer-fire-south-bend-indiana/ (accessed April 8, 2023).
26. R. Keefe, Explosion at Illinois ethanol plant causes grain silos to collapse, Dust Safety Science. (2022). https://dustsafetyscience.com/ethanol-plant-explosion-peoria-illinois/ (accessed April 8, 2023).

27. R. Keefe, Firefighters investigate explosion and fire at ethanol production plant, Dust Safety Science. (2022). https://dustsafetyscience.com/grain-dryer-explosion-lawlor-iowa/ (accessed April 8, 2023).
28. Didion Milling Company explosion and fire, CSB. (n.d.). https://www.csb.gov/didion-milling-company-explosion-and-fire-/ (accessed April 8, 2023).
29. Journal of the American Chemical Society, (n.d.). https://pubs.acs.org/journal/jacsat (accessed April 8, 2023).
30. 2019 Bagish Jha / TNN / Updated: Jun 11, Company director, 2 others booked for Boiler Blast, are 'untraceable': Gurgaon News - Times of India, The Times of India. (n.d.). https://timesofindia.indiatimes.com/city/gurgaon/company-director-2-others-booked-for-boiler-blast-are-untraceable/articleshow/69732310.cms (accessed April 8, 2023).
31. E. Interact, Infosis / as-er / detail, Infosis. (n.d.). https://www.infosis.uba.de/index.php/en/site/13947/aser/index/1316.html (accessed April 8, 2023).
32. iDNES.cz, Mu, IDNES.cz. (2013). https://www.idnes.cz/plzen/zpravy/mrtvy-muz-v-chrici-na-plzensku.A131110_170721_plzen-zpravy_maq/tisk (accessed April 8, 2023).
33. Vaurio- ja Onnettomuusrekisteri Varo, VARO. (n.d.). https://varo.tukes.fi/ExtranetHome/Incident/4469 (accessed April 8, 2023).
34. R. Keefe, Grain dryer significantly damaged in dust explosion at ethanol plant, Dust Safety Science. (2022). https://dustsafetyscience.com/grain-dryer-explosion-coshocton-ohio/ (accessed April 8, 2023).
35. Natural gas + ethanol = explosion, Natural Gas + Ethanol = Explosion | Energy Justice Network. (n.d.). http://www.energyjustice.net/content/natural-gas-ethanol-explosion (accessed April 9, 2023).
36. /author/veselina-petrova-18/, Green Plains Ethanol plant fire caused by pump failure, Renewablesnow.com. (n.d.). https://renewablesnow.com/news/green-plains-ethanol-plant-fire-caused-by-pump-failure-409393/ (accessed April 9, 2023).
37. Biomass accident investigations – missed opportunities for learning and ..., (n.d.). https://www.researchgate.net/publication/319292319_Biomass_accident_investigations_-_missed_opportunities_for_learning_and_accident_prevention (accessed April 9, 2023).
38. C. Cloney, One killed and one injured in grain elevator explosion in South Sioux City, Nebraska, Dust Safety Science. (2019). https://dustsafetyscience.com/grain-elevator-explosion-sioux-city-nebraska/ (accessed April 9, 2023).
39. E. Palmer, Amgen facility has its second explosion in 9 months, Fierce Pharma. (2014). https://www.fiercepharma.com/supply-chain/amgen-facility-has-its-second-explosion-9-months (accessed April 9, 2023).
40. Adobe Acrobat: PDF Edit, convert, sign tools, Google. (n.d.). https://chrome.google.com/webstore/detail/adobe-acrobat-pdf-edit-co/efaidnbmnnnibpcajpcglclefindmkaj?hl=en-GB (accessed April 10, 2023).
41. Towards an inherently safer bioprocessing industry - icheme.org, (n.d.). https://www.icheme.org/media/16974/hazards-28-paper-68.pdf (accessed April 10, 2023).
42. Biogas and corrosion, La Référence Du Retour D'expérience Sur Accidents Technologiques. (2018). https://www.aria.developpement-durable.gouv.fr/flash/aria-news-flash/biogas-and-corrosion/?lang=en (accessed April 10, 2023).

43. Aria, La Référence Du Retour D'expérience Sur Accidents Technologiques. (2021). https://www.aria.developpement-durable.gouv.fr/ (accessed April 10, 2023).
44. Fire in a non-hazardous waste landfill, La Référence Du Retour D'expérience Sur Accidents Technologiques. (-1). https://www.aria.developpement-durable.gouv.fr/accident/45976_en/?lang=en (accessed April 10, 2023).
45. Biogas release, La Référence Du Retour D'expérience Sur Accidents Technologiques. (-1). https://www.aria.developpement-durable.gouv.fr/accident/38485_en/?lang=en (accessed April 10, 2023).
46. Dust explosion in a dryer, La Référence Du Retour D'expérience Sur Accidents Technologiques. (-1). https://www.aria.developpement-durable.gouv.fr/accident/34319_en/?lang=en (accessed April 10, 2023).
47. Aria - la référence du retour d'expérience sur accidents technologiques, (n.d.). https://www.aria.developpement-durable.gouv.fr/ (accessed April 10, 2023).
48. Aria - la référence du retour d'expérience sur accidents technologiques, (n.d.). https://www.aria.developpement-durable.gouv.fr/ (accessed April 10, 2023).
49. R. Keefe, One worker killed in grain silo explosion at Brazilian warehouse, Dust Safety Science. (2022). https://dustsafetyscience.com/grain-warehouse-explosion-sorriso-brazil/ (accessed April 10, 2023).
50. H. Regan, Explosion and fire break out at Russian lab known for housing deadly smallpox virus, CNN. (2019). https://www.cnn.com/2019/09/17/health/russia-lab-explosion-smallpox-intl-hnk/index.html (accessed April 10, 2023).
51. R. Keefe, Dust explosion affects grain elevator at Agricultural Cooperative, Dust Safety Science. (2022). https://dustsafetyscience.com/grain-elevator-explosion-sawyer-kansas/ (accessed April 10, 2023).
52. R. Keefe, Dust explosion occurs at biomass thermal power plant in Germany, Dust Safety Science. (2022). https://dustsafetyscience.com/dust-explosion-sinsheim-germany/ (accessed April 10, 2023).
53. L. Cordeiro, Silo com várias toneladas de milho estoura no nortão, Só Notícias. (2021). https://www.sonoticias.com.br/geral/silo-com-milho-estoura-e-fica-destruido-no-nortao/ (accessed April 10, 2023).
54. R. Keefe, Explosion at Idaho Grain Silo leaves one person seriously injured, Dust Safety Science. (2021). https://dustsafetyscience.com/dust-explosion-mountain-home-idaho/ (accessed April 10, 2023).
55. Biogas leak in a treatment plant, La Référence Du Retour D'expérience Sur Accidents Technologiques. (-1). https://www.aria.developpement-durable.gouv.fr/accident/47989_en/?lang=en (accessed April 10, 2023).

**Appendix 1 Dataset considered for the analysis**

| S.No. | Date of Incident | Location | Type of Industry | Fatalities | Injuries | Root Cause | References |
|---|---|---|---|---|---|---|---|
| 1 | 2016 | France | Biogas | 0 | 0 | Mechanical Integrity, Preventive Maintenance | 55 |
| 2 | 2013 | India | Biogas | 2 | 1 | Operating Procedures, Work Permit Syste, Hazard Awareness and Identification m | 20 |
| 3 | 2009 | Mexico | Waste Treatment | 6 | 3 | Personnel Training, Operating Procedures, Hazard Awareness and Identification | 19 |
| 4 | 2009 | India | Biogas | 4 | 3 | Pre-Startup Safety Review, Work Permit System, Hazard Awareness and Identification | 19 |
| 5 | 2007 | Germany | Biogas | 0 | 2 | Hazard Awareness and Identification, Personnel Training | 19 |

| S.No. | Date of Incident | Location | Type of Industry | Fatalities | Injuries | Root Cause | References |
|---|---|---|---|---|---|---|---|
| 6 | 2006 | Germany | Biogas | 0 | 0 | Mechanical Integrity | 19 |
| 7 | 2005 | Germany | Waste Treatment | 4 | 13 | Personnel Training, Hazard Awareness and Identification | 19 |
| 8 | 2004 | USA | Biogas | 0 | 0 | Hazard Awareness and Identification, Safeguards, Controls and Layers of Protection | 19 |
| 9 | 2001 | Japan | Other | 0 | 0 | Safety Culture, Hazard Awareness and Identification | 21 |
| 10 | 2009 | UK | Biogas | 1 | 0 | Operating Procedures, Hazard Awareness and Identification, Personnel Training | 19 |

| S.No. | Date of Incident | Location | Type of Industry | Fatalities | Injuries | Root Cause | References |
|---|---|---|---|---|---|---|---|
| 11 | 1998 | Italy | Biogas | 1 | 1 | Operating Procedures, Hazard Awareness and Identification, Personnel Training | 19 |
| 12 | 2006 | Finland | Other | 0 | 0 | Hazard Awareness and Identification | 22 |
| 13 | 2019 | USA | Ethanol Production | 0 | 2 | Dust Explosion | 23 |
| 14 | 2018 | USA | Ethanol Production | 0 | 0 | Dust Explosion | 25 |
| 15 | 2021 | USA | Ethanol Production | 0 | 2 | Dust Explosion | 24 |
| 16 | 2022 | USA | Ethanol Production | 0 | 2 | Dust Explosion | 26 |
| 17 | 2022 | USA | Ethanol Production | 0 | 0 | Dust Explosion | 27 |
| 18 | 2017 | USA | Food Products Manufacturing | 5 | 14 | Dust Explosion | 28 |
| 19 | 2015 | Denmark | Waste Treatment | 0 | 2 | Hazard Awareness and Identification, Personnel Training | 29 |

| S.No | Date of Incident | Location | Type of Industry | Fatalities | Injuries | Root Cause | References |
|---|---|---|---|---|---|---|---|
| 20 | 2019 | India | Biopharmaceuticals | 3 | 0 | Safety Culture, Operating Procedures, Personnel Training | 30 |
| 21 | 2002 | Germany | Waste Treatment | 1 | 0 | Operating Procedures, Personnel Training, Hazard Awareness and Identification | 31 |
| 22 | 1997 | Italy | Biogas | 2 | 1 | Work Permit System | 19 |
| 23 | 2013 | Czech Republic | Biogas | 1 | 0 | Hazard Awareness and Identification, Personnel Training | 32 |
| 24 | 1999 | France | Wood Pellet Production. | 0 | 0 | Dust Explosion, Process Hazard Analysis (PHA) | 19 |
| 25 | 2007 | France | Biodiesel | 0 | 0 | Operating Procedures | 33 |
| 26 | 2022 | USA | Ethanol Production | 0 | 0 | Dust Explosion | 34 |
| 27 | 2014 | USA | Ethanol Production | 0 | 0 | Dust Explosion | 35 |

| S.No. | Date of Incident | Location | Type of Industry | Fatalities | Injuries | Root Cause | References |
|---|---|---|---|---|---|---|---|
| 28 | 2014 | USA | Ethanol Production | 0 | 0 | Preventive Maintenance, Mechanical Integrity | 36 |
| 29 | 1990 | Denmark | Waste Treatment | 0 | 4 | Safeguards, Controls and Layers of Protection, Hazard Awareness and Identification | 37 |
| 30 | 2005 | Denmark | Biogas | 0 | 0 | Work Permit System, Hazard Awareness and Identification | 37 |
| 31 | 2002 | Denmark | Wood Pellet Production. | 0 | 1 | Dust Explosion, Hazard Awareness and Identification | 37 |
| 32 | 2010 | Denmark | Wood Pellet Production. | 1 | 1 | Dust Explosion, Hazard Awareness and Identification | 37 |

| S.No. | Date of Incident | Location | Type of Industry | Fatalities | Injuries | Root Cause | References |
|---|---|---|---|---|---|---|---|
| 33 | 2012 | Denmark | Other | 0 | 1 | Dust Explosion, Hazard Awareness and Identification | 37 |
| 34 | 2009 | Germany | Biogas | 1 | 2 | Other | 38 |
| 35 | 2014 | USA | Biopharmaceuticals | 0 | 2 | Hazard Awareness and Identification | 39 |
| 36 | 2015 | USA | Ethanol Production | 1 | 1 | Hazard Awareness and Identification | 41 |
| 37 | 2013 | France | Waste Treatment | 0 | 0 | Other | 40 |
| 38 | 2012 | France | Other | 0 | 0 | Preventive Maintenance | 42 |
| 39 | 2013 | France | Waste Treatment | 0 | 0 | Preventive Maintenance | 42 |
| 40 | 2019 | France | Biogas | 0 | 0 | Preventive Maintenance | 43 |
| 41 | 2019 | France | Waste Treatment | | | Mechanical Integrity, Preventive Maintenance | 43 |

| S.No. | Date of Incident | Location | Type of Industry | Fatalities | Injuries | Root Cause | References |
|---|---|---|---|---|---|---|---|
| 50 | 2019 | Russia | Research | 0 | 1 | Other | 50 |
| 51 | 2022 | USA | Grain Processing | 0 | 0 | Dust Explosion | 51 |
| 52 | 2022 | Germany | Biogas | 0 | 0 | Dust Explosion | 52 |
| 53 | 2021 | Brazil | Grain Processing | 0 | 0 | Dust Explosion | 53 |
| 54 | 2021 | USA | Grain Processing | 0 | 1 | Work Permit System, Work Permit System, Hazard Awareness and Identification | 54 |

**Appendix 2 Some industrial fermentation processes[1]**

| Product | Microorganism |
|---|---|
| Citric Acid | A. niger |
| Ethanol | S. cerevisae |
| Glutamate | C. glutamicum |
| Lactic Acid | Lactobacillus sp. |
| Lysine | C. glutamicum |

**Appendix 3 Major applications of bioprocessing industry[1]**

| Period | Application |
|---|---|
| Pre-1940s | • Baker's Yeast<br>• Solvents (methanol, ethanol, propanol, butanol etc.)<br>• Organic acids (citric, lactic, gluconic, itaconic etc.)<br>• Amino acids<br>• Beverage |
| Pre-1980s | • Probiotics<br>• Antibiotics<br>• Enzymes<br>• Biopolymers<br>• Vaccine<br>• Biosurfactants |
| Post-1980s | • Biopharmaceuticals<br>• Recombinant Proteins (somatostatin, Interferon-α, Interferon -β, Coagulation factor VII, Coagulation factor IX etc.)<br>• Mab (Monoclonal antibodies) trastuzumab (Herceptin), pertuzumab (Perjeta), bevacizumab (Avastin), rituximab (Mabthera) etc.)<br>• Plant Bioactive compounds (carotenoids, flavonoids, carnitine, choline, coenzyme Q, dithiolthiones, phytosterols, phytoestrogens, glucosinolates, polyphenols, and taurine 6+ etc.)<br>• Biodiesels<br>• Lab-grown meat<br>• Agriculture |