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Hazop Analysis of a Bioprocess for Polyhydroxyalkanoate (PHA) Production from Organic Waste: Part A

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Abstract: the number of bioprocesses for the circular economy of organic waste has grown in recent years. Implementation of new processes and technologies should consider occupational health and safety issues from the initial design stages. Among the process hazards analysis techniques, HAZard and OPerability (HAZOP) methodology is widely used for studying both the process's hazards and their operability problems, by exploring the effects of any deviations from design conditions. In the present study, a modified version of HAZOP methodology has been applied to a three-steps process developed at pilot scale in the Treviso municipal wastewater treatment plant in order to produce polyhydroxyalkanoate (PHA) as the final high value product. This paper shows the results of HAZOP analysis applied to the first process step (acidogenic fermentation) aimed at volatile fatty acids production. The analysis has been applied to the process conditions corresponding to the maximum PHA content in the biomass. The HAZOP study results showed that this methodology allowed a comprehensive exploration of conventional chemical engineering process hazards and biological hazards. Final piping and instrumentation diagrams (P&IDs) for acidogenic fermentation have been designed, identifying all prevention measures aimed at managing the hazard and operability issues. The P&ID shows the interconnection of equipment and the instrumentation required for controlling the process.

Keywords: bioprocess; polyhydroxyalkanoates; HAZOP analysis; occupational health and safety; operability; P&ID; deviation; cause; consequence.

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1. Introduction

Industrial biological processes are widely used in the chemical industry, ranging from pharmaceutical to food or energy production. The growth of bioprocess facilities is generating interest in the field of occupational health and safety (OH&S) risks. In the framework of circular economy strategy, biomass is increasingly used for the production of energy and high value products. However, the assessment of potential OH&S issues is still limited. The biomass could involve potential exposures to biological and chemical agents, which could be released in the workplace during the industrial handling [1–3]. In particular, the biofuel (bioethanol, biogas, biomethane, etc.) production is characterized by hazards, such as the formation of potentially explosive atmospheres and pool fire.

These accidental releases can also derive from failures of process plants components (tanks, flanges, valves, pumps, compressors, etc.) [4].

INAIL research activity in the field of industrial biotech processes is focused on assessing and controlling the occupational risks related to the biomass use for the production of sustainable bioproducts and biofuels [5–8]. There are no specific techniques for hazard identification in bioprocesses, especially addressed to process safety. The conventional hazard identification techniques may often overlook the specific issues posed by biological reactions [9]. In the last years, specific checklists have been created, tested and tuned on real case studies to screen the possible criticalities related to bioprocesses. In the framework of occupational health and safety, a tailored methodology for the hazard identification (biological and chemical hazards) has been applied to the anaerobic digestion of animal manure for biogas production [10].

Among the process hazard analysis techniques, the hazard and operability (HAZOP) methodology is diffusely used for studying both the process's hazards and its operability problems by exploring the effects of any deviations from design conditions.

A HAZOP study is a highly disciplined procedure aimed at identifying how a process may deviate from its design intention. This method is able to distinguish between hazard (any operation that could cause a catastrophic release or that could result in harm to workers) and operability (any operation within the project that could cause a plant shutdown with possible impact on safety or profits). It is defined as the application of a formal, systematic and critical examination of the process and the engineering intentions of new or existing facilities to assess the outcomes of possible operating failures of individual equipment pieces and the consequential effects on the facility [11]. The HAZOP safety-analysis technique is applied worldwide and recognized by legislation, since it has demonstrated its effectiveness in identifying environmental, safety and health hazards. The HAZOP methodology was developed in the 1970s, when the growing size of chemical plants made a preventive approach necessary. In subsequent years, this methodology was applied to pharmaceutical [12], nuclear [13] and transportation [14] sectors and in continuous chemical processes [15,16].

HAZOP has been widely applied in combination with other analysis techniques [17,18] and efforts have been made for automating the analysis [19–21].

It is applicable to all types of installations at any stage of their life, particularly for new installations, where the operational experience is lacking. This analysis highlights the risks and weaknesses in a systematic way. It is particularly important for complex installations, at different stages of their life:

- During the design phase in order to check that safety objectives are correctly met;
- Before start-up in order to verify the adequacy of operational and emergency procedures;
- During the operation in order to assess the impact on safety of maintenance operations or any modification to the installation.

Among others, the reasons for HAZOP analysis may be:

(1) To verify the project safety;
(2) To check operating and safety procedures;
(3) To increase the safety of an existing system;
(4) To verify that safety equipment is working in the best possible way.

The study result may lead to a project revision and/or check through the following actions:

- Operating procedures development;
- Verification of design values, process parameters and possible modifications;
- Request for additional alarms;
- Request for unforeseen alarms or blocks;
- Useful information for assessing and managing the risk associated with the accidental identified events.

The HAZOP methodology, applied to the present case study, is based on a specific layered approach, which allows us to identify the industrial bioprocess hazards. It consists of two levels: a checklist and a HAZOP analysis, modified to consider both engineering and biotechnological aspects, and their interactions (BioHazOp). The tool goal is the integration of biotechological aspects and typical chemical processes hazards, and the identification of their cause–consequence relations.

In the paper, the modified version of HAZOP methodology has been applied to a three-steps process aimed at producing polyhydroxyalkanoate (PHA) as final high value product. The process has been developed at pilot scale in Treviso municipal wastewater treatment plant (WWTP) [22]. In more detail, the PHA production by mixed microbial culture (MMC), using pretreated organic fraction of municipal solid waste (OFMSW) and secondary sludge (SS), applies the feast−famine approach in the traditional three-steps process scheme. The analysis has been focused on the process conditions corresponding to the maximum PHA content in the biomass [23]. The HAZOP methodology output has been used to draw a piping and instrumentation diagram (P&ID), which shows the interconnection of equipment and the instrumentation required for controlling the process. The P&ID drawing is a fundamental tool for the scaling-up from pilot to industrial plant.

The paper is focused on the results of BioHazop analysis applied to the first step (acidogenic fermentation) of the PHA production process. The results of BioHazOp analysis, applied to the second step of the PHA production process, are reported in another paper (*Hazop analysis of a bioprocess for polyhydroxyalkanoate (PHA) production from organic waste: part B*) in the same Special Issue.

2. Materials and Methods

2.1. HAZOP Analysis

The HAZOP method is based on the strength in process flow diagrams (PFDs) and P&ID, breaking the design into manageable sections with definite boundaries called nodes, so ensuring the analysis of each piece of process equipment. A small multi-disciplinary team undertakes the analysis, whose members should have sufficient experience and knowledge to answer most questions. The team members should be expert in the most relevant areas for plant operation.

The method relies on the use of guidewords (such as no, more, less) combined with process parameters (e.g., temperature, flow, pressure), which aim at revealing deviations (such as less flow, higher temperature) from the process intention or normal operation. This procedure is applied to a particular node as part of the system characterized by nominal intention of the operative parameters. After the deviations have been determined, the experts team explores their feasible causes and their possible consequences. For every cause–consequence pair, safeguards, which could prevent, detect, control or mitigate the hazardous situation, must be identified. Finally, if the safeguards are insufficient to solve the problem, additional prevention and protection measures and recommendations must be considered [11].

The HAZOP analysis objectives should be made as clear as possible; in general, the goal is to identify risks and operational failures.

The BioHazOp analysis, applied to the case-study, has been split into five stages:

1. Definition of the purpose and objectives of the study;
2. Team selection;
3. Study preparation;
4. Carrying out the analysis;
5. Recording the results.

2.2. HAZOP Methodology

The basic requirements of the HAZOP and BioHazOp analysis are:

- The examination must be systematic;
- The analysis must be carried out with a degree of formality (forms, etc.), so that the reasons for each decision made during the analysis can be clearly identified by different people at different times;
- All working on the implementation of the project must be involved in the analysis.

In biotech processes as well as in traditional chemical ones, the hazard identification plays a critical role, since all unidentified hazards could generate uncontrolled risks.

Based on the plant documentation (diagrams, cause-effect matrices, data sheets, etc.), all lines/items are analyzed through the iterative process shown in Figure 1.

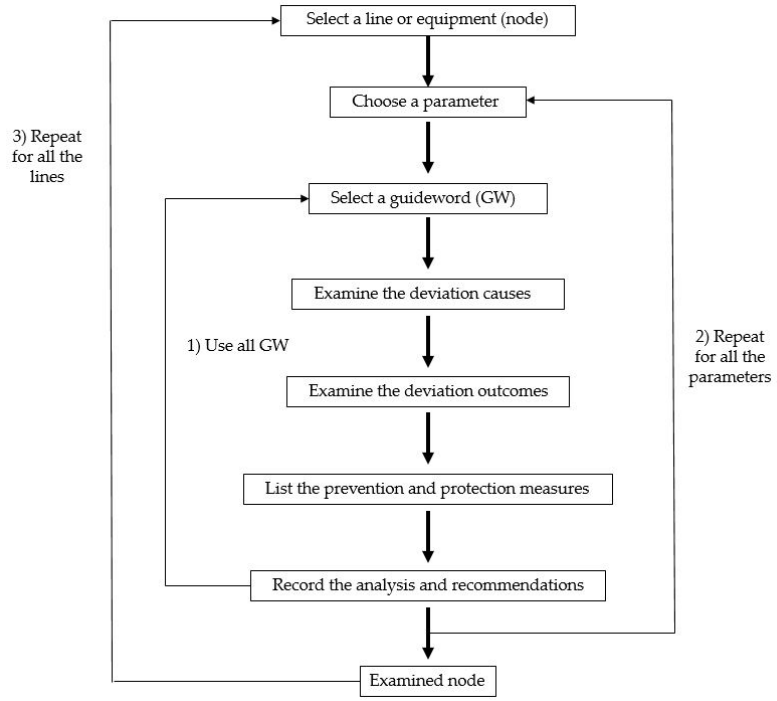

Figure 1. HAZOP flowsheet.

Both chemical and bioprocesses hazards can be simultaneously analyzed by an integrated procedure [24].

In the case study, two specific tools were used:

1. A checklist;
2. An ad hoc HAZOP method (BioHazOp).

It should be highlighted that residual risk has to be monitored and periodically reviewed, in the framework of a dynamic process updated to the knowledge of hazards and the early available warnings [25,26].

2.2.1. The Checklist

The first step of applied methodology consists of a checklist designed to identify criticalities issues associated with the engineering and biotechnological aspects of the process. The checklist is an input tool for the BioHazOp analysis; it is aimed at identifying the role of the process parameters. Its main outcome is the creation of a list of fundamental parameters of bioprocess under analysis.

The proposed checklist consists of two sections:

1. Process specification section (substances hazard classification, biohazard, flammability, explosivity, relevant parameters, etc.);
2. General section (management outline): operating procedures, plant layout, emergency response/on-going programs and process hazard analysis.

The checklist has been designed on the Directive 2000/54/EC [27] and Regulation EC N° 1272/2008-CLP Regulation [28] in order to ensure that the process is complied with the European legislation and standards.

2.2.2. BioHazOp

The second layer of the methodology is based on a modified HAZOP procedure. Deviations from design intent could induce consequences which are in some way novel with respect to conventional chemical processes. The relationship between causes and consequences (bio and not) requires a specific investigation. In common with HAZOP, the analysis is based on the knowledge of the process parameters and the plant operations. Even in this case, an interdisciplinary team is required, and a leader guides the analysis of the relationship between guidewords and operating parameters of the node under analysis in order to identify the deviations.

In BioHazOp, no new guidewords were necessary since the approach of conventional HAZOP studies allowed the satisfactory performance of the analysis.

In the present study, the team prepared the relevant deviations matrices (RDMs) as a result of the brainstorming sessions. The RDM combines a standard set of guidewords with the identified process parameters for the node, which has to be analyzed. This matrix consists of a list of the possible combinations of guidewords and process parameters to be used in the BioHazOp study.

Table 1 shows the BioHazOp flowsheet scheme. For each node the deviations from normal operating condition are initially analyzed from an engineering standpoint, looking for causes, consequences and countermeasures. The biotechnological process aspects are successively taken into account, highlighting causes and outcomes related to the microbial consortia and their behavior (they are indicated as "biocauses" and "bioconsequences").

Table 1. BioHazOp worksheet scheme.

Process Unit				
Engineering process causes consequences	Biotechnology process causes consequences	Existing counter measures	Biohazard	Proposed counter measures
process parameter				
deviation 1				
deviation 2				
deviation n				

The flowsheet supports the discussion on the causes and consequences of biotech process deviations. It should be highlighted that a biocause can also induce a consequence on bioprocess parameter variations and vice versa. The BioHazOp approach leads the multidisciplinary team to a more in-depth analysis of the relationship between bio and not-bio parameters, raising more questions than traditional HAZOP analysis. In the study, the presence of biohazards as a consequence of each specific deviation has been assessed. This requirement comes through the introduction of a specific column in the BioHazOp worksheet (Table 1). The column content goal is to highlight the presence of specific biosafety problems, depending on the risk group of the organism involved in the process unit, classified according to the Directive 2000/54/EC [27].

2.3. Piping and Instrumentation Diagram (P&ID)

The piping and instrumentation diagram is an articulate plant drawing, which includes the piping and process equipment with its instrumentation and control systems. In particular, it shows how process equipment is connected and represents flows directions, safety and control systems, pressure ratings and instrumentation details by the specific

symbols use. The symbols must be simple and easy to remember, while, at the same time, clearly depicting the represented equipment function. A P&ID should generally include:

- Mechanical equipment (vessel, tanks, pumps, etc.);
- Valves and their identification;
- Process piping;
- Flows directions;
- Vents;
- Drains;
- Physical sequence of equipment;
- Interconnections;
- Instrumentation (level, temperature and pressure transmitters, etc.).

Plants P&IDs are developed by process design engineers and are followed by instrumentation and piping engineers. The P&ID is also used for assistance for construction of the corresponding plant and for its correct operating. The P&ID is normally developed from a process flow diagram, which captures the basic flows at the plant design stage.

Modern software for creating a P&ID typically offers wide symbols libraries and is able to automatically check the entire design in order to avoid errors during the drafting process. The latest P&ID systems generate a detailed list of problem areas and highlight them on the piping and instrumentation diagram. It follows that the designer can make corrections in a quick and easy way and prevent the issues from affecting subsequent project phases. Furthermore, P&ID plays a significant role in the maintenance and modification of the process described. During the design stage, the diagram also provides the basis for the safety systems development. In the case study, the software M4 P&ID FX has been used to represent the process.

3. Case-Study: Pilot Plant for the PHA Production

The BioHazOp analysis has been applied to a pilot plant for the PHA production from municipal organic waste. This plant is located at the municipal wastewater treatment plant (WWTP) of Treviso.

The three-stages process consists of :

1. Acidogenic fermentation of the organic feedstock for volatile fatty acids (VFA) production;
2. PHA-storing microorganisms' selection from MMC;
3. PHA accumulation maximization.

Previous studies demonstrated that the polymer composition changed with respect to the used substrate, feedstock composition, feeding strategies and strategy adopted for the accumulation performance, including the important role which is played by environmental factors (temperature, pH, dissolved oxygen, byproduct inhibition and nutrient concentrations) [12]. The parameters, which can influence the selection performances, are the solid retention time (SRT), the hydraulic retention time (HRT), the cycle length (CL), the organic loading rate (OLR) and the dissolved oxygen (DO). Different temperatures and feedstock compositions were initially tested, as well as the effect of thermal hydrolysis. The mesophilic fermentation (37 °C) on thermally hydrolyzed feedstock ensured stability in terms of VFA production at high concentration (30 ± 2 $gCOD_{VFA}/L$) and COD_{VFA}/COD_{SOL} ratio (0.86 ± 0.09) [7]. Figure 2 shows the process flow diagram (PFD).

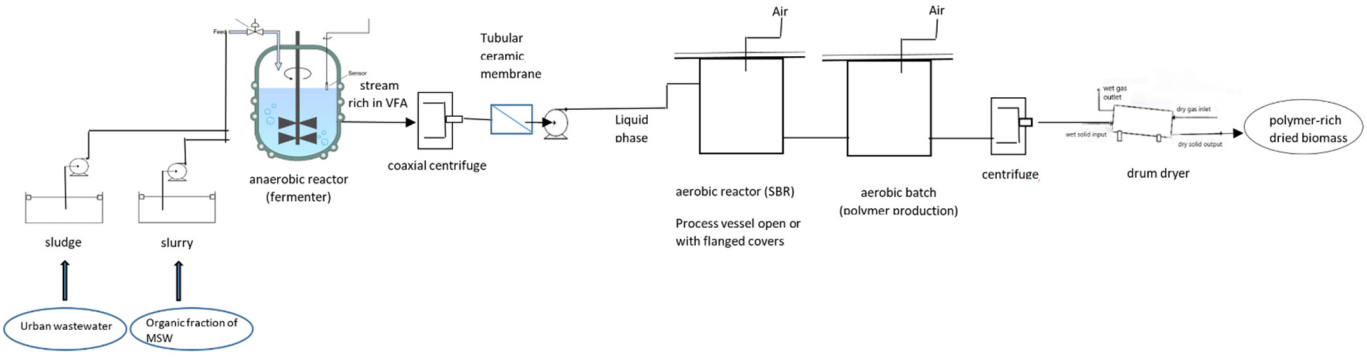

Figure 2. Process Flow Diagram (PFD) of integrated pilot plant platform for PHA production.

A thermal pre-treatment is applied; it consists of the application of a high temperature (72 °C) for 48 h to the feedstock mixture inside the fermentation reactor. After this time, the reactor temperature is decreased and maintained at 37 °C for four days.

The BioHazOp analysis has been applied to process conditions corresponding to the maximum PHA content in the biomass. This choice has led to the adoption of the following operating parameters and feedstock composition (Table 2).

Table 2. Fermenter operating parameters.

HRT	Window Time (d)	Feedstock (v/v%)	T (°C)	pH	Hydrolysis (°C, h)
5–6	140–420	SS(30%)-OFMSW(70%)	37	uncontrolled	72 °C, 48 h

The first stage consisted of the acidogenic fermentation of a mixture composed by 30% *v*/*v* SS and 70% *v*/*v* OFMSW for VFA production, followed by a solid/liquid separation stage for the fermented stream refinement. The fermenter (Figure 3) was a continuous stirred tank reactor (CSTR). The refined VFA-rich stream was successively fed to the sequencing batch reactor (SBR) for PHA selection and also to the PHA accumulation reactor. Both reactors for selection and accumulation worked under a fully aerobic regime and were automatically operated by a programmable logic controller (PLC, MyRio Labview from National Instrument), which also acquired real-time signals from immersion probes. The acidogenic fermentation was carried out in batch mode in a 400 litres (L) reactor equipped with a mechanical stirrer (HRT equal to 6 days). During each SBR operation, roughly 8–10 batch fermentation runs were conducted. The temperature was controlled by a thermostatic jacket and maintained at 72 °C for 48 h for the thermal mixture pretreatment. It was successively set at 37 °C for 4 days. The thermal pretreatment was used to foster the substrate solubilization. The fermenter pH was left uncontrolled and maintained at about 5.0–5.5 for the whole duration of the study. The solid/liquid separation was divided into two stages:

1. Caxial centrifuge equipped with a 5.0 μm porosity nylon filter bag (first stage);
2. Ultrafiltration membrane with 0.2 μm porosity (second stage).

Figure 3. Fermenter of pilot plant.

The PHA selection from MMC was performed in a 100 L working volume reactor equipped with Bibus EL-S-250 linear membrane blowers (ensuring a continuous maximum DO level equal to 8.0 mg O_2/L and a complete stirring of the mixed liquor) and immersion probes (DO, pH and temperature). The SBR temperature was maintained between 25 °C and 28 °C by an immersion heater. Since no settling phase was programmed, the HRT was equal to the SRT in all runs. The stirring inside the SBR is ensured by a blower (it is placed outside the reactor), which distributes the air to four diffusion plates located inside the reactor, at the bottom of the liquid column. The most efficient run (Ae3) in terms of storage yield and maximum PHA content has been selected. Table 3 shows the operating parameters of Ae3 run.

Table 3. Operating parameters applied to Ae3 run.

run	HRT (d)	SRT (d)	OLR (g COD/L d)	CL (d)	SRT/CL (d/d)	Feeding Frequency (d^{-1})	Operation Length (d)	Load Per Cycle (g COD/L)
Ae3	1	1	4	0.5	2	2	45	20

The HAZOP analysis was focused on acidogenic fermentation and thermal pre-treatment (hydrolysis reactor).

4. Results

4.1. Relevant Deviation Matrices and BioHazOp worksheets

For the purposes of the BioHazOp analysis, all the information on biological and chemical hazard as well as on process parameters and the prevention measures implemented at the Treviso pilot plant have been collected for the node under analysis. Table 4 shows the main analysis results, while Table 5 contains information referred to the overall process (whole plant).

Table 4. Node 1: CSTR (collection of useful information for BioHazOp analysis).

The anaerobic reactor (fermenter) contains VFA with acetic and butyric acids as the main components. It continuously works by processing up to 380 L/5 days of mixture. Some substances in the mixture are hazardous from a toxic or fire standpoint. More in detail, the mixture contains:
- Biological agents including bacteria, microfungi and protozoa. With reference to infective risk, the biological agents have been classified according to the Annex III of Directive 2000/54/EC [27] as amended by Directives 2019/1833/EC [29] and 2020/739/EC [30];
- Chemical agents consisting of VFA (acetic acid, propionic acid, butyric acid, valeric acid, isobutyric acid, isovaleric acid, caproic acid, isocaproic acid and heptanoic acid) and gases (carbon dioxide, hydrogen, methane, hydrogen sulphide).
Such substances pose physical hazards and/or health hazards according to the [28] EC Regulation 1272/2008 (CLP Regulation) classification criteria.
The products of any dangerous reactions may include alcohols, organic macromolecules and gaseous products. A biowaste pre-treatment is applied to the process.
Unwanted reactions may be caused by abnormal process conditions (e.g., temperature, pH), abnormal flow rates, system failure (oxygen inlet), electric equipment malfunction (e.g., sensors), mechanical failure (e.g., pump or stirrer trip), etc. Moreover, hazard can be also due to loss of containment.
The process equipment includes relief systems (hydraulic seal protected against freezing through antifreeze liquid) and drains (sampling points).
The process does not work in or near the flammability range.
Further detailed information has been acquired with respect to the following:

- BIOHAZARD: the biological agents, involved in the hydrolytic and acidogenic steps, include bacterial genera such as Clostridium, Bacillus, Peptococcus, Vibrio, Enterococcus, Lactobacillus, Ruminococcus, Butyribacterium, Propionibacterium, Micrococcus, etc., fungi (Phycomicetes, Ascomycetes) and protozoa. The thermal biowaste pretreatment (70 °C) and mixing are able to inactivate completely fecal indicator bacteria (fecal coliform, Salmonella spp., and fecal Streptococcus), within 80 min.
According to Directive 2000/54/EC [27], regarding the infectious risk, the microorganisms, which take part in the acidogenic fermentation, are mainly assigned to the risk group 1 and to a small extent of the risk group 2 (low pathogenicity). Some of the risk group 2 microorganisms should be considered opportunistic agents. Furthermore, one should take into account the sensitizing and toxic risk related to the exposure to biological agents in bioaerosol and biological components conveyed such as particulates (i.e., bacterial endotoxins, fungal spores) during some process activities.
The organic waste is automatically fed to the fermenter continuously or semi-continuously (once a day) in containment conditions. During acidogenic fermentation, the microbial reactions take place in the closed bioreactor and, therefore, there is no workers exposure. However, activities, such as sampling for the monitoring of the fermentation process, could involve the workers exposure. Furthermore, some specific operations, such as handling of the electromechanical pumps, pipes, compressors, valves, drainage, cleaning and maintenance tasks, may pose exposure risks to bioaerosol. Therefore, the workers' activities should be checked to define the exposure characteristics. The ways of penetration into the body are the gastrointestinal one (eg. hand-to-mouth contact), skin (eg contact through cuts), mucous membranes (splashes on nose, mouth, eyes) and inhalation (bioaerosol).
The potential occupational exposure could be identified and documented through the biological environmental monitoring plan, but it is not mandatory according to Directive 2000/54/EC [27]. In order to prevent infectious workers risk, effective personal hygiene measures are sufficient, including the provision of adequate hand washing facilities. The sensitizing and toxic effects can be controlled by minimizing the generation of bioaerosols or dusts in the workplace. Where residual hazards/risks cannot be controlled by collective measures, the employer should provide for appropriate personal protective equipment, such as suitably fitted respiratory devices, when working in areas close to where bioaerosol is generated.

-TOXICITY and ECOTOXICITY: the toxic agents in the main quantity are CH_3COOH (acetic acid, CAS No. 64-19-7) and $CH_3CH_2CH_2COOH$ (butyric acid, CAS No. 107-92-6), having potential health effects both acute and chronic. The substances entry routes are inhalation, skin contact and eye contact. Specific toxic effects are associated with each substance. So, the main toxic effects of acetic acid are:

(1) Acute effects: increasing concentration involves increasing corrosive effects on skin and mucous membranes, and exposure to high concentrations causes severe damages to the eyes and the lungs. The oral intake of high concentrations can cause chemical burns in the digestive tract, metabolic disorders, blood impairment, cardiovascular reactions and renal damage; (2) Chronic effects: skin changes, chronic inflammation of eyes and respiratory tract, erosive tooth damage. Some substances (valeric acid, hydrogen sulfide) are ecotoxic for the aquatic systems. Preventive and protective measures include the use of gas detectors and of personal protective equipment for tasks involving the chemicals handling.
- FLAMMABILITY & EXPLOSIVITY: the main components of the gaseous mixture are: CH_4 (methane, CAS No. 74-82-8, H_2S (hydrogen sulfide, CAS No. 7783-06-4) and H_2 (hydrogen, CAS No. 1333-74-0). The operating overpressure and temperature are 20 mbar and 37 °C. The main condition, which has to be avoided, consists in the intake of O_2 into the fermenter from the outside, even though there is no ATEX zone in the plant. The main preventive measure consists in the use of gases detection devices.

Table 5. Overall process (whole plant)-collection of useful information for BioHazOp analysis.

Operating Procedure
There is no specific written procedure to maintain the on-going integrity of the process equipment. Nevertheless, good operating practices have been applied to carry out plant operations, such as cleaning of coaxial centrifuge and ultrafiltration membranes, and taking samples for laboratory analysis.
Plant layout
There are buffer zones between the plant and the external public (population). The pilot plant operators are potentially exposed to hazards from the wastewater treatment plant within which the PHA production process takes place. The OFMSW transfer to digester is carried out under containment conditions and therefore it has no impact on the environment and operators. The workplace layout includes the location of control rooms, laboratories and offices, drainage areas and sampling points.
Emergency/ongoing program
A number of steps in the production process is managed by programmable logic controller (PLC). There is no system which ensures the plant is currently kept and periodically tested.
Management-Process Hazard Analysis (PHA)
No risk analysis techniques other than BioHazop analysis (FTA, FMEA, What if, etc.) have been applied to the plant. BioHazop addresses the following: - Hazards of the process; - Process equipment; - Engineering and administrative controls; - Consequences of failure for engineering or administrative controls, including consequences of deviation and steps required to correct or avoid the deviation; - A qualitative evaluation of safety and health effects (of failures) on employees in the workplace. The BioHazop analysis has been performed by a team that had the expertise: - In engineering and process operations; - In the BioHazop evaluation methodology; - In biological and chemical (health and safety issues) risk assessment.

A list of relevant deviations for the process under analysis has been drawn up for the node. As a consequence of the brainstorming sessions, the original lists have undergone several revisions. The team has discussed all non-bio parameters (i.e., flow of substrate, operating temperature, pressure and composition of the substrate and products), which may be responsible for deviations leading to hazardous situations. The same parameters may also be responsible for operability problems, along with the level and the pH of substrate, the mixing inside the anaerobic reactor and the substrate residence time. In the

discussion, it became evident that some of the deviations related to the parameters under analysis could also have bioconsequences. With regard to the bio-parameters, the ones usually taken into account in the study of bioprocesses (enzymatic activity, foam, biochemical oxygen demand, oxidation-reduction potential, conductivity, osmolality and turbidity) have been discussed with respect to both hazard and operability, but none has been considered relevant for the case study. Specifically, the analysis has been focused on the main parameters of the process under study and exploration of all the combinations between the guidewords and the operating parameters to be applied to the BioHazOp analysis. Thus, a RDM has been created for the node to show the set of relevant deviations, which have to be analyzed by the multidisciplinary team. The tailored matrix is reported in Table S1. Based on the information collected for the whole plant as well as for the node and the RDM, the HAZOP team conducted the BioHazOp analysis. The filled worksheet is reported in Supplementary Material (Table S2).

4.2. Piping and Instrumentation Diagrams

Figures S1 and S2 (Supplementary Material) show the P&IDs of examined node. Figure S1 represents the existing pilot plant, whereas Figure S2 shows the design intention of the hydrolysis reactor and the fermenter. Particular attention has been addressed to the acidogenic fermentation because it produces flammable gases, such as methane (CH_4), hydrogen (H_2) and hydrogen sulphide (H_2S). Figure S2, which is derived from BioHazop analysis, minutely reports the instrumentation, which is able to measure parameters such as flow, temperature, pressure, gases concentrations, etc., and alarm levels linked to main operating parameters. With reference to Figure S2, the following letters are used to describe the control devices involved in the PHA production process. In particular, each device is labeled with two or three letters. The first letter describes the parameter, which is controlled:

- F (flow rate);
- P (pressure);
- L (level);
- T (temperature).

The second or the third letter indicates the type of control device:

- I (indicator);
- C (controller);
- T (transmitter).

Further details on the diagrams are reported in Supplementary Material.

5. Discussion

The BioHazOp analysis has been focused on the most efficient run in terms of storage yield and maximum PHA content among those conducted in Treviso pilot plant [23]. The most significant result of the BioHazOp analysis is the identification of prevention measures which have to be applied to the plant in order to ensure its operability over time and occupational risks mitigation. As shown in the last right-hand column of Table S2 (Supplementary Material) for almost every pair of cause–consequence, safeguards, which could prevent, detect, control or mitigate the hazardous situation, have been identified. Finally, if the safeguards have been considered insufficient to solve the problem, additional prevention measures and recommendations have been proposed. In filling the worksheets arranged for the BioHazOp analysis during the brainstorming sessions, some of the selected parameters, originally considered guidewords and deviations have been eliminated or modified in accordance with the in-depth analysis. Table 6 shows some of the parameters not included in the analysis for the node together with the reasons for exclusion.

Table 6. Parameters not included in the BioHazOp analysis of node 1.

Parameter	Reasons for Exclusion
Run duration	The fermentation continues until the feedstock input mixture is consumed. In order to produce representative data, the fermentative process must last for at least 20–25 days (corresponding to 3–4 HRT)
Chemical oxygen demand (COD)	The parameter is not definable. It depends on the feedstock (OFMSW) carbon content variability throughout the year and therefore it is out of control for the process operability
Oxidation-reduction potential	the parameter is not measured in the pilot plant

A very important outcome of BioHazOp analysis is the attention, which has to be addressed to valves (valves equipped with electric actuators and non-return valves) maintenance in order to ensure the process efficiency and safety.

In particular, the maintenance and its timing depend on service conditions (temperature, pressure, fluid typology, etc.). Periodic cleaning is recommended, and the actuator must not be cleaned by aggressive solvents or highly flammable and injurious detergents to health. During and after cleaning, the sealing points on the actuator should be inspected. In case of lubricant loss and accumulated dirty, the sealing elements have to be repaired. The valves components have to be examined for excessive wear.

In case of hydraulic non-return valves provided with dampers, a specific maintenance is required. It consists of:

- Periodic check of their side covers. In case of leakages, the screws must be re-tightened, or gaskets must be replaced;
- Hydraulic damper check (lubricant level). In particular, it is recommended to use lubricant, which has a kinematic viscosity ranged between 30 and 50 mm^2/s (ISO grade);
- Check of hydraulic circuit connections (all the components have to be perfectly tightened).

Once a month, it is recommended to check the correct valves operating by their opening and closure. Before carrying out the valves maintenance, the line has to be drained and depressurized and the electric supply must be disconnected. The non-observance of these precautions could cause injuries to workers. In particular, it is extremely important that the operators responsible for the valves installation, operation and maintenance are properly qualified and trained. With reference to the pilot plant, another analysis outcome consists in the need of monitoring the VFA concentration. A common system to measure VFA concentrations is to apply chromatographic techniques to sample aliquots taken in the mixture. In order to check the fermenter efficiencies and VFA yields, a gas chromatography equipped with a capillary column and flame ionization detector has been widely and successfully applied. VFAs are present in a wide range of matrices, i.e., wastewater, landfill leachates, human and animal fluids, food and environmental systems. The difference in nature and complexity of the matrices has resulted in the development and publication of a great number of procedures for quantification and speciation of VFAs [31]. An innovative analytic approach is presented for determining the total volatile fatty acids concentration in anaerobic digesters [32]. The new approach analyzes the total inorganic carbon concentration (CT) on the same sample, but separately from the titration method, which is performed to determine the total VFA concentration. Once CT is determined (e.g., via a TOC -Total Organic Carbon- analyzer), a 2-point titration procedure can be performed to yield accurate concentration results. The titration method consists of only two points, carried out to pH values, which are close to 5.25 and 4.25. This technique is adequate for the purpose of determining the total VFA and for alarming operators in case of process deterioration and imminent failure. It is simple to execute, may be used by researchers working on anaerobic processes, but it is also appropriate as a routine tool for

controlling full-scale anaerobic digesters. For the purpose of occupational risk management, further investigation should be conducted with respect to operations routinely carried out at the plant, such as the feeding and discharging of fluid mixtures by pumps, the sampling of the liquid mixture for laboratory analysis, membrane filtration operations and more generally solid-liquid separation steps, membrane washing by chemical agents (sodium hydroxide, sodium hypochlorite, phosphoric acid) and plant shutdown for the pumps and filters maintenance.

As mentioned in 4.2, the P&ID is a plant drawing including the piping and process equipment with its instrumentation and control systems. The final pilot plant P&ID shows all prevention measures identified as necessary to address hazard and operability problems. The P&ID plays a strategic role in the maintenance and modification of process, which it describes. During the design stage, the diagram provides the basis for the development of system control schemes, allowing safety and operational investigations. Therefore, it represents a fundamental milestone in order to ensure the best performance of industrial processes in terms of operability, efficiency and safety. With reference to examined pilot plant, the P&ID allows us to improve its safety level and efficiency in terms of VFA production. These goals can be achieved by the rigorous choice of instrumentation and alarm levels linked to main operating parameters (reagents flow, temperature, gases concentration, etc.). Indeed, in order to control the PHA production process and avoid parameters deviations from design intention, the instrumentation and alarm levels play a particularly relevant role.

All hazards not identified in the HAZOP analysis are not considered in terms of risk and this is particularly relevant for the biological risks, whose assessment is difficult in the workplace. Containment losses from fermenter could cause the release of biological agents into the working environment. The biological risk assessment in non-safety-level activities is seriously hampered, since neither universally approved criteria for assessing the exposure to biological agents nor agreed dose-response estimates and occupational exposure levels (OELs) are yet available [33]. With regard to the host response, the role which is played by the biological agents in the development or aggravation of symptoms and diseases is poorly understood. The human response to exposure to biological agents depends on the organic involved material and individual susceptibility. In order to overcome the current knowledge gaps in the biological risk assessment, the potential risk should be managed in a precautionary manner, taking into account, by an expert in microbiology, the biological agents involved in the biotech process and biohazards related to plant areas and workers activity [6]. The design of workplaces and work processes and the choice of adequate equipment and working methods allow the control of occupational biohazard in the biotech production plant.

6. Conclusions

BioHazOp is a very useful tool for improving the industrial process's safety and operability because it allows us to find the adequate countermeasures which are able to avoid parameters deviations from design intention. The deviations could cause a process efficiency decrease and hazardous scenarios (flammable and toxic gases releases or exposure to biological agents) for workers health and safety. The obtained results clearly show that the applied methodology allows an in-depth analysis of conventional and biological hazard. Indeed, the conventional hazards can be easily overlooked by lab-scale process experts. On the other side, it is harder to identify biohazards, well known only by biotechnological processes experts, for those who are are less familiar with safety issues in processes scaled up to industrial size. Thus, the BioHazOp methodology promotes the integration of the different expertise of the multidisciplinary team and is aimed at a deeper and more comprehensive analysis of process hazard and operability.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation9020099/s1. Table S1: Relevant Deviations Matrix (RDM) listing the possible combinations of guidewords and process parameters to be used in BioHazop study, Table S2: Worksheet obtained by BioHazOp analysis of node 1, Figure S1: P&ID of node 1 (hydrolysis reactor and CSTR) and Figure S2: Final P&ID of node 1.

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