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Innovative Mixed Microbial Culture Processes for PHA Production at Pilot Scale: Professional Chemical Risk Assessment

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Identification and fine-tuning of a process that allows the effective conversion of organic carbon from urban waste into valuable bio-based products is the main goal of “REsources from URban BIowaSte”- RES URBIS (GA 7303499) project in the European Horizon2020 program.
In this frame, the Mixed Microbial Culture (MMC) process for polyhydroxyalkanoates (PHA) production has not been exploited at full scale yet and the process is presently under investigation only at pilot scale.
The successful development and the industrial implementation of a safe biochemical conversion process is essential for meeting sustainable development goals. Although the health and safety risks posed by bioprocesses for the valorisation of biomass are usually expected to be lower than the traditional chemical and petrochemical processes, there is still a lack of information about these aspects of biorefinery plants, especially for novel bioprocesses under development.
Taking steps to meet this challenge, the work presents early results of professional chemical risk assessment in application to the MMC-PHA production process at pilot plant scale. The assessment has been performed applying a guideline drawn up by Italian National System for Environmental Protection for the protection of operators from risks to their safety and health arising, or likely to arise, from the effects of chemical agents that are present at the workplace or as a result of any work activity involving chemical agents. Meeting the requirements of Community legislation and the updates introduced by EC Regulation 1907/2006 (REACH, 2006) and EC Regulation 1272/2008 (CLP, 2008), the guideline provides a methodology for chemical risk assessment distinguishing between risks for safety, related to reactivity and chemical-physical properties of chemicals, and risks for health, mainly ascribable to toxicological properties of chemical agents.
Starting from the analysis of the Process Flow Diagram of a pilot scale plant for PHA production, a lot of information (hazardous properties of chemical agents, circumstances of work involving such agents, including their amount, level, type and duration of exposure, relevant material safety data sheets, occupational exposure limit values, effect of preventive measures taken or to be taken) has been collected in order to apply the guideline criteria and the joined IT tool. The paper presents the results of the assessment attempt.

1. Introduction

In the context of REsources from URban BIowaSte - RES URBIS (GA 7303499) project in the European Horizon2020 program, a combined acidogenic fermentation and anaerobic treatment have been developed on pilot scale for urban bio-waste conversion into volatile fatty acids (VFA) and biogas. The piloting facilities for polyhydroxyalkanoates (PHA) production are located in Treviso (northeast Italy) municipal wastewater treatment plant (Valentino et al., 2019). The process consists in three phases: acidogenic fermentation, biomass selection and PHA production. The feedstock is a mixture of secondary sludge and liquid fraction from solid/liquid separation after squeezing of organic fraction of municipal solid waste.
The first process phase is the acidogenic fermentation in a continuous stirred reactor (fermenter) fed by the feedstock. The anaerobic phase produces an effluent rich in VFA, which are used for the two following steps.

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Before reaching the sequencing batch reactor (SBR), the fermented stream is subjected to a double-stages liquid/solid separation (using a coaxial centrifuge and a tubular ceramic membrane). The separation allows to reduce as much as possible solid impurities in the PHA production line (SBR and accumulation reactor). The solid fraction goes to the anaerobic digestion for the energy generation, whereas the liquid stream (rich in VFA) is conveyed to the SBR (aerobic reactor) and aerobic batch. The second phase is aimed at enriching the mixed microbial culture (PHA-accumulating bacteria), which is the feedstock for PHA production. SBR is fed by air and stream rich in VFA. The third phase, which occurs in an aerobic batch, allows to produce the biopolymer.
In the framework of the European Community, according to Directive 98/24/EC (European Union, 1998), art. 4, the determination and assessment of risks to the safety and health of workers arising from the presence of hazardous chemical agents have to take into account several factors. They consist in: substances hazardous properties, information on safety and health that shall be provided by the supplier (e.g. the relevant Safety Data Sheet), the level, type and duration of exposure, the circumstances of work involving such agents including their amount, any occupational exposure limit values or biological limit values established by Law, the effect of preventive measures taken or to be taken, and, where available, the conclusions to be drawn from any health surveillance already undertaken. Provided that the employer is always obliged to apply the general principles for prevention of risks associated with hazardous chemical agents (art. 5.2), the results of the assessment referred to art. 4 may reveal a risk that requires to apply stricter measures including specific protection and prevention measures (art. 6), arrangements to deal with accidents, incidents and emergencies (art. 7) and health surveillance (art.10). Otherwise, where the results of the risk assessment show that there is only a *slight* risk to the safety and health of workers, and the general adopted measures are sufficient to reduce that risk, the provisions of articles 6, 7 and 10 do not apply.
Occupational risk assessment may be carried out through the application of dedicated algorithms: special tools are designed for supporting the employer in carrying out the assessment and setting prevention measures.
In this paper, risk assessment has been performed applying a guideline drawn up by Italian National System for Environmental Protection (SNPA) for the protection of operators from risks arising from chemical agents (SNPA, 2017). The guideline meets the requirements of Community legislation and the updates introduced by EC Regulation 1907/2006 - REACH Regulation (European Parliament and Council of the European Union, 2006) and EC Regulation 1272/2008 - CLP Regulation (European Parliament and Council of the European Union, 2008) and provides a methodology for chemical risk assessment distinguishing between risks for safety, related to reactivity and chemical-physical properties of chemicals, and risks for health mainly ascribable to toxicological properties of chemical agents. The guideline has been specifically developed for risk assessment in research and didactic laboratories of SNPA, but it has been applied to Treviso plant because of the low amount of chemicals handled at pilot scale. This assumption complies with the definition of a Process category (PROC) of *Chemical Safety Assessment* under REACH Regulation. In fact, PROC 15 corresponds to *Use as laboratory reagent* and defines workplaces, where substances are used in amount less than or equal to 1 l or 1 kg (ECHA, 2015).

2. The risk assessment methodology

2.1 Methodological approach for evaluation

Risk assessment is the overall process, that includes risk analysis and evaluation, which are characterized by a series of logical stages (Figure 1) aimed at making a judgement on risks the workers are exposed to.
Following the approach above described, risks for safety have to be distinguished from risks for health. All factors listed in Directive 98/24/EC (art. 4) are taken into account; in case of working activities involving exposures to a number of different chemical agents, the assessment shall be based on the combination of all these agents.

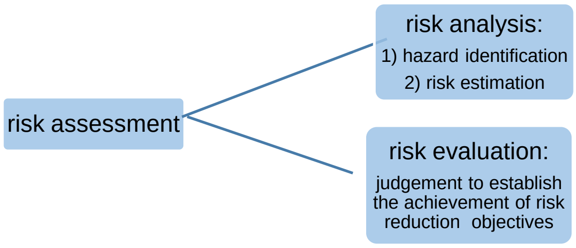

Figure 1: The risk assessment process

2.2 Risk for health

The risk assessment for health comprises three steps: hazard assessment, exposure assessment and risk characterisation.
The hazard assessment requires the collection and the evaluation of any available information on every substance and its properties in order to identify danger, potential effects on human health and occupational limit value.
The assessment of substance and mixtures classified as *dangerous* consists in estimate and/or measurement of the substance concentration, which workers are or may be exposed to as a consequence of handling it.
The last step of risk assessment is the risk characterization (exposure level) with regard to exposure limit values and protection measures taken to reduce it. This characterization can be carried out by the calculation model shown below. The calculation model compares the risk factors with all prevention and protection measures adopted to reduce the risk. The methodology employed for the assessment of risks for health focuses on identification of the workers exposure level to dangerous chemical agents and adopts the assessment criteria defined by the REACH-compatible ECETOC TRA (Targeted Risk Assessment) tool.
The assessment procedure starts with collection of information on:
1) chemical agents present at the workplace;
2) analysis of tasks, activities and workplaces;
3) adopted preventive and protection measures.
The procedure has to be applied to each worker or Similar Exposure Group (SEG: group of workers having the same general exposure profile for the chemical agents) of workers.
An algorithm has been employed for the calculation of risk index or exposure level L, based on all parameters listed in Directive 98/24/EC, art. 4:

$$L = \sum_{i=1}^{n} \frac{H_i\, T_i\, S_i\, E_i\, Q_i\, U_i\, D_i\, A_i}{K_i\, VL_i} \quad (1)$$

where:
-L is the worker exposure level to the n dangerous chemical agents;
-H_i is the sum of the factors of danger characterizing risky properties H of the i-th dangerous chemical agent (H phrases under CLP Regulation);
-T_i is the sum of exposure factors T characterizing the route of exposure (dermal and/or by inhalation) of the i-th dangerous chemical agent;
-S_i is the physical state factor corresponding to the i-th substance physical state;
-E_i is the exposure length of time factor corresponding to the duration of exposure to the i-th substance in a reference week;
-Q_i is the amount factor corresponding to the i-th chemical agent amount used in a reference week;
-U_i is the condition of use factor taking into account the possibility of release into the air of the i-th substance;
-D_i is the stored amount factor corresponding to the i-th chemical agent amount present in the workplace (excluding the amount contained in safety cabinets and in specific storage areas) in a reference week;
-A_i is the work factor depending on the type of work activity (usual work, maintenance, cleaning, waste management);
-K_i is the worker prevention and protection factor corresponding to all the measures (fume hood and extraction systems, collective and personal protection devices, written procedures, specific training of workers etc.) taken to reduce the risks;
-VL_i is the threshold limit value (ppm), if any, set by European Directives for the i-th substance.
For each i-th dangerous substance handled by an operator, the IT tool calculates a L_i value; all L_i values are summed for calculating overall L exposure for the operator under the conservative assumption that all chemicals employed in a week are handled in a single day. If, according to the parameters used in the calculation of risk index, the overall exposure level (L) for a worker or a SEG is lower than 1, it can be stated that prevention and protection measures at the workplace are sufficient to contain the risks for health, or better, that the risks have been reduced to an acceptable level. In this case there is no obligation to carry out a deeper risk assessment in order to apply, if necessary, specific protection and prevention measures, arrangements to deal with accidents, incidents and emergencies, health surveillance and health and exposure records. Higher L values clearly signify higher-risk situations.

2.3 Risks for safety

The assessment of risk for safety is performed through qualitative observations on chemical-physical properties of the substances employed by the operators and on workplace characteristics. The assessment is required

whenever dangerous chemical agents characterized by hazard statements (phrases H2XX) and by supplemental hazard information on fire, explosion and corrosion danger (phrases EUHXXX), are handled in a workspace. Specifically, risk assessment entails the evaluation of:
-risk of fire;
-risk from potentially explosive atmospheres (ATmosphere EXplosive - ATEX);
-risk from incompatibility among chemicals.
Risk of fire associated with handling of dangerous chemical agents is considered *low* if all the following conditions are simultaneously verified at the workplace: the presence of an efficient and effective gas detection system; the provision of suitable fire-fighting equipment; the presence of an emergency team, properly formed and trained; the absence of unchecked ignition sources.
According to the guideline, risk from potentially explosive atmospheres (ATmosphere EXplosive - ATEX) has to be evaluated. If no area of workplace is classified as *hazardous zone* due to the presence of potentially explosive atmospheres, this risk for safety can be considered low.
Risk from incompatibility among chemicals is due to their reactivity. It's well known that the consequences of an unintentional mixing of different substances may be: a fast chemical reaction or an explosion; the generation of flammable or toxic gaseous products; the generation of dangerous products upon contact with the skin.
In order to evaluate the dangerous situations, the software uses two-dimensional arrays analysing the interactions of chemicals with other substances in the workplace, besides air and water.

3. Results and discussion

3.1 Risk for health

PHA production process in Treviso pilot plant has been analysed in order to apply the calculation model.
The following assumptions have been made:

- a single SEG corresponding to *plant managment attendant* has been identified in the workplace;
- the process doesn't imply handling of carcinogen and mutagenic substances: the assessment of risk for health covers only dangerous chemical agents;
- the chemical agents present at the plant are those present in biowaste feedstock and those formed as a result of fermentation step. They mainly consist of a VFA (acetic, propionic, butyric, valeric, isobutyric, isovaleric, caproic, isocaproic and heptanoic acids) mixture. Some chemical substances (NaClO, H_3PO4 e NaOH) haven't been considered in the assessment, because they are employed only in clean-up steps in small amount and sporadically (once or twice a month);
- the fermenter is loaded every two weeks: fermentation mixture exiting from it contains a maximum VFA concentration;
- the composition of the feedstock was set at OFMSW (Organic Fraction of Municipal Solid Waste) volumetric fraction of 30-35% and thickened WAS (Waste Activated Sludge) volumetric fraction of 65-70%, as optimal percentages (in terms of fermentation process management). This mixture composition corresponds to conditions of process stabilization under mesophilic condition;
- the fermenter operating temperature has been assumed equal to 37° C; such choice depends on optimized conditions in terms of VFA production and VFA/CODsol;
- the fermenter is refilled approximately every two weeks with 380 L of feedstock mixture. The average amount of VFA in the fermented mixture is about 15 gCOD/L per week which corresponds to 9.35 g/L. For each acid it has been assumed the maximum amount between the two measured at the inlet and the outlet of the fermenter. The value corresponds to the maximum amount of each chemical agent handled, which the operator may be exposed to on a weekly basis. The assumption is even more conservative if we consider that at least half the amount of each acid in the fermenter is present in form of salt or ionic species into the acid water solution (pH 4.5-5.5);
- for the flammable gaseous (composed by H_2, CH_4, H_2S and CO_2) mixture into the fermenter the daily average concentration values (as measured) have been assumed. These concentration levels correspond to process stabilisation under mesophilic condition. The %(v/v) to mass (g) conversion has been done through density values;
- with respect to the condition of use, it has been assumed that the system is closed. Under such assumption chemical substances are used or stored into reactors or gas-tight housings and transferred from one vessel to another via airtight pipes;
- the duration of exposure for a worker employed in conducting the plant is assumed to be 1 hour per day per 5 working days;
- referring to the workers' tasks, the transmission/intake paths involved are by inhalation or through the skin (skin exposure may occur by accidental contact);

- the type of work activity carried out by the plant management attendant can be identified as usual work (maintenance, cleaning, waste management have been considered residual activities and therefore they have been neglected);
- the plant is placed outdoors so that the effectiveness of dilution of pollutants into the air can be considered good. Furthermore, the plant management attendant wear personal protection devices (gloves, glasses, antiacid coats).

For each dangerous chemical agent, CLP classification and H statements have been assumed. Hazard statements form part of the Globally Harmonized System of classification and labelling of chemicals (GHS).
On the basis of the above assumptions, the calculated values (L_i), referred to chemical agents, have been found to be lower than 1 (Table 1) and the overall L value calculated for *plant management attendant* has turned out pairs to 0.814.

Table 1: Risk index or exposure level L_i for chemical agents handled at Treviso plant

Substance	L_i
Acetic acid	0.008
Propionic acid	0.011
Butyric acid	0.054
Valeric acid	0.011
Isobutyric acid	0.179
Isovaleric acid	0.048
Caproic acid	0.139
Isocaproic acid	0.152
Heptanoic acid	0.210
Carbon dioxide	0
Methane	0
Hydrogen	-
Hydrogen sulfide	0.002

3.2 Risk for safety

Hazard from potentially explosive atmospheres is related to flammable gases (CH_4, H_2S, H_2) generating during fermentation step and to some of the acids (acetic, propionic and isobutyric acids) composing the VFA mixture. Risk from potentially explosive atmospheres (ATEX) hasn't been evaluated so far in terms of classification of *hazardous zones*. However, the lower flammability limit (LFL) of gaseous mixture has been calculated as a function of the chemical composition of flammable gaseous mixture, which depends, in turn, on the fermenter operating temperature and the feedstock. The investigation showed that the formation of potentially explosive atmospheres is extremely improbable in the fermentation reactor.
The conditions posed by the calculation model for controlling risk of fire haven't been all verified at the workplace so that no judgement can be given about the containment of the risk. Incompatibility among chemicals doesn't pose a risk for safety in the plant. Corrosion danger is only related to the presence of NaClO, H_3PO4 e NaOH, whose CLP classification is H290 (*Corrosive to metals*) and H314 (*Causes severe skin burns and eye damage*). These chemical agents are employed in small amount for the cleaning of coaxial centrifuge and tubular ceramic membrane used for solid/liquid separation step of the process. Even though they haven't been considered in the risk assessment, their use in clean-up steps of the process should be governed through *ad hoc* written procedures.

4. Conclusions

Any methodological approach for chemical risk assessment, both at pilot and industrial scale, has to be consistent with the preliminary implementation of general principles and measures for the protection of health and safety of workers (Directive 98/24/EC, art. 5). Risk evaluation resulting from the application of calculation models, as every adoptable calculation methodology, has always to be considered preliminary, has not absolute ma only comparative value and generally provides conservative risk evaluations. However, this methodology may provide the way to move from a qualitative to a semi-quantitative approach in chemical risk assessment taking into account all the information required for it according to Directive 98/24/EC, art. 4. The value of the synthetic single index by the algorithm corresponds to a risk judgement, which can be used to implement preventive and protective measures. In this context, the use of models may help to understand how the

prevention and protection measures work in the risk containment. Furthermore, in order to assess the effectiveness of the adopted measures, the algorithm should be applied again in any in-depth evaluation phase. Hazardous properties of substances handled at Treviso pilot plant bear a danger both for health and for safety of the operators. On the basis of the model chosen and of the assumptions made for Treviso pilot plant, L_i values and overall L value for plant management attendant have been found to be less than 1 (*slight risk* for health). Even at industrial scale, the fermenter has to be placed outside the plant in order to ensure the dilution of pollutants at least at the feedstock and fermentation steps. According to the model output, inside the plant the risk arising from the effects of chemical agents involved in the process, is contained by a closed system for substances use and installing and maintaining efficient collective protection devices (environmental forced ventilation and localized suction systems). Risk for safety, related to the presence of flammable substances, can be considered *low* (under control) adopting all the measures which can contain the risk from potentially explosive atmospheres and the risk of fire. At the pilot-scale plant level, the formation of potentially explosive atmospheres is extremely improbable in the fermentation step. This is due to the oxygen absence during the acidogenic fermentation, which is an anaerobic process. However, moving to industrial scale plant, some technical devices including gases concentrations monitoring and safety valves against the overpressures can be considered essential in order to ensure safe conditions. Risk of fire can also be fully contained applying all the measures listed by the software: these measures are usually taken in an industrial scale plant.
Despite the different amounts of chemical agents involved, the analysis focused on a pilot plant is also valid for a full-scale plant in showing the methodology usually employed by algorithms dedicated to chemical risk assessment in the framework of the European Community Directives.

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References

ECHA, Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.12: Use description. Version 3.0. December 2015.

European Union, Directive 98/24/EC of 7 April 1998 on the protection of the health and safety of workers from the risks related to chemical agents at work (fourteenth individual Directive within the meaning of Article 16(1) of Directive 89/391/EEC). Official Journal of the European Communities L 131/11, 5. 5. 98.

Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC.

Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on Classification, Labelling and Packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

Sistema Nazionale per la Protezione dell'Ambiente (SNPA), 2017, Manuale per la valutazione del rischio da esposizione ad agenti chimici pericolosi e ad agenti cancerogeni e mutageni. Linee Guida SNPA | 05 2017, terza revisione.

Valentino F., Moretto G., Gottardo M., Pavan P., Bolzonella D., Majone M., 2019, Novel routes for urban bio-waste management: A combined acidic fermentation and anaerobic digestion process for platform chemicals and biogas production, Journal of Cleaner Production 220, 368–375.