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# Phycoremediation of paper mill effluents by waste grown microalgae

P. Dutta[1], S. Bhakta[2] and A. K. Bastia[1Ψ]
1. P.G. Department of Botany, MSCB University, Baripada-757003, Mayurbhanj, Odisha.
2. Botanical Survey of India, Western Regional Centre, 7-Korean Road, Pune-411001, Maharashtra.

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ABSTRACT

Microalgae gained a lot of attention in recent years because of their possible use in pollution management. They can be cultivated in unproductive habitats like nutrient rich wastewater as microalgae require high amount of nutrients for their growth that helps to reduce the pollution load from the environment. During this study six waste grown microalgal species, viz. *Limnothrix planctonica, Hapalosiphon hibernicus, Scytonema hoffmanii, Dolichospermum affine, Oocystis polymorpha* and *Tetradesmus obliquus* sourced from different wastewater habitats were utilized for remediation of wastewater collected from effluent treatment pond (ETP) of Emami Paper Mill, Balasore. To measure efficiency of wastewater utilization, the physico-chemical parameters of standardised wastewater were ascertained before and after utilization by all the tested species of algae under controlled culture conditions in laboratory. The phycoremediation results revealed that *H. hibernicus, O. polymorpha* and *L. planctonica* played better role for removal of pollutants from wastewater followed by *T. obliquus* and *S. hoffmanii. H. hibernicus* showed better performance by completely removing (100%) colour, turbidity, iron and phosphate from the wastewater. pH was corrected to normal after treatment by all the test algal species except *S. hoffmanii.* Moreover, the cyanobacterial species were found to be more efficient in terms of removal of nutrients like nitrate, silica, potassium and sodium as compared to green algae.



## 1. Introduction

Pollution is a man-made phenomenon which become a major concern of today's society due to increase in both natural and artificial compounds in the environment. The release of industrial and municipality wastewater to various water bodies makes them fully contaminated by depositing huge organic and inorganic pollutants such as heavy metals, xenobiotics, microplastics, phosphates, nitrates, carbon compounds etc. (Chowdhury *et al.,* 2016; Mencio *et al.*, 2016; Sausa *et al*., 2018; Eerkes-Medrano *et al*., 2019). The treatment of wastewater can not be done by a single methodology due to its extremely variable scales, types of pollutants and regional conditions. But this global problem can be solved by using microalgae as they are capable to perform phototrophic, mixotrophic or heterotrophic metabolism (Hu *et al*., 2018; Subashchandrabose *et al*., 2013) that represent a biological system for remediation of various types of wastewaters. Presence of nitrate and phosphate in wastewater make it a good feed stock for microalgae as both are essential for their growth. On the other hand, with growth it can remove maximum quantity of toxic materials from wastewater. Various reports showed that microalgae (cyanobacteria and green algae) can remove organic and inorganic nutrients from domestic and industrial wastewater up to 96% (Oswarld and Gotaas, 1957; Olguin, 2003; Chinnasamy *et al*., 2010; Kong Q-x *et al*., 2010; Wang *et al*., 2010) and efficiency of this technique is promising. However, very few researches have been carried out on treatment of paper mill effluents by using microalgae (Shruthi *et al*., 2012; Sasi *et al*., 2020). Being eco-friendly and cost effective, in the present investigation an attempt has been made to remediate wastewater of paper industry by using

Ψ *Corresponding author, Email:* bastianou@gmail.com

microalgae (cyanobacteria and green algae) sourced from wastewater.

## 2. Materials and methods

### *2.1. Sampling of paper mill wastewater; isolation and culture of dominant waste grown microalgae as test organisms*

Field visits were made to the Baripada municipality area, Mayurnhanj and Emami Paper Mill, Balasore for the collection of algal samples along with wastewater from Emami Paper Mill during the period March, 2016 to May, 2017 (Plate-1). Six dominant microalgal species isolated from wastewater habitats viz. *Limnothrix planctonica*, *Dolichospermum affine*, *Hapalosiphon hibernicus*, *Scytonema hoffmanii*, *Oocystis polymorpha* and *Tetradesmus obliquus* were selected for remediation of industrial wastewater collected from effluent treatment ponds of Emami Paper Mill (ETP). The standardised wastewaters in which the test species showed maximum growth in comparison to inorganic medium were used as medium for phycoremediation process. The remediation experiment was carried out in culture vessels under cool fluorescent light (7.5 $W/m^2$) at 26 ±1°C temperature up to 20 days of inoculation.

### *2.2. Analysis of wastewater*

The physicochemical parameters of wastewaters were analysed before and after utilization using the standard procedure of APHA (1992), IS: 3025 (1984, 1986, 1988 and 2003), testing kit and spectrophotometer.

## 3. Results

### *3.1. Physico-chemical properties of paper mill wastewater*

The partially treated effluent (ETP) of Emami Paper Mill was analysed for its physico-chemical parameters (Table 1). Due to organic load in paper industry, the colour of the effluent was orange and up to 32.4 HU. At the time of wastewater collection, the temperature was recorded to be 29°C and the nature of effluent was alkaline having pH 8.68. BOD and conductivity of the effluent were very high, approximately 230 mg/L and 2199μS/cm respectively. The effluent was little turbid with turbidity 10.9 NTU, total hardness 720 mg/L and alkalinity 40 mg/L. TSS and TDS were noted to be 78 and 195 mg/L respectively. The nutrient load in the wastewater found higher than the normal value and maximum value recorded for sulphate (147.9 mg/L) followed by calcium (125.63 mg/L), silica (57.18 mg/L), sodium (56.9 mg/L), magnesium (45.56 mg/L), nitrate (20.52 mg/L) and potassium (15.4 mg/L) but minimum phosphate (1.81 mg/L) and iron (1.23 mg/L).

### *3.2. Utilization of wastewater by test organisms*

For nutrient utilization, *H. hibernicus* showed better removal of calcium (80%) than *L. planctonica* (63%) but less than that of *D. affine* (44%), *S. hoffmanii* (16%), *O. polymorpha* (12%) and *T. obliquus* (10%). Maximum reduction of magnesium was brought about by *L. planctonica* (58%) followed by *O. polymorpha* (57%), *S. hoffmanii* (41%) and less in *T. obliquus* (10%), *H. hibernicus* (17%) and *D. affine* (14%). Except *T. obliquus* (26%), all the test algal species showed better performance in terms of removal of sodium as in *D. affine* (88%) followed by *T. obliquus* (87%), *O. polymorpha* (60%), *H. hibernicus* (55%) and *S. hoffmanii* (51%). Potassium reduction was maximum (88%) in *L. planctonica* followed by *D. affine* (84%) and *O. polymorpha* (56%), *S. hoffmanii* (46%), *T. obliquus* (34%) and *H. hibernicus* (33%). Complete utilization (100%) of iron was observed in *L. planctonica*, *H. hibernicus*, *O. polymorpha* and *T. obliquus* followed by *D. affine* (70%) and *S. hoffmanii* (66%). Regarding sulphate, *L. planctonica*, *H. hibernicus*, *T. obliquus* and *D. affine* showed less utilization (percentage of reduction) than *S. hoffmanii* (60%) and *O. polymorpha* (56%). *L. planctonica*, *H. hibernicus*, *O. polymorpha* and *T. obliquus* completely utilized (100%) phosphate from wastewater than *S. hoffmanii* (88%) and *D. affine* (48%). However, maximum nitrate usage was executed by cyanobacterial species viz. *L. planctonica* (95%) followed by *H. hibernicus* (77%), *S. hoffmanii* (60%), *D. affine* (47%). But green alga showed less reduction of nitrate i,e 25% in *T. obliquus* and 15% in *O. polymorpha*. For silica, *S. hoffmanii* showed maximum utilization (81%) followed by *D. affine* (62%), *H. hibernicus* and *O. polymorpha* (60% in both), whereas less in *L. planctonica* (41%) and *T. obliquus* (18%) (Table 2, Plate 2).

## 4. Discussion

The above findings emphasized that all the test alga species are effective agents for amelioration of polluted habitats. They have potential to remove physical, chemical and nutritional loads from wastewaters (Rajasulochana *et al*., 2009; Rao *et al*., 2011; Umamaheswari & Shanthakumar, 2017). This highlighted the possibility and prospects of an alternative, cost effective and eco-friendly approach for wastewater utilization. The physico-chemical analysis of paper mill industry revealed that most of the parameters examined in assessing quality of wastewater are below permissible limit except colour, conductivity, turbidity, hardness, BOD and silica. All the waste grown test microalgal species could effectively remove all the physical and chemical impurities, remediate to permissible limit as reported earlier (Pathak *et al*., 2014; Brar *et al*., 2017; Das and Deka, 2019). Moreover, higher values of nitrates, phosphates and

BOD in the effluent favour growth of cyanobacteria (Palmer, 1969; Boominathan, 2005; Sanjaya *et al*., 2011) than green algae.

In Emami Paper Mill wastewater, iron and phosphates were fully removed (100%) by both the green algae, *O. polymorpha* and *T. obliquus* (Sengar *et al*., 2011) followed by cyanobacterial species like *L. planctonica* and *H. hibernicus.* Cyanobacterial species were proved to be more efficient in removal of nutrients like nitrate, silica, potassium and sodium as compared to green algae. *S. hoffmanii* and *T. obliquus* showed better performance inremoval of TSS, TDS and BOD from wastewater followed by *H. hibernicus* and *L. planctonica.* Except *S. hoffmanii*, other algal species were found effective for removal of turbidity from wastewater with satisfactory performance (100%) in *H. hibernicus*. Colour reduction and removal of hardness from wastewater was more in *H. hibernicus, L. planctonica.* pH was corrected to normal after treatment by all the test algal species except *S. hoffmanii.*

This establishes the versatility of microalgae as an important agent for remediation of industrial wastewater and as an important biological tool for assessment vis-a-vis monitoring of environmental toxicants (Chu, 2012). Assertion of their phycoremediation potential will definitely boost future research on development of algae-based technologies for better exploitation of their bioremediation potential with production of value-added by-products.

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**Plate 1 (Figs. 1-6):** Collection of waste grown algae and wastewater from Emami paper mill, Balasore, 1-3: Industrial area of the paper mill with effluent treated pond, 4-5: Surrounding rice fields of the paper mill, 6: Collection of waste grown algae from municipal sewage wastewater, Baripada.

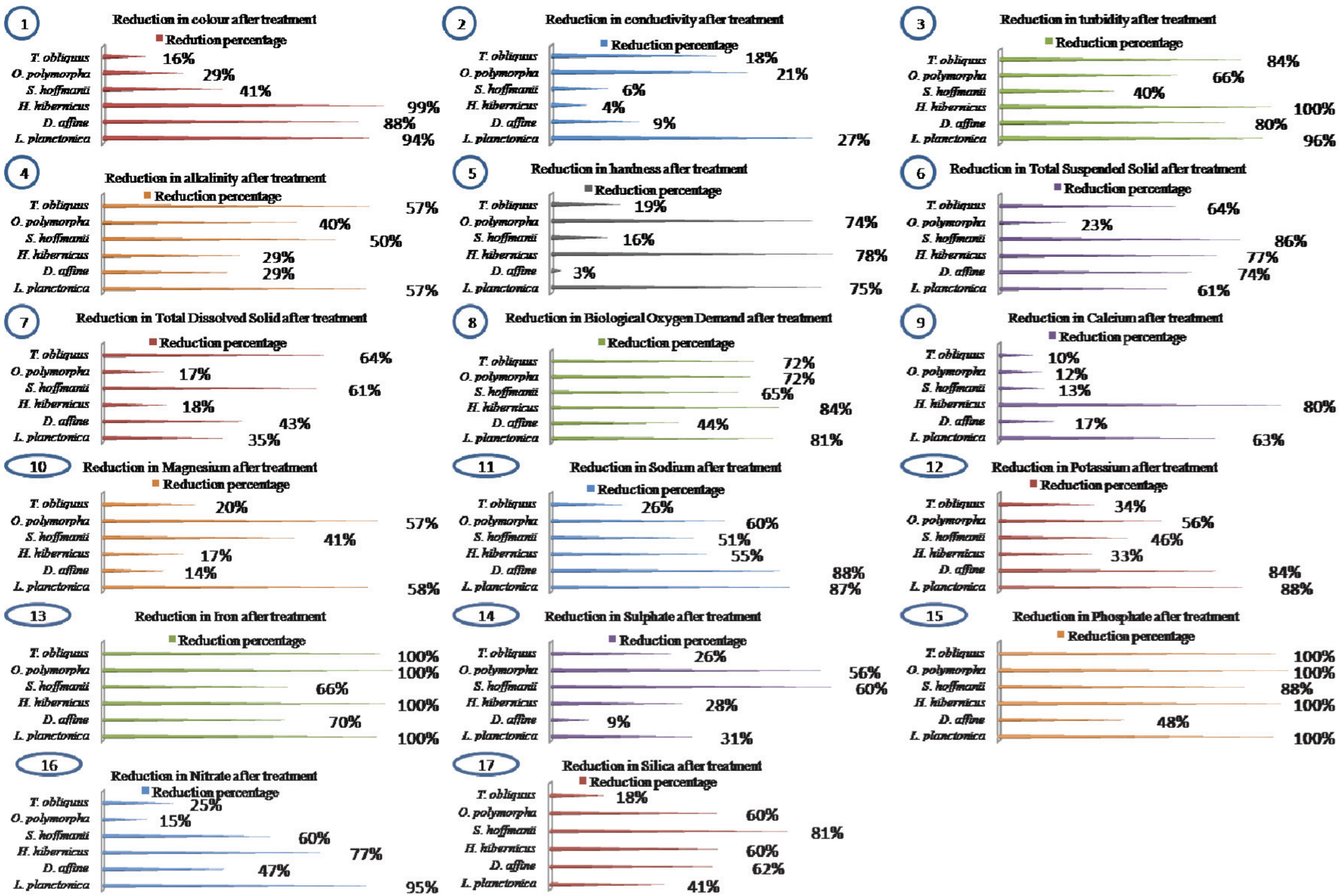


**Plate 2 (Figs. 1-17):** Comparative utilization (%) of different physicochemical parameters (colour, conductivity, turbidity, alkalinity, hardness, TSS, TDS, BOD, calcium, magnesium, sodium, potassium, iron, sulphate, phosphate, nitrate and silica) of selected concentrations of Emami paper mill wastewater by test microalgae.

Table-1

Physico-chemical characteristics of wastewater collected from effluent treatment pond (ETP) of Emami Paper Mill, Balasore.

| Colour (HU) | pH | Conductivity (µS/cm) | Turbidity (NTU) | Total alkalinity (as $CaCO_3$) (mg/L) | Total hardness (as CaCO3) (mg/L) | TSS (mg/L) | TDS (mg/L) | BOD (mg/L) | Calcium (mg/L) | Magnesium (mg/L) | Sodium (mg/L) | Potassium (mg/L) | Iron (mg/L) | Sulphate (mg/L) | Total phosphate (mg/L) | Nitrate (mg/L) | Silica (mg/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 32.4 | 8.68 | 2199 | 10.9 | 40.0 | 720.0 | 78 | 195 | 230 | 125.63 | 45.56 | 56.9 | 15.4 | 1.23 | 147.9 | 1.81 | 20.52 | 57.18 |

Table-2

Physico-chemical characteristics of selected concentrations of Emami Paper Mill wastewater (before and after utilization) collected from effluent treatment plant (ETP) suitably remediated by specific test algal species incubated at 26±1°C under continuous light of 7.5 W/m$^2$ up to 20 days.

| Test Algal species | | Colour (HU) | pH | Conductivity (µS/cm) | Turbidity (NTU) | Total alkalinity (as $CaCO_3$) (mg/L) | Total hardness (as CaCO3) (mg/L) | TSS (mg/L) | TDS (mg/L) |
|---|---|---|---|---|---|---|---|---|---|
| *Limnothrix planctonica* (75% effluent) | Before | 24.2 | 7.6 | 1649.3 | 7.8 | 30.0 | 540.0 | 58.5 | 146.3 |
| | After | 1.5 | 7.8 (-) | 1209.3 | 0.34 | 13.0 | 135.0 | 23.0 | 95.0 |
| | (% reduc.) | **(94%)** | | (27%) | **(96%)** | **(57%)** | **(75%)** | **(61%)** | (35%) |
| *Dolichospermum affine* (75% effluent) | Before | 24.2 | 7.6 | 1649.3 | 7.8 | 30.0 | 540.0 | 58.5 | 146.3 |
| | After | 3.0 | 7.7 (-) | 1506.9 | 1.6 | 21.3 | 526.5 | 15.0 | 83.0 |
| | (% reduc.) | **(88%)** | | (9%) | (80%) | (29%) | (3%) | **(74%)** | (43%) |
| *Hapalosiphon hibernicus* (50% effluent) | Before | 16.2 | 7.2 | 1099.5 | 5.5 | 20.0 | 360.0 | 39.0 | 97.5 |
| | After | 0.2 | 7.3 (-) | 1055.6 | 0 | 14.2 | 81 | 9.0 | 80.0 |
| | (% reduc.) | **(99%)** | | (4%) | **(100%)** | (29%) | **(78%)** | **(77%)** | (18%) |
| *Scytonema hoffmanii* (100% effluent) | Before | 32.4 | 8.7 | 2199.0 | 10.9 | 40.0 | 720.0 | 78.0 | 195.0 |
| | After | 18.9 | 7.4 | 2061.0 | 6.5 | 20.0 | 608.0 | 11.0 | 75.0 |
| | (% reduc.) | (41%) | **(15%)** | (6%) | (40%) | **(50%)** | (16%) | **(86%)** | **(61%)** |
| *Oocystis polymorpha* (50% effluent) | Before | 16.2 | 7.2 | 1099.5 | 5.5 | 20.0 | 360.0 | 39.0 | 97.5 |
| | After | 11.5 | 7.8 (-) | 867.0 | 1.9 | 12.0 | 93 | 30.0 | 81.0 |
| | (% reduc.) | (29%) | | (21%) | **(66%)** | (40%) | **(74%)** | (23%) | (17%) |
| *Tetradesmus obliquus* (75% effluent) | Before | 24.2 | 7.6 | 1649.3 | 7.9 | 30.0 | 540.0 | 58.5 | 146.3 |
| | After | 20.3 | 7.8 (-) | 1354.4 | 1.2 | 13.0 | 435.0 | 21.0 | 53.0 |
| | (% reduc.) | (16%) | | (18%) | **(84%)** | **(57%)** | (19%) | **(64%)** | **(64%)** |

| Test Algal species | | BOD (mg/L) | Calcium (mg/L) | Magnesium (mg/L) | Sodium (mg/L) | Potassium (mg/L) | Iron (mg/L) | Sulphate (mg/L) | Total phosphate (mg/L) | Nitrate (mg/L) | Silica (mg/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Limnothrix planctonica* (75% effluent) | Before | 172.5 | 94.2 | 34.2 | 42.7 | 11.6 | 0.9 | 110.9 | 1.4 | 15.4 | 42.9 |
| | After | 32.2 | 35.3 | 14.2 | 5.7 | 1.4 | 0 | 76.5 | 0 | 0.7 | 25.3 |
| | (% reduc.) | **(81%)** | **(63%)** | **(58%)** | **(87%)** | **(88%)** | **(100%)** | (31%) | **(100%)** | **(95%)** | (41%) |
| *Dolichospermum affine* (75% effluent) | Before | 172.5 | 94.2 | 34.2 | 42.7 | 11.6 | 0.92 | 110.9 | 1.4 | 15.4 | 42.9 |
| | After | 96.6 | 78.6 | 29.5 | 5.3 | 1.9 | 0.28 | 101.0 | 0.7 | 8.2 | 16.5 |
| | (% reduc.) | (44%) | (17%) | (14%) | **(88%)** | **(84%)** | **(70%)** | (9%) | (48%) | (47%) | **(62%)** |
| *Hapalosiphon hibernicus* (50% effluent) | Before | 115.0 | 62.8 | 22.8 | 28.5 | 7.7 | 0.61 | 73.9 | 0.91 | 10.3 | 28.6 |
| | After | 18.4 | 12.4 | 19.0 | 12.9 | 5.2 | 0 | 53.3 | 0 | 2.3 | 11.5 |
| | (% reduc.) | **(84%)** | **(80%)** | (17%) | **(55%)** | (33%) | **(100%)** | (28%) | **(100%)** | **(77%)** | **(60%)** |
| *Scytonema hoffmanii* (100% effluent) | Before | 230.0 | 125.6 | 45.6 | 56.9 | 15.4 | 1.2 | 147.9 | 1.8 | 20.5 | 57.2 |
| | After | 80.5 | 108.9 | 26.8 | 27.9 | 8.3 | 0.42 | 59.3 | 0.21 | 8.3 | 10.9 |
| | (% reduc.) | **(65%)** | (13%) | (41%) | **(51%)** | (46%) | **(66%)** | **(60%)** | **(88%)** | **(60%)** | **(81%)** |
| *Oocystis polymorpha* (50% effluent) | Before | 115.0 | 62.8 | 22.8 | 28.5 | 7.7 | 0.6 | 73.9 | 0.9 | 10.3 | 28.6 |
| | After | 32.5 | 55.3 | 9.8 | 11.3 | 3.4 | 0 | 32.4 | 0 | 8.8 | 11.5 |
| | (% reduc.) | **(72%)** | (12%) | **(57%)** | **(60%)** | **(56%)** | **(100%)** | **(56%)** | **(100%)** | (15%) | **(60%)** |
| *Tetradesmus obliquus* (75% effluent) | Before | 172.5 | 94.2 | 34.2 | 42.7 | 11.6 | 0.9 | 110.9 | 1.4 | 15.4 | 42.9 |
| | After | 48.3 | 84.5 | 27.3 | 31.5 | 7.6 | 0 | 82.1 | 0 | 11.5 | 35.3 |
| | (% reduc.) | **(72%)** | (10%) | (20%) | (26%) | (34%) | **(100%)** | (26%) | **(100%)** | (25%) | (18%) |

(-) = No reduction; (reduc.)= Reduction

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